Host-finding behaviour
of *Trichogramma brassicae*
in maize

Bastiaan Paul Suverkropp

Host-finding behaviour of *Trichogramma brassicae* in maize

aan mijn vader

Cover: *Trichogramma brassicae* on an *Ostrinia nubilalis* egg mass. Photo: FAL Zürich-Reckenholz

Stellingen behorende tot het proefschrift van B.P. Suverkropp.

1. Als aangetoond kan worden dat een parasitoïde onder laboratoriumomstandigheden op een bepaalde prikkel reageert, wil dat nog niet zeggen dat die prikkel het zoekgedrag van de parasitoïde in het veld beïnvloedt.

    Frenoy, C.; Durier, C.; Hawlitzky, N., 1992. Journal of chemical ecology, 18: 761-773.
    Frenoy, C.; Farine, J. P.; Hawlitzky, N.; Durier, C., 1991. 6th European Workshop on Insect Parasitoids. Perugia 3-5 April, 1991. Redia, 74: 143-151.
    Renou, M.; Hawlitzky, N.; Berthier, A.; Malosse, C.; Ramiandrasoa, F., 1989. Entomophaga, 34: 569-580.
    Dit proefschrift.

2. Gezien de zoekstrategie van *Trichogramma brassicae* is het voor deze sluipwesp essentieel dat veel planten onderzocht kunnen worden. Verlenging van de gemiddeld korte verblijftijden per blad leidt tot een vermindering van de kans om eimassa's van de gastheer te ontmoeten.

    Dit proefschrift.

3. Op planten met kairomonen blijft *Trichogramma brassicae* langer zoeken dan op schone planten, maar ze wordt niet door kairomonen aangetrokken tijdens de vlucht, zoals geconcludeerd is door Smits (1982).

    Smits, P. H., 1982. Les Trichogrammes, Antibes, Les Colloques de l'INRA, 9: 139-150.
    Dit proefschrift.

4. Theorieën over optimale zoekstrategieën die zijn gebaseerd op het vergelijken van diverse plekken met gastheren door parasitoïden zouden alleen gebruikt mogen worden als men een indicatie heeft van de ontmoetingskans van de betreffende soort met gastheren in een veldsituatie. Tot nu toe ontbreekt deze informatie.

    Godfray, H.C.J., 1994. Parasitoids. Princeton University Press, Princeton, NJ: 26-81.

5. Genetische manipulatie van nuttige organismen kan leiden tot efficiëntere biologische bestrijding, maar dit weegt op korte termijn niet op tegen het verlies van het beeld van biologische bestrijding als een natuurlijke en natuurvriendelijke manier van gewasbescherming.

    Hoy, M., 1994. In: Insect Parasitoids: Biology and Ecology. Proceedings from the 5th European Workshop on Insect parasitoids, Biri, Norway, 24-28 May 1994: 9-39.

6. Voor een effectief gebruik van simulatietechnieken dient men zich voortdurend te realiseren dat simulatiemodellen een middel en geen doel zijn.

7. Kwaliteitscontrole helpt een brug te slaan tussen sluipwespen als project en sluipwespen als produkt.

8. De opmerking "Man should stop fighting among themselves and start fighting insects" moet tegenwoordig luiden "Man should stop fighting among themselves and make insects fight each other."

    Naar Luther Burbank (1849-1926) In: Seldes, G. (ed.), 1960. The Great Quotations. Lyle Stuart, New York.

9.   Er zijn genoeg theorieën voor afwijkende sex-ratio's bij parasitoïden, maar nog niemand heeft de oorzaken van de extreme ondervertegenwoordiging van vrouwen bij de amateurentomologen onderzocht.

Godfray, H.C.J., 1994. Parasitoids. Princeton University Press, Princeton, NJ: 151-211.
Ellis, W.N., 1995. In: Koomen, P., Ellis, W.N., van der Geest L.P.S. (eds.), 1995. Insekten onderzoeken. Nederlandse Entomologische Vereniging, Amsterdam: 189-196.

10.   Het is merkwaardig dat veel mensen insecten als 'vieze beesten' classificeren, terwijl insecten om te kunnen overleven doorgaans grondig schoon moeten zijn en zich daarom voortdurend poetsen.

Bernard Werber, B., 1991. Les Fourmis. Albin Michel, Paris.

11.   Het falen van de verkoop van Extenso graanproducten (graan uit geïntegreerde produktie) in de winkel in Zwitserland geeft aan dat de consument landbouwprodukten slechts in 'biologisch' en 'niet biologisch' kan of wenst te onderscheiden.

12.   De tijd die men wint door goed met software om te kunnen gaan, gaat weer verloren aan hulp aan collega's die softwareproblemen hebben.

13.   Als je gelooft dat je iets niet kunt, dan lukt het je ook niet, hoe simpel het ook is.

Urd, 1992. In: Fujishima, K., 1992. Aa Megami-sama. Kodansha, Tokio. 188 pp.

14.   Het is merkbaar dat beslissingen over het openbaar vervoer grotendeels gemaakt worden door mensen die het zelf niet gebruiken.

15.   Iedereen die graag in tweedehands boekwinkels rondsnuffelt, kent de efficiëntie van lukraak zoeken.

Contents

Voorwoord

It is because you are a very small animal that you will be Useful in the adventure before us.

A.A. Milne "Winnie the Pooh"

Dit proefschrift is het resultaat van vijf jaar werk met *Trichogramma brassicae* aan de Eidgenössische Forschungsanstalt für landwirtschaftliche Pflanzenbau, tegenwoordig Eidgenössische Forschungsanstalt für Agrarökologie und Landbau Zürich-Reckenholz, en aan de vakgroep entomologie van de Landbouwuniversiteit Wageningen. Tijdens deze tijd heb ik van vele mensen assistentie en ondersteuning gekregen, die het onderzoek gemaakt hebben tot wat het is, en die ik bij deze hartelijk wil bedanken voor hun bijdrage.

Allereerst Joop van Lenteren, die bereid was als promotor voor het proefschrift te fungeren. Hoewel hij in Wageningen zat en ik in Zwitserland, waardoor we elkaar niet zo vaak zagen, heeft de 'begeleiding op afstand' goed gefunctioneerd. Hij was altijd goed op de hoogte van mijn werk, voorzag mijn artikelen snel van gedegen kritiek, en hield de grote lijn van mijn onderzoek in de gaten.

Franz Bigler war zuständig für die tägliche Betreuung von meiner Dissertation. Er war immer offen für anregende Diskussionen und stellte seine reiche Erfahrung, Praxiskenntnisse und unpublizierte Daten über *Trichogramma* und *Ostrinia* zur Verfügung. Er hat auch das Stipendium von Ciba-Geigy organisiert, dass dieses Projekt ermöglicht hat. Stephan Bosshart hat Riesenmengen *Trichogramma* und *Ostrina* für mich gezüchtet. Hätte ich die Zuchten selber aufbauen und betreuen müssen, dann hätte ich mindestens ein Drittel weniger Zeit für meine Forschung gehabt. Auch sonst hat er mir immer mit gutem Rat und in praktischen Dingen geholfen. Auch Mario Waldburger, immer parat um mein Deutsch zu korrigieren, und Werner Jossi haben mir bei vielen praktischen Fragen zur Seite gestanden. Ana Dutton war einen Sommer lang meine Mitarbeiterin und hat in dieser Zeit viele Experimente mitgemacht. Auch inhaltlich hat sie einen wichtigen Beitrag zu meiner Dissertation geleistet. Ausserdem hat sie mir beigebracht, Huitlacoche (*Ustilago maydis* = Beulenbrand) zu kochen. Joel Schwarz bracht in het kader van zijn stage vele nachten in het insectarium door met het observeren van *Ostrinia*, en vele middagen in de kas met het observeren van *Trichogramma*. Professor Wolfgang Nentwig von der Universität Bern war bereit, mein Schweizer Doktorvater zu sein. Mein 'roommate' Fabio Cerutti hat mir an Anfang der Dissertation sehr geholfen, das Reckenholz kennen zu lernen. Die anregende Diskussionen mit ihm, und mit Stephan Blanché und Jerome Casas von der ETH Zürich, habe ich sehr geschätzt. Padruot Fried hatte als Abteilungsleiter immer eine offene Tür und hat sich sehr interessiert für meine Forschung. Im Reckenholz haben mir nicht nur viele Leute geholfen, es war auch schön mit meinen 'roommates' und Kollegen innerhalb und ausserhalb die Arbeitszeit zusammen zu sein. Also, Gerhard, Angelica, Dani, Andrea, Barbara, Padruot, Ruth, Andrea, Ursula, Annette, und alle andere: es isch d'Plausch gsii!

Das Projekt wurde finanziert von Ciba-Geigy (jetzt Novartis). Ich möchte meinen Kontaktpersonen Philip Newton und Claude Brassel für ihr Interesse danken.

De laatste fase van het onderzoek, de simulaties, werd in Wageningen uitgevoerd. Dankzij het internet hoefde ik maar weinig tijd daadwerkelijk in Wageningen te zijn. Op de vakgroep Entomologie werd ik bij mijn sporadische bezoeken als de verloren zoon ingehaald. Herman van Roermund van de vakgroep Entomologie stelde zijn simulatiemodellen voor

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Encarsia formosa ter beschikking als uitgangspunt voor het *Trichogramma*-model, stond altijd klaar voor discussies over simulatie en heeft zeer nuttige kritiek op de simulatiehoofdstukken geleverd. Daniël van Kraalingen (AB-DLO, Wageningen) richtte de TTUTIL-simulatieomgeving in. Yke Syb Ykema (Vakgroep I&D, Wageningen) wijdde mij in in de geheimen van de Alpha-Vax en hielp om de simulaties draaiende te houden terwijl ik terug in Zürich was. Hij en mijn andere D&D-vrienden uit Wageningen waren ook een gewaardeerde bron van afleiding als ik mijn werk helemaal uit mijn hoofd wilde zetten.

Mijn ouders hebben mij de kans gegeven te studeren, en mijn interesse in insecten altijd gestimuleerd. Het spijt me dan ook meer dan ik kan zeggen dat mijn vader de voltooiing van dit proefschrift niet meer mee heeft kunnen maken. Mijn moeder en schoonouders hebben me bij de eindspurt nog zeer geholpen door in Zürich en Wageningen op Zina te passen. Tenslotte, Jannie, samen zijn we aan het 'avontuur Zwitserland' begonnen. Inmiddels zijn we een dochter, anderhalf proefschrift, twee trouwringen en vele ervaringen rijker. Ik had het niet zonder je willen doen.

Ik hoop dat dit proefschrift kan bijdragen aan de verdere ontwikkeling van de biologische bestrijding als deel van een milieuvriendelijke landbouw, en dat ik ook zelf op dit gebied werkzaam kan blijven, want, naar de woorden van Lafcadio Hearn:"All that nightmare ever conceived of faceless horror, and all that ecstasy ever imagined of phantasmal pulchritude, can appear but vapid and void by comparison with the stupefying facts of entomology."

Chapter 1

A simple tritrophic system: *Trichogramma brassicae-Ostrinia nubilalis-Zea mays.*

INTRODUCTION

Trichogramma brassicae Bezdenko is a parasitic wasp that reproduces by ovipositing in the eggs of other insects, mainly lepidopterans. In Switzerland and other European countries, it is released in maize to control the pest *Ostrinia nubilalis* Hübner. Together with the primary host plant of *O. nubilalis*, maize, the herbivore and the parasitoid form a tritrophic system. The main events in this system during the growing season in Switzerland are as follows: Maize is planted in spring. In May *O. nubilalis*, which overwinters in the larval stage inside maize stubbles, pupate, hatch and mate. The females then start ovipositing in the maize crop. *O. nubilalis* egg masses can be found in the field from mid-June to mid-August, depending on the weather. At the first appearance of *O. nubilalis* egg masses in the field, cards with moth eggs parasitized by *T. brassicae* are placed twice in maize fields with a 10-14 day interval between the releases. The parasitoids hatch, mate, and the females start searching for lepidopteran eggs. In maize fields, the only available eggs are egg masses of *O. nubilalis*. Ten to twenty days after parasitization, depending on the weather, the first *T. brassicae* start hatching from the parasitized egg masses. During the rest of the *O. nubilalis* oviposition period, the released *T. brassicae* and their descendants form a population in the maize field that parasitizes most of the available *O. nubilalis* egg masses. Unparasitized host eggs hatch, and the *O. nubilalis* larvae start eating the maize plants, boring into the stems and eventually moving to the base of the stem where they will overwinter.

It is clear that this tritrophic system is artificial and relatively recent. Its three elements came together only after the introduction of maize in Europe. *O. nubilalis* might have been a host of *T. brassicae* before the introduction of maize, but this is not known. In some regions, high natural parasitism of *O. nubilalis* egg masses in maize fields by *T. brassicae* occurs (Maini *et al.*, 1982; Uzun, 1995). Mass releases of T. brassicae have been made in Switzerland, where this research project was conducted, since 1978 (Bigler, 1983), but natural parasitism by *T. brassicae* is negligible. The reason for this is still unclear, as *T. brassicae* is able to find alternative hosts and to overwinter in Switzerland (Suverkropp, unpubl.).

It is assumed that host-finding of parasitoids in their natural environment is optimized because it is under constant evolutionary pressure (Godfray, 1994). However, in an artificial tritrophic system where the parasitoid has to be reintroduced every year, this natural selection does not occur. *T. brassicae* is also quite polyphagous (Li, 1994; Pak, 1988; Suverkropp, unpubl.). This means that the host finding behaviour of *T. brassicae* is not necessarily adapted especially to either *O. nubilalis* or maize. Still, because parasitoid, host and host plant interact, the behaviour of *T. brassicae* can only be understood and manipulated in the context of the tritrophic system. I will therefore give a short description of the three elements of the system.

Trichogramma brassicae

Description and taxonomy of Trichogramma

Trichogramma brassicae belongs to the chalcidoid wasp family Trichogrammatidae. The species has no common name. It is a small (0.5 mm) parasitoid that parasitizes the eggs of lepidopterans. Both males and females have rather chunky brown to dark brown bodies, wings with simple venation and a hairy fringe, and bright red eyes. The sexes can be distinguished by their antennae, which are hairy and rigid in the male and hairless and mobile in the female.

    T. brassicae was first found in the former soviet republic of Moldavia and described by Bezdenko (1968), in a relatively obscure publication. Unfortunately, the type is missing (Sorokina, 1992). In the 1970s, breeding material described as *T. evanescens* was collected from *Ostrinia nubilalis* (European corn borer) egg masses in Moldavia and exported for biocontrol purposes to Antibes, France (Voegelé *et al.*, 1975). Pintureau and Voegelé found that this material was sufficiently different from *T. evanescens* to constitute a different species, which they named *Trichogramma maidis* Pint. et Voeg. (Pintureau and Voegelé, 1980). In 1990, it became clear that *T. maidis* Pint. et Voeg. had already been described as *T. brassicae* Bezdenko. The different names *T. evanescens* Westwood (Moldavian strain), *T. maidis* Pint. et Voeg. and *T. brassicae* Bezdenko have all been used for wasps descending from the same breeding material. The Swiss *T. brassicae* Bezdenko stock was taken from the Antibes population in 1975 (Bigler, pers. comm).[1]

    The genus *Trichogramma* was first described by Westwood (1833). The genus *Trichogramma* can be found in all ice-free habitats, but their relative abundance and species richness in the different regions is mostly unknown (Nagarkatti and Nagaraja, 1977). Up to date, 140-160 species have been described, depending on the author (Nagarkatti and Nagaraja, 1977; Voegelé and Pintureau, 1982; Viggiani and Laudonia, 1989). Species identification is difficult, and many species descriptions are not universally accepted, which complicates the selection of *Trichogramma* for biological control programmes (Knutson, 1981; Goulet and Huber, 1993; Pinto and Stouthamer, 1994).

Biological control with Trichogramma

Trichogramma species have long been used for biological control. The first use of *Trichogramma* in classical biological control (i.e. inoculative releases) was made by Radeckij (1911) in Russian Turkestan, using *T. evanescens* against *Cydia pomonella* in orchards. Inundative methods in cabbage against *Pieris brassicae* and *Barathra brassicae* were tried in Germany by Voelkel (1925). The first breakthrough in *Trichogramma* technology was made by Flanders (1927), who developed a method for rearing *T. minutum* on eggs of Sitotroga

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[1]A new species found in France by Pintureau and Voegelé in 1982 was called *Trichogramma brassicae* Pint. et Voeg. (Voegelé, 1982), because the authors were not aware that the name had already been used by Bezdenko. The species was later renamed *T. buesi* (Voegelé, 1985).

cerealella. Rearing *Trichogramma* on factitious hosts greatly lowered the costs and efforts of production and made inundative releases possible. From the 1930's, *Trichogramma* have been used more or less successfully in many countries against pests in arable crops, orchards, forests and stored products (Kot, 1964). A second breakthrough in the use of *Trichogramma* for biocontrol was the development of storage techniques, using the parasitoids natural diapause and quiescence behaviour (Voegelé, 1976). Two relatively recent developments with great potential for further increasing effectiveness and economic competitiveness of *Trichogramma* biocontrol are artificial host eggs and the development of selection and quality control procedures. Artificial host eggs would abolish the need for rearing host eggs, which could result in cheaper production. At the moment, large-scale production of *Trichogramma* species on artificial eggs is only used in China (Grenier, 1994). Selection and quality control procedures are both based on the same principle: that certain parameters of the parasitoids behaviour can be measured in the laboratory and give an indication of the parasitoids potential performance in the field as a biocontrol agent. Selection concerns itself with selecting the right *Trichogramma* for application in a specific agricultural ecosystem from the large number of species and strains available worldwide (Hassan, 1994). Quality control involves measuring the quality of *Trichogramma* after rearing. This allows producers to check the quality of their product before release, farmers or farmers organisations to check the quality of products sold and governments to set quality standards for biocontrol producers (Bigler, 1994).

  Trichogramma species are used for biological control in many countries, the largest users being the former USSR, China and Mexico (Li, 1994). *T. brassicae* is mainly used against *Ostrinia nubilalis*, but also against *Pieris* spp. and *Mamestra brassicae* in Europe and the Middle East (Li, 1994). In Switzerland, *T. brassicae* is released yearly on about 7000 ha of maize against *O. nubilalis*, and in the whole of Western Europe on 33.000 ha.

Ostrinia nubilalis

Biology of O. nubilalis

Ostrinia nubilalis (former synonyms *Botys zealis, Pyrausta nubilalis* and *Pyralis nubilalis* (Caffrey and Worthley, 1927)) is a lepidopteran of the family Pyralidae, which was first described by Hübner (1796). The common name is European Corn Borer. Females are yellowish with some brown coloration, with a wing spread between 25 to 35 mm and a body length of 15 mm. Males are brown with a wing spread of 22 to 28 mm and a body length of 14 mm. Apart from size and colouration, the abdomen clearly differentiates the sexes. In the males it is pointed and extends beyond the wings, in the female it is shorter and thicker. The overall impression of male and female is quite different and it is not surprising that at first male and female were described as different species (Hübner, 1796).

  It is widely distributed in the northern hemisphere, occurring in Europe, West Asia and the northern part of China, northern Africa and North America. It can adapt itself to many habitats from arid to humid and from warm to cold. Its origin is unknown but probably southern Europe (Beck, 1987). It was introduced in North America around 1912 but spread

very rapidly (Caffrey and Worthley, 1922). In Switzerland, the pest was endemic in some traditional maize growing areas. It spread rapidly in the 1970's when hybrid cultivars made maize growing possible in most parts of the country. Now, it is present in most of the maize-growing areas north and south of the Alps (Bigler and Bosshart, 1992).

The original host plants are unknown, but may have been millet (*Panicum miliaceum* L.) or hops (*Humulus* spp.). *O. nubilalis* is quite polyphagous and has been found on over 200 different plants (Poos, 1927; Hodgson, 1928; Lewis, 1975; Beck, 1987). Its preferred host is maize on which it is an important pest in the whole area of its distribution. In western Europe north of the Loire and the Alps, and the northern states of the USA and in Canada, there is only one generation per year. In warmer areas, two or even more generations per year are possible (Beck, 1987).

The first two larval instars feed mainly on the leaves. The third to fifth instars bore into the stem and work their way down, thus severely weakening the stem of the maize plant, which often breaks (Beck, 1956; Guthrie *et al.*, 1971). Weight of cobs and plant dry weight is reduced by larval feeding. The stem might break as a result of tunnelling, resulting in an even stronger reduction (Bigler *et al.*, 1990). The holes made by the larvae also increase fungal infections (*Botrytis* spp. and *Fusarium* spp.). The acceptable level of damage depends on the use of the maize; In grain and silage maize the threshold levels are higher than in sweet maize and seed maize (Boivin *et al.*, 1988).

O. nubilalis can also be a pest of economic importance in beans, peppers, apple, sunflower, hops, cotton and other plants (Elliott *et al.*, 1982; Eckenrode *et al.*, 1983; Straub *et al.*, 1986; Savinelli *et al.*, 1986; Legg *et al.*, 1986). In Switzerland, *O. nubilalis* is a pest of maize, but also of beans.

Natural enemies and control of O. nubilalis

The egg masses of *O. nubilalis* are readily eaten by polyphagous predators such as coccinellids, chrysopids, nabids, anthrocorids and mirids. The eggs are parasitized by several trichogrammatid parasitoids, such as *Trichogramma brassicae* Bezdenko, *T. evanescens* Westwood, *T. ostriniae* Pang et Chen, *T. dendrolimi* Matsumura, *T. nubilale* Ertle et Davis, *T. pretiosum* Riley, *T. minutum* Riley, *T. exiguum* Pinto et Platner ,*T. pintoi* Voeg. and *T. fuentesi* Torre (Baker *et al.*, 1949; Yin *et al.*, 1983; Manojlovic, 1984a; Birova, 1988; Thomson and Stinner, 1989; Maini and Burgio, 1990; Wilson and DuRant, 1991; Hintz and Andow, 1990; Li, 1994; Fursov, 1995). Larvae are parasitized by Hymenopteran and Dipteran parasitoids (Wressell, 1973; Voegelé *et al.*, 1975; Manojlovic, 1984b; Barbattini, 1989; Hudon *et al.*, 1989; Grenier *et al.*, 1990; Maini and Burgio, 1990), and eaten by generalist insect, arachnid and avian predators (Whitman, 1975; Quiring and Timmins, 1988). Two diseases, the fungus *Beauveria bassiana* Vieillemin (Winnie *et al.*, 1981) and the protozoan *Nosema pyrausta* Paillot (Lewis and Lynch, 1976; 1978) also cause significant mortality in the field.

Ostrinia nubilalis is controlled in a number of countries with inundative releases of trichogrammid parasitoids (Li, 1994). The larvae of *O. nubilalis* can also be controlled with pesticides. *Bacillus thuringiensis* Berliner var. *kurstaki* has been used as a spray or granular

pesticide (Hudon and Martel, 1977; Lynch *et al.*, 1980). Experiments have been made with the confusion method through application of the male sex pheromone (Buechi *et al.*, 1981) and the sterile male technique (Zanaty and Shenishen, 1988). Ploughing of winter stubble can be very effective for corn borer control. If all maize stubble can be ploughed under to a depth of at least 20 cm, the larvae overwintering in the stalks will not be able to emerge in spring (Hudon *et al.*, 1989). This can kill up to 90% of the overwintering population (Langenbruch, 1981).

Breeding of maize for resistance has only been partially successful (Guthrie, 1980; Guthrie, 1988). A new approach in resistance breeding are genetically engineered maize plants that can produce *Bacillus thuringiensis* toxins. Corn borer larvae on these plants will usually die within 48 hours (Koziel *et al.*, 1993).

Zea mays

Zea mays, the only species of the genus *Zea*, is a gramineaous plant of the subfamily *Panicoideae*, known as Maize or Corn. The plant reaches a height of about 2.5 meter in Switzerland, with up to 14 leaves and a total leaf area of 5000 cm^2 (Garibay, unpubl.). It originates from Central America. Due to its high yield and adaptability to many climates and soil types, it is now grown worldwide on 134.606.000 ha (FAO, 1995), mainly as forage, although it is also used for human consumption.

In Switzerland, maize was introduced in the 18th century (Schütt, 1972). After the second world war, it became a popular forage crop and now covers approximately 60000 ha (FAO, 1995). It is planted in April and harvested in August (Schütt, 1972), with a density of 8.9 plants/m^2. The soil underneath the maize plants is usually barren, although other systems are now being considered (Bigler *et al.*, 1995). The main insect pests attacking maize in Switzerland are *Ostrinia nubilalis*, aphids, *Oscinella frit*, elaterids and tipulids. Aphid populations are usually kept in check by natural enemies. Elaterids and tipulids are controlled with soil granulates. Since *O. nubilalis* is biologically controlled with *T. brassicae*, no other insecticides are normally used in Swiss maize cultivation.

RESEARCH AIMS

The host-finding behaviour of *T. brassicae* is an important object of study for two reasons. By studying host finding behaviour of *T. brassicae*, our understanding of parasitoid behaviour in general can be increased. Although there is an extensive literature on searching behaviour by parasitoid wasps, research has tended to concentrate on larval parasitoids (Godfray, 1994). Much less is known about searching by generalist egg parasitoids such as *T. brassicae.*

Studying host finding behaviour of *T. brassicae* can also improve biological control with *Trichogramma*. *Trichogramma* species have been used in biological control for a long time. However, most applications of *Trichogramma* are based on trial and error experiments and on experience (Hassan, 1994). It has been tried to base selection of *Trichogramma* for biocontrol on scientific criteria, and also to establish quality parameters for *Trichogramma* production. Because detailed and quatitative information about the searching system is lacking, the effect of any selection or quality parameter on *Trichogramma* performance as a biocontrol agent is hypothetical. Also, it is not possible to establish what the constraints are that make *Trichogramma* unsuccessful in some biocontrol systems.

The process which leads parasitoids to oviposition can be divided in host-habitat location, host location and host acceptance (Doutt, 1964). Host-habitat location is not important in the *T. brassicae-O. nubilalis-Z. mays* system, because the parasitoids are released within the crop. Host acceptance has been studied quite extensively and both qualitative and quantitative data are available (Schmidt, 1994). This study will therefore concentrate on the host-location behaviour, which is relatively unknown.

Many factors have been shown to influence *Trichogramma* parasitism in the field. First, the propensities of the released parasitoids themselves. Walking speed (Bigler *et al.*, 1988) and overall quality, which include longevity, fecundity and host-acceptance (Dutton *et al.*, 1996) have a direct effect on *T. brassicae* parasitism in the field. Second, the behaviour of the host, as the density of the host will influence how many egg masses individual parasitoids can find, and because *T. brassicae* reacts to *O. nubilalis* kairomones (Kaiser *et al.*, 1989a; Renou *et al.*, 1989; Bieri *et al.*, 1990; Frenoy *et al.*, 1991; 1992). Third, host plant aspects. The effect of crop size has been studied for *T. maidis* (=*brassicae*) (Maini *et al.*, 1991) and *T. nubilale* (Need and Burbutis, 1979) in maize. Fourth, environmental effects like weather. A correlation between field temperatures and parasitism in *T. brassicae* was shown by Dutton *et al.* (1996). Finally, the numbers released *of T. brassicae*, will influence parasitism. The effects of all these factors have usually been described qualitatively only.

The aim of this study is to describe the searching behaviour of *T. brassicae* in maize fields, both qualitatively and quantitatively. More specifically, data will be collected on movement behaviour of *T. brassicae* on plants and between plants. Using these data, a simulation model will be developed to quantify what the chances are that the parasitoid encounters an egg mass during a certain period of time in the field. With the simulation model, the effect of parasitoid, host, host plant and environmental factors on the encounter probability will be quantified.

OUTLINE OF THE THESIS

The current knowledge about the oviposition behaviour of the host *O. nubilalis* is summarized in Chapter two. Some experiments are made to provide additonal data. Egg masses are collected in the field to determine the egg mass distribution on maize at the leaf level. Direct observations of *O. nubilalis* are made to establish its movement patterns on maize plants and its oviposition behaviour. The distribution of host scales, which *O. nubilalis* leaves on the plant when ovipositing, is also studied.

In Chapter three, the current knowledge of dispersal and host-finding of *T. brassicae* and of other trichogrammids is reviewed.

The dispersal behaviour of *T. brassicae* is studied in the field, using glue-sprayed trap plants (Chapter four). Dispersal speed in the field is estimated. The results of trapping *T. brassicae* on plants are compared with parasitism on plants in the same field, to see whether there is a good correlation between dispersal and parasitism. The distance of the first flight from the release plant is also measured.

The landing behaviour of *T. brassicae* is studied in the field and in the greenhouse in Chapter five. In the greenhouse, landing is observed directly and by using glue-sprayed plants. In the field, landing positions are determined using glue-sprayed plants. The effect of row position of the plant, release height, and presence of host kairomones on the plant on the number and position of parasitoids landing is also studied.

In Chapter six, the behaviour of *T. brassicae* on the plant is studied. Movement on the plant is observed directly to measure residence times, number of leaves visited, and between-leaf movement patterns. The effect of *O. nubilalis* scales and volatiles on host-finding and residence times is also studied.

The influence of temperature on walking speed and walking activity of *T. brassicae* is assessed in Chapter seven.

In Chapter eight, a model is described that integrates the information of previous chapters. Searching of *T. brassicae* is simulated at leaf and plant level. Sensitivity analyses are performed to find out which parasitoid, host and environmental factors have the strongest influence on host encounter probability.

The plant model from the previous chapter is extended to a crop model in Chapter nine, to estimate encounter probability of *T. brassicae* with host egg masses in the field. The effect of parasitoid, host, host plant and environmental factors on encounter probability is simulated.

In the final Chapter, the results are summarized and discussed. Based on the simulation results, recommendations for biological control and quality control are made. Implications of the simulation results for parasitoid foraging theory are also discussed.

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Chapter 2

Mating and oviposition behaviour of *Ostrinia nubilalis*, the European corn borer

ABSTRACT

A review of *Ostrinia nubilalis* oviposition behaviour is given. The position of egg masses on the maize plants and the leaf were sampled in the field. Most egg masses were found on the lower leaf side, on the middle part of the leaf or closer to the stem, and close to the mid-rib. Direct observations of oviposition behaviour were made in climate rooms and field cages. *O. nubilalis* moved very little on the plants and only 10 % of the females that landed on the plants oviposited. Females oviposited where they landed, and walked only a few centimetres if at all. Near the egg masses, scales of the adult were not abundant, 37% had no scales in the vicinity, and 45% only a few scales. Many scales were found on other places of the plants. Thus, scales are not an indicator for the presence of egg masses in their immediate vicinity. At the leaf and plant level, scales might serve as a useful host-cue to *Trichogramma brassicae*, an egg parasitoid of *O. nubilalis*.

INTRODUCTION AND REVIEW OF OSTRINIA NUBILALIS OVIPOSITION BEHAVIOUR

Ostrinia nubilalis Hübner (Lepidoptera: Pyralidae), the European corn borer, is an important pest of maize in most temperate regions of the northern hemisphere. In order to understand the host-finding behaviour of its egg parasitoid *Trichogramma brassicae* Bezdenko, it is necessary to know the behaviour of its host. *T. brassicae* is not specialized on *O. nubilalis* or maize. It parasitizes other lepidopteran eggs on other host plants (Suverkropp, unpubl.). The egg stage of lepidopterans is hard to detect, because it does not feed and thus produces no frass and excrements. Parasitoids can improve their chances to detect hosts by using host-cues produced by a stage of the host that is more detectable than the stage that the parasitoid is searching for (Vet and Dicke, 1992). This infochemical detour is used by *T. brassicae*, which reacts to scales and sex pheromones of the adult *O. nubilalis* (Smits, 1982; Kaiser *et al.*, 1989; Renou *et al.*, 1989, 1992; Bieri *et al.*, 1990; Frenoy *et al.*, 1991, 1992). Whether these adult cues are reliable depends on the behaviour of the adult, which determines where these cues will be found in time and space. Learning is used by parasitoids to connect stimuli that are easy to detect but unreliable with stimuli that are hard to detect but reliable cues to host presence. *T. brassicae* can associate the odour of maize with the presence of *O. nubilalis* egg masses after an oviposition experience (Kaiser *et al.*, 1989), but the reaction to host scales of *Mamestra brassicae* was not influenced by oviposition experience (Gardner and van Lenteren, 1986). A generalist might have a strong fixed response to cues that are common to all its hosts (Vet and Dicke, 1992), but in that case it is unable to optimize its responses by learning. The more fixed the responses to scales, the stronger differences in scale distribution between host species will affect the parasitoid.

Flight and mating

In Switzerland and southern Germany, *O. nubilalis* adults are present from the end of June till August, with a peak in July. In Switzerland south of the Alps, a second generation has its peak in August and September (Bigler and Brunetti, 1986). Because of crop rotation in Switzerland, the fields in which *O. nubilalis* emerge will usually not be maize fields. Following emergence, the moths spend the first night in the fields of emergence where they mate (Buechi *et al.*, 1981; Cordillot, 1989). Although a single mating suffices to fertilize all the eggs of the female, multiple matings are possible (Poos, 1927; Cordillot, 1989). The second night, females will migrate to the maize fields (Cordillot, 1989). The males stay behind but will follow the next few days, attracted by the sex pheromones of still unmated females. From then on, the moths migrate daily in and out of the field. Daytime is spend in the grassy borders of the fields (Derridj *et al.*, 1986; Cordillot, 1989). During the night *O. nubilalis* enters the maize field to oviposit, and to drink the dew from maize plants, which contains sugars (Derridj et al., 1986). Flight takes place in the first three hours of darkness (Broersma *et al.*, 1976). The moths fly low, usually less than one meter above the vegetation (DeRozari *et al.*, 1977). Mating and flight are inhibited by low temperatures (Hudon *et al.*, 1989), heavy rains (Oloumi-Sadeghi *et al.*, 1975) and strong winds (Loughner and Brindley, 1971; Sappington and Showers, 1983). Light rains stimulates *O. nubilalis* activity (Everett *et al.*, 1958; Barlow and Mutchmore, 1963; Kira *et al.*, 1969).

Oviposition

The oviposition period ranges from one to 28 days with an mean of 12.8 days (Poos, 1927). Oviposition, which has an endogenous circadian rhythm (Skopik and Takeda, 1980), and is influenced by the weather, mainly takes place in the first two hours after dark (Caffrey, 1928; Cordillot, 1989). Various factors stimulate or deter oviposition. Certain growth stages of the maize plant are preferred for oviposition (Derridj *et al.*, 1989), and the largest and most developed plants are preferred (Patch, 1942; Horber, 1961). Plant extracts were shown to have some attraction for *O. nubilalis* (Cutright and Huber, 1928; Moore, 1928; Cantelo and Jacobson, 1979). Compounds on the leaf surface, such as glucose, will stimulate oviposition (Derridj and Fiala, 1983; Fiala *et al.*, 1985; Derridj *et al.*, 1992). Pentane extracts of the whorl and tassel stages of maize stimulate oviposition, but extracts of an earlier and later developmental stage do not (Udayagiri and Mason, 1995). Eggs (Thiery and le Quere, 1991), larval frass (Dittrick *et al.*, 1983) and damaged maize plants (Schurr and Holdaway, 1966) emit volatiles which repel gravid females. Thus, females avoid oviposition on plants where egg masses or larvae are already present. When egg-laying is about to begin, the moths hover about one meter above ground (Hudon and LeRoux, 1986a).

Oviposition itself was observed by Poos (1927) and Caffrey and Worthley (1927). To oviposit, the female sits on the leaf with its head upward. The end of the abdomen is bend down and the ovipositor extruded. When the ovipositor touches the leaf, the abdomen is pulsed rapidly until an egg emerges. The round egg is pushed against the leaf, and is flattened with the tip of the ovipositor. Eggs are laid in an overlapping pattern. Females do

not walk during the oviposition of an egg mass, unless they produce a narrow egg mass with many eggs. Laying a normal egg mass will take about 5 minutes. Leaf folds seem to be preferred for oviposition (Abel *et al.*, 1995).

The size of egg masses varies from 1 to 64 eggs, with an mean of 17 eggs (Poos, 1927; Caffrey and Worthley, 1927). The size is not influenced by temperature (Gohari and Hawlitzky, 1986) or position on the plant (Vaillant and Hawlitzky, 1990). During its lifetime, the female can produce about 350 eggs (Caffrey and Worthley, 1927; Reh and Ohnesorge, 1988). Egg masses are held together and stuck to the leaf with a compound produced by the abdominal glands (Beck and Stauffer, 1950).

Distribution of egg masses

Within-field egg mass distributions
Most of the work on egg mass distribution in the field has been done in order to establish accurate and inexpensive sampling procedures for predicting *O. nubilalis* population densities. All three types of theoretical distribution have been observed for *O. nubilalis*. If *O. nubilalis* would choose maize plants at random, the number of egg masses per plant should be Poisson distributed, which is actually the most common distribution found in the field (McGuire *et al.*, 1957; Chiang and Hodson, 1959; Calvin *et al.*, 1986; Coll and Bottrell, 1991; Sorenson *et al.*, 1993). If *O. nubilalis* would have a clear preference for certain plants within the field, an aggregated egg mass distribution should be found. Hudon and LeRoux (1986b) found a negative binomial distribution of egg masses over the plants, indicating some clustering. If *O. nubilalis* would avoid plants on which egg masses were laid, a uniform distribution should be the result. At higher egg mass densities, indications for such uniform distributions can be found: more plants have only one egg mass then would be expected from a Poisson distribution (Vaillant and Hawlitzky, 1990; Hawlitzky *et al.*, 1994). If the egg mass density is low, it is hard to detect differences between these three types of distribution. The location of a plant in the field can also have some influence. The number of egg masses can be higher at the field edge (Lee, 1988). In general, the distribution of *O. nubilalis* egg masses in the field is not always completely random, but can be adequately described as a Poisson distribution, especially if densities are low (Shelton *et al.*, 1986).

Within-plant egg mass distributions
Egg masses are mainly laid on the leaves. Depending on the source, between 0 and 6% of the egg masses are found on the stem (Everly, 1959; Burgstaller, 1974; Windels and Chiang, 1975; Shelton *et al.*, 1986; Milani *et al.*, 1988; Coll and Bottrell, 1991). One reason for this might be that the higher glucose levels on the leaf surface result in increased oviposition by *O. nubilalis* (Fiala *et al.*, 1985).

An overview of height distribution data from the literature on egg mass presence is presented in Table 1. Although the mean leaf level of oviposition varies greatly from year to year and from area to area (Klinger, 1979), most egg masses are found in the middle leaf levels.

Table 1. Distribution of *O. nubilalis* egg masses on maize plants. Literature data.

position	plant size	source
Most egg masses close to silking ear	in mid-summer	Beck, 1987
Mainly lower part of plants		Burgstaller, 1974
Symmetrical distribution over leaf 2-12, maximum on leaves 6-7	13-14 leaves	Calvin *et al.*, 1986
76.7% on middle leaves near silking ear		Coll and Bottrell, 1991
82% on leaf 1-4, 98% on leaf 1-6	8-10 leaves	Despins and Roberts, 1986
Lower part of plant	15 leaves	Despins and Roberts, 1986
6-15% on ear leaf		Everly, 1959
95% of leaf 4-10, maximum on leaf 7	13 leaves	Hawlitzky *et al.*, 1994
87% on leaf positions 1-8 (17.9% on leaf 6, 16.9% on leaf 7)		Huber *et al.*, 1928
11% on the husks of the ear		Hudon and LeRoux, 1986a
Leaf 5 to 15, maximum on leaf 8	17 leaves	Ismail, 1989
95% on leaf 5-9 (28% on leaf 7)	12-14 green leaves	Lee, 1988
Leaf 8-13	14-16 leaves	Lupoli *et al.*, 1990
83% in leaf 3-7		Ohnesorge and Reh, 1987
85% in 5 leaves around primary ear		Sorenson *et al.*, 1993
Leaf 2-8, 70.7% on leaf 4-6, maximum on leaf 5	10 leaves	Vierling, 1985
43.3% in ear leaf and two adjacent leaves (field maize)		Windels and Chiang, 1975
34.1% in ear leaf and two adjacent leaves (sweet maize)		Windels and Chiang, 1975

Exceptions to this were found by Burgstaller (1974) and Despins and Roberts (1986), who found most egg masses in the lower third of the plant. Humidity and temperature influence the leaf level of oviposition (Chiang and Hodson, 1972; Despins and Roberts, 1986; Ohnesorge and Reh, 1987). The distribution over the leaf levels is symmetrical with most egg masses on the middle leaves. *O. nubilalis* apparently moves between the plants at a horizontal level to oviposit. The number of egg masses per unit surface area is higher in the upper leaves, due to the smaller leaf size.

Within-leaf egg mass distribution

The literature is very clear about the distribution of egg masses over the leaf sides: most egg masses are found on the lower leaf side (Table 2). This is not only true for maize, but

Table 2. Leaf side position of *O. nubilalis* egg masses in maize and other crops. Literature data.

lower side	crop	source
52.2%	maize	Milani *et al.*, 1988
62.4%	maize	Milani *et al.*, 1988
73.4%	maize	Sorenson *et al.*, 1993
79.1%	maize	Ismail, 1989
87.2%	maize	Coll and Bottrell, 1991
87.2%	field maize	Windels and Chiang, 1975
92.6%	end of season maize	Everly, 1959
95.1%	sweet maize	Windels and Chiang, 1975
95.6%	maize	Lee, 1988
96.3%	early season maize	Everly, 1959
97.6%	maize	Poos, 1927
99%	maize	Hudon and LeRoux, 1986a
100%	maize	Jarvis and Guthrie, 1987
100%	maize	Shelton *et al.*, 1986
Almost all	cotton	Savinelli *et al.*, 1986
>90%	sunflower	Legg *et al.*, 1986
50%	maize, insectary	Hudon and LeRoux, 1986a

also for other crops (Legg *et al.*, 1986; Savinelli *et al.*, 1986); Only in one case there was an equal amount of egg masses on both leaf sides in the field. The next season in the same area, 62% of the egg masses were on the lower leaf side (Milani *et al.*, 1988). In an insectary experiment, the moth oviposited equally on both leaf sides (Hudon and LeRoux, 1986a), but in insectaries the leaves may touch the netting, which increases the chance of landing on the upper side by *O. nubilalis* flying along the cage.

Less is known on the distribution of egg masses over the leaf (Table 3). The parts closer to the stem have more egg masses. Poos (1927) and Beck (1987) mention that *O. nubilalis* prefers to oviposit close to the mid-rib. Legg *et al.* (1986) observed that on sunflower the large veins are also preferred for oviposition.

Table 3. Position of *O. nubilalis* egg masses on the maize leaf. Literature data.

position	source
49% on base, 35% middle, 16% on point	Burgstaller, 1974
22% on base, 49% middle, 16% on point	Vierling, 1985
42% on base, 40% middle, 18% on point	Klinger, 1979
Less egg masses on point than on middle and base	Shelton *et al.*, 1986
Most near middle or base, few at point	Poos, 1927

In conclusion, it appears that sufficient data on egg mass distribution within the field and the plant are available, but information at the leaf level is lacking. In this paper, the within-plant and within-leaf distribution of egg masses of *O. nubilalis* is presented. Observations on oviposition behaviour of *O. nubilalis* were made to obtain an idea of the potential distribution of scales and other contact host-cues over the visited plants.

MATERIALS AND METHODS

Materials

Moths
Ostrinia nubilalis Hübner reared at the Swiss Federal Research Station for Agroecology and Agriculture were used for the climate room observation experiments. The colony was started in 1990 with 500 diapausing larvae collected at Cadenazzo (Ticino, Switzerland). Larvae were reared on an artificial medium for approximately 20 generations. Pupae were moved to cages (45x45x45 cm) where the adults could emerge and mate. On top of the cage, a sheet of wet filtration paper was placed for the females to oviposit.This sheet was then removed to collect the egg masses and start a new rearing cycle. Material for the observations was obtained by collecting pupae and letting them emerge. The freshly emerged *O. nubilalis* males and females were left to mate for four days in plexiglass containers provided with honey-water in a 25°C climate room. For the field cage observations, *O. nubilalis* emerged from pupae collected from stubble at the Swiss Federal Research Station for Agroecology and Agriculture and *O. nubilalis* from second generation pupae collected near Cadenazzo (Ticino, Switzerland) were used.

Plants
For the climate room observation experiments, greenhouse grown maize plants (cultivar LG11, 1.50 m high) were used. For the insectary observations, field-grown plants were potted (cultivar: Atlet, 1.70 m high) for use. Field grown plants have thicker stems and a much larger leaf area compared to greenhouse grown plants of the same height.

Methods

Oviposition in the field
Egg masses were counted during the summer of 1993 in Schinznach (AG) and Wasterkingen (ZH) in Switzerland. A total of 18 maize fields (9 from each region) were used for the study. A total of 144 and 110 plots, each plot containing 10 plants, were sampled in Schinznach and Wasterkingen respectively: a total of 2540 plants were carefully examined for *O. nubilalis* egg masses. Position of egg masses within plants and within leaves were recorded. For within-plant distribution, leaves of the maize plants were numbered with the lowest leaf on the plant as leaf one. To record within-leaf distribution of egg masses the position of each egg mass was marked on a sketch of the leaf. Relative position of the egg mass to the maize stem and to the mid-rib were later measured with a ruler on the leaf sketch. Plants at Schinznach were checked on 22 June and 7, 21 July and at Wasterkingen on 23, 30 June and 13, 27 July. This time covered most of the oviposition period of *O. nubilalis*. All data were combined to determine *O. nubilalis* oviposition distribution on the leaf.

Observations in the climate room
A maize plant was placed in a plexiglass cage (58x58x126 cm). Observations were made in a 25°C climate room (16L/8D) during eight nights. Light was turned off at 17.00, observations started at 20.00 and finished at 22.30. Illumination for the observer was provided by low-intensity red light. A container with 6 to 28 *O. nubilalis*, of which about half were female, was placed on the bottom of the cage and the lid removed. The movement of *O. nubilalis* over the plant was recorded for 2.5 hours on a diagram of the plant and the leaves. The maize plants were left in the cage overnight and checked the next day for egg masses. *O. nubilalis* was observed for eight nights in the climate room.

Observations in the insectary
Four maize plants were placed in an 2.5 x 2.5 x 2 m field insectary with a metal frame covered with fine plastic netting. Fresh *O. nubilalis* were released in the cage as they became available, resulting in a population of mixed ages. About 20 *Ostrinia* were in the cage during observations, of which about 2/3 were females. After sunset, behaviour was observed for two hours during ten nights.

Distribution of moth scales
To see whether *O. nubilalis* left any visible host-cues (scales) close to oviposition sites and on other places of the plant, plants were checked under the microscope for moth scales. Four of the plants used for observations in the climate room, and four plants from the field cage were checked for scales.

RESULTS

Distribution of O. nubilalis egg masses on the plant
A total of 397 egg masses were recorded in the field, which means one egg mass for every 6.4 plants checked. Table 4 gives date, place, number of egg masses found on the lower and upper part of the leaf and total number of egg masses found.

Except for one egg mass found on the stem, all other egg masses were found on the leaves. Only 3% of the egg masses were found on the upper leaf side. The egg masses were mainly found on leaves three, four, five, six and seven (18, 25, 23, 16 and 8% respectively), as illustrated in Figure 1.

Table 4. Number of *Ostrinia nubilalis* egg masses found in 18 maize fields at various dates in Schinznach and Wasterkingen (Switzerland).

date	place	side of leaf lower	upper	total
22.6	Schinznach	25	0	25
23.6	Wasterkingen	36	2	38
30.6	Wasterkingen	51	3	54
7.7	Schinznach	130	2	132
13.7	Wasterkingen	49	2	51
21.7	Schinznach	69	2	71
27.7	Wasterkingen	26	0	26
Total number of egg masses		386 (97%)	11 (3%)	397 (100%)

Figure 1. Distribution of 396 *O. nubilalis* egg masses over the leaf levels of maize in the field. Level one is the lowest leaf.
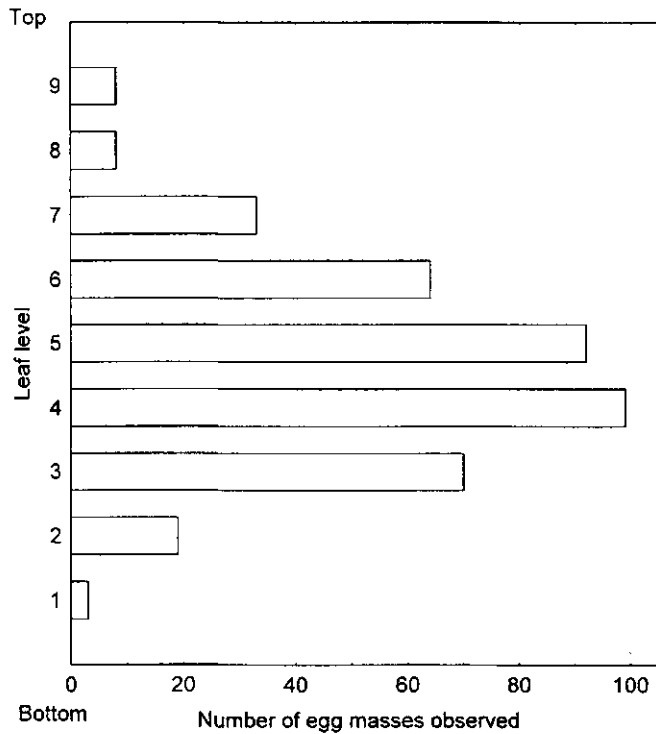

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Figure 2 illustrates the position of egg masses relative to the leaf mid-rib. Forty-two percent of the egg masses were found on or very close to the leaf mid-rib (10% of total leaf width), and 64 percent in 20% of total leaf width. Very few egg masses are found close to the edge of the leaf.

Position of egg masses relative to the plant stem are represented in Figure 3. A large proportion of egg masses were found on the middle section of the leaf. Approximately 80% of the egg masses were found between the relative distance of 20 and 60% from the stem.

Observation of oviposition of O. nubilalis
Results of climate room and field insectary observations are shown in Table 5.

Table 5. Observation of *Ostrinia nubilalis* landing on maize plants in a climate room cage and a field insectary.

|	climate room	field insectary
Number of observation nights	8	10
Number of landings	93	76
Female	69	71
Male	24	5
Number of moths landing	57	33
Mean number of landings per moth	1.63	2.30
Percentage of landings on lower side	66	84
Mean leaf level of female landings	4.9 ± 0.2*	5.5 ± 0.3*
Mean time on plant (min)	20.5 ± 4.2*	11.6 ± 2.8*
Median time on plant (min)	2	1
Number walking	15	9
Number of females walking	12	8
Mean distance walked (cm)	4.9 ± 1.7*	2.5 ± 0.3*
Median distance walked (cm)	2	3
Probing with abdomen observed	44	12
Ovipositions	7	8
Mean oviposition leaf level	4.4 ± 0.8*	5.8 ± 0.6*

*Mean ± s.e.
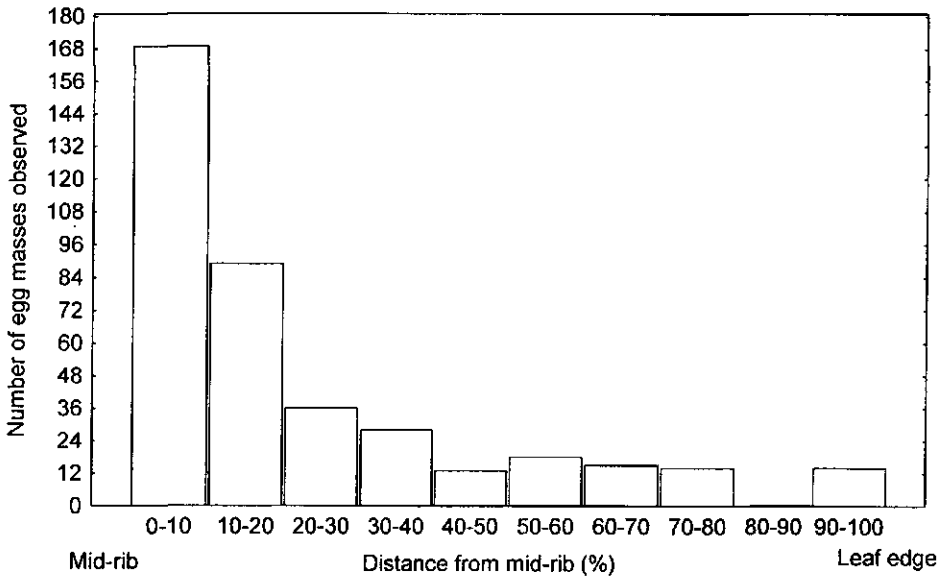
Figure 2. Distribution of 396 *O. nubilalis* egg masses over the leaf width of maize leaves in the field. Distance relative to the mid-rib.
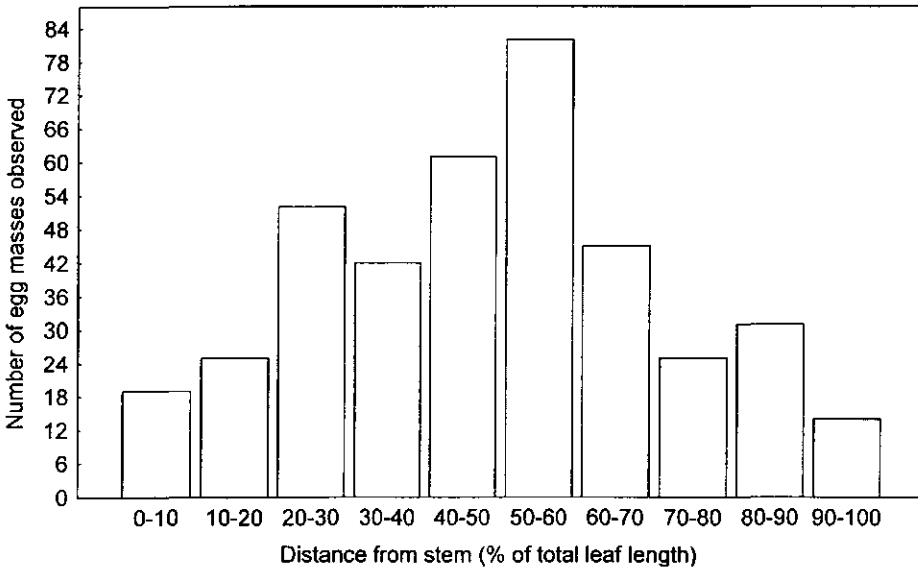
Figure 3. Distribution of 396 *O. nubilalis* egg masses over the leaf length of maize leaves in the field. Distance relative to the stem.

In both experiments, part of the moths were not active or just flew against the walls of the cage. In the climate room, the cage was small so accidental contact with the plant was quite likely, which might explain the higher number of contacts observed. Females landed proportionally more than males. A few of the females were very active, and made many short visits to the plant. In the climate room, 27 landings had a duration of only five seconds or less, while in the insectary, 38 landings had this duration. This explains why the median duration of a visit was so much lower than the mean duration. In the climate room, one female landed ten times and walked much further over the leaf than all other females observed. In the insectary, the largest number of landings by a single female was eight times.

Walking after landing was observed in only a few of the cases, both for males and females. However, some of the *O. nubilalis* were discovered on the leaves without the landing being observed. In these cases, it was not possible to say whether they had walked or not. The distance walked was usually short. The mean distance in the climate room was higher only because of the behaviour of one female. Walks occurred only on the leaves. *O. nubilalis* stayed on the same leaf side while walking, except for two cases in the climate room. In the climate room, probing with the abdomen was observed in many cases. In the insectary, light conditions were less good making such detailed observations harder. The number of actual ovipositions was quite low compared to the number of landings. In both the climate room and the insectary, the mean leaf level of oviposition was close to the mean leaf landing level (4.4 in the climate room and 5.8 in the field insectary). The distribution of landings of females over the leaf levels is shown in Figure 4.

Distribution of moth scales
Scales of *Ostrinia* found could be divided into large (length 0.234-0.167 mm, width 0.033-0.007 mm) and small (length 0.067, width 0.033-0.007 mm) size scales. Clusters of scales can be detected with the naked eye because of their "shiny" appearance. Single large scales were easily seen on the leaf under a microscope, but single small scales were hard to detect. Results in Table 6 show that most of the scales found were not directly associated with egg masses. When a single scale or scale cluster was closer than 3 cm to an egg mass, the two were assumed to be associated. Of the 62 egg masses, 82% had no or only a few scales (1-10) on or around them, while only 18% had an area with many large and small scales around. The number of dense scale patches without egg masses was almost the same as those associated with egg masses. Such patches had sizes from 2 to 20 mm in diameter, and were irregularly formed. Five very long and narrow scales patches were found (20-100 mm long), none of which was associated with an egg mass. Large scales were sometimes found single or in groups (1-10 scales) on the leaves. In four cases, single scales (both large and small) were spread over a large part of one leaf side.
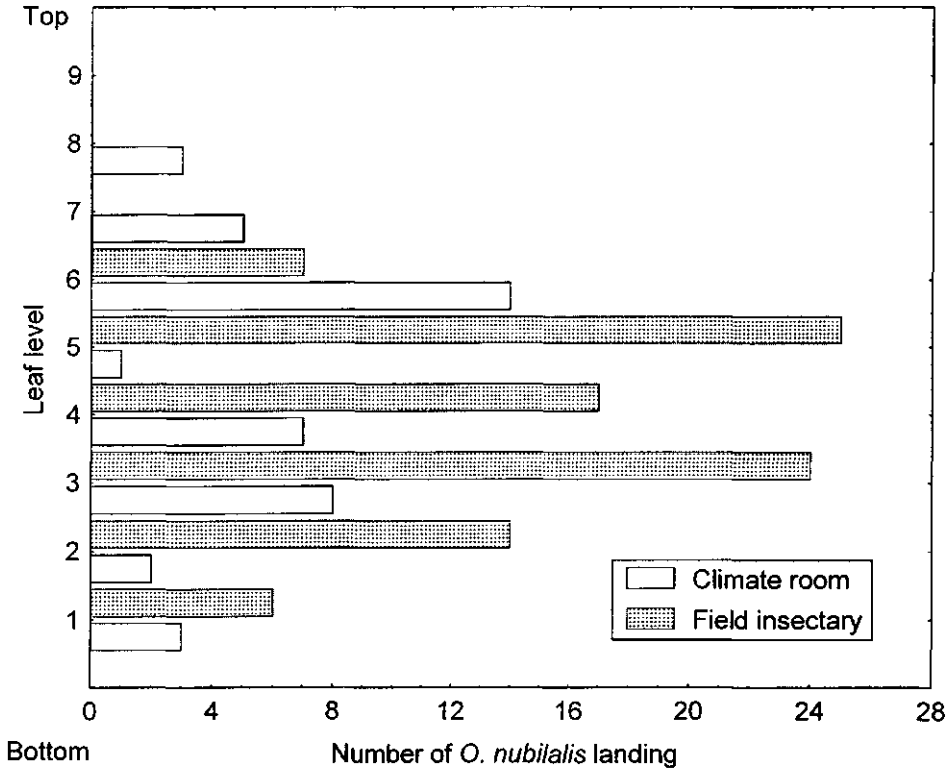
Figure 4. Distribution of female *O. nubilalis* landings over the leaf levels of maize in a climate room cage and a field insectary. Level one is the lowest leaf.

Table 6. Distribution of *Ostrinia nubilalis* egg masses and scales observed on maize plants in a climate room cage.

	number	percentage
Number of plants checked:	8	
Number of egg masses	62	
Egg masses on lower side	39	65
Total scales associated with egg masses	39	45
Total scales not associated with egg masses	48	55
Egg masses		
No scales	23	37
Few scales on or near egg mass	28	45
Dense scale area around egg mass	11	18
Scales not associated with egg masses		
Single scales or few scales	29	60
Large area with single scales	4	8
Scales track	5	10
Dense scale area	10	21

The combination of the behavioural observations and the scales found showed that some of the dense patches of scales resulted from oviposition and abdominal probing. From the number of egg masses with little or no scales, it is clear that probing or ovipositing will not necessarily leave scales on the leaf. For each oviposition in the direct observations, 9.33 landings were made. Combined with the scale data, it is found that a landing resulting in oviposition has a 63 % chance of leaving scales behind, while landings not resulting in oviposition have only a 9% chance of leaving scales on the leaf. The distribution of egg masses and scales over the leaf levels was similar to the *O. nubilalis* landing distribution shown in Figure 4.

DISCUSSION

Distribution of *O. nubilalis* egg masses in the field shows that egg masses in maize plants are mainly found on the middle leaves. As previously noted by other researchers, very few egg masses were laid on the upper leaf side. The within-leaf distribution of egg masses in relation to the mid-rib shows that most egg masses were laid close to the mid-rib. A large proportion of egg masses were found on the middle section of the leaf relative to the plant stem, and oviposition on the tip of the leaf was rare.

The results of the observation experiments show clearly that there is a close correlation between landing and oviposition. Few *O. nubilalis* move over the plant by walking, and those that do walk do so only for short distances. This means that the distribution of egg masses found on the plant also indicates the landing distribution over the plant. In our observations, 34% of landings in the climate room cage and 16% of the landings in the field insectary were on the upper leaf side. Since upper side oviposition is found in insectaries, but rarely in the field, it is likely that in field situations the percentage of landings on the upper side of leaves is lower. Although landing and oviposition sites are similar, the number of landings is much higher than the number of ovipositions. In the experiments, the walls and ceiling of the cage or the insectary were often used as a resting place. In more than half of the landings in the cage, abdominal probing was observed. The abdomen contains most of the receptors for perceiving plant substances indicating plant quality, such as fructose, saccharose, malate and aconitate (Baur *et al.*, 1994). This suggests that after most landings the plant is not used as a resting place only.

The egg parasitoid *Trichogramma brassicae* shows a distinctive landing and searching pattern on clean maize plants. *T. brassicae* lands on both sides of the leaf, and landing position is not correlated with the mid-rib, but landing distributions over the length of the leaf are quite similar with those of *O. nubilalis* egg masses (Chapter 5). Both sides of the leaf are searched, but *T. brassicae* spends up to 10% of time close to the mid-rib and also spends more time searching the middle and base part of the leaf (Chapter 6). Apparently, the searching and landing pattern of *T. brassicae* is quite similar with the *O. nubilalis* egg laying pattern, apart from the time that *T. brassicae* spends on the upper leaf side. Since both the amount of egg masses and scales on upper leaf sides is low in the field, it would be more efficient for *T. brassicae* to spend more time on the lower leaf side. But it is unlikely that the behaviour of *T. brassicae* is a result of co-evolution with *O. nubilalis*, since it is a generalist parasitoid. Landing distribution and movement patterns of *T. brassicae* have probably evolved to provide an efficient way to search many different plants for lepidopterans.

T. brassicae, like many *Trichogramma* species, reacts to host-cues. Scales of *O. nubilalis* elicit an arrestment response (Bieri *et al.*, 1990). So it can be questioned whether scales of *O. nubilalis* could be used to find egg masses more efficiently. The data show that at distances of one to three centimetres (i.e. the maximum walking distance of *O. nubilalis* on the leaf), there is not a good correlation between egg masses and scales. Many egg masses had little or no scales in the vicinity, while on the other hand many scales and patches of scales were found on leaves with no egg masses. The density of *O. nubilalis* in the cage and insectary experiments was unnaturally high. In natural field conditions, the number of egg masses and scale spots will be lower, with a maximum of two or three egg masses per plant. The position of the scales will be different too. Since moth landings in the field will be mostly on the lower leaf side, the host-cues will also be more concentrated on the lower leaf side than in the observation experiments. Scales in the field will indicate that there probably is an egg mass on the plant or on the leaf, but this egg mass might not be close to the point where the scales are encountered.

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Chapter 3

Trichogramma dispersal and host-finding behaviour

INTRODUCTION

As a result of the worldwide use of *Trichogramma* species in biological control, it is probably the most intensively studied parasitoid genus in the world. In this chapter, the aspects of *Trichogramma* behaviour that are most relevant to the host-finding process are reviewed, with an emphasis on *T. brassicae* behaviour on maize. First, a short description of mating behaviour is given, because mating may influence dispersal. Dispersal and host-finding behaviour and the factors influencing them are then described in detail. Although host acceptance itself is not the topic of this thesis, it is shortly reviewed because it has a strong impact on the interpretation of experiments cited in the literature which deal with dispersal or host-finding. Finally, the missing information essential for understanding and describing the host-finding process in the field, is listed.

Earlier reviews on *Trichogramma* movement were made by Keller *et al.* (1985) and Noldus (1989). The reviews in Wajnberg and Hassan (1994) contain up to date literature of most aspects of *Trichogramma* biology and utilization, but the information on *Trichogramma* host-finding is very sparse.

MATING BEHAVIOUR

Trichogramma emerge in the morning (Zaslavski *et al.*, 1995), and male *Trichogramma* emerge before females. Males stay on the host eggs close to where they emerged and mate with the emerging females (Waage and Ming, 1984; Lee *et al.*, 1986; Forsse *et al.*, 1992; Pompanon *et al.*, 1995), so brother-sister matings often occur in *Trichogramma*. If the host egg is large enough to contain numerous *Trichogramma*, mating will sometimes even take place inside the host egg (Lee *et al.*, 1986). On egg patches of *Mamestra brassicae*, *T. evanescens* lays males and females in such a sequence that an optimal sex ratio is reached. The number of males is lower than the number of females, because each male mates with different females (Waage and Ming, 1984; Waage *et al.*, 1984). Sex ratios of up to one male per seven females are found. If isolated small host eggs are parasitized in the field, like those of *Pieris rapae* or *P. napi*, the males will have to search for the females by moving to other leaves and plants. Large antennae are a male characteristic in most *Trichogramma* species, indicating that sex pheromones are involved. For *T. maidis* (*=brassicae*) olfactometer tests showed that males were attracted to females by odour (Pintureau and Toonders, 1983). *T. brassicae* is arrhenotokous, which means that unmated females can produce offspring, but only males. Although thelytokous parthenogenesis (unmated females producing females) was found in nine *Trichogramma* species (Pintureau, 1994), it has not yet been observed for *T. brassicae*. When *T. brassicae* parasitizes *Ostrinia nubilalis* (European corn borer), it will usually find egg masses, so in this case dispersal for mating is essential.

DISPERSAL

Direct and indirect measurement

Dispersal of *Trichogramma* has usually been studied to answer questions important for biocontrol (Keller *et al.*, 1985). For inundative *Trichogramma* releases, it is crucial to know how far from a release point *Trichogramma* still gives an acceptable level of parasitism. On the other hand, it is also important to know how many *Trichogramma* stay in the field, tree or orchard in which they were released. Recently, concerns have been voiced about the impact of *Trichogramma* releases on populations of rare butterflies. To assess these risks, more information on long-range dispersal of *Trichogramma* is needed (Andow *et al.*, 1993).

Insects that exploit temporary habitats need to disperse, while in stable or isolated habitats dispersal often is disadvantageous (Roderick and Caldwell, 1992). *Trichogramma* species are in general not specialized on one host and thus they cannot or do not have to synchronize their life cycle with that of a single host species. Host eggs are often not available close to the point of emergence, and *Trichogramma* must disperse to reach other host-habitats.

Measuring dispersal in the field of tiny insects like *Trichogramma* is difficult. Direct observation of flight behaviour is impossible. The presence of *Trichogramma* at some point can be measured directly or indirectly. Direct methods employed are sticky card traps (Hendricks, 1967; Kolmakova and Molchanova, 1981; Van den Berg *et al.*, 1987), yellow coloured water traps (Frei, pers. com), suction traps and sweep nets (Stern *et al.*, 1965). If *Trichogramma* are already present in the habitat, it is necessary to mark the released *Trichogramma*, for example, with radioactivity (Stern *et al.*, 1965) or fluorescent powder. Advantages of direct measurement are a clear record of the presence of *Trichogramma* and the possibility of studying both female and male dispersal behaviour, but usually only a very small part of the released *Trichogramma* are recaptured. Van den Berg *et al.* (1987) recaptured only 36 of 60.000 *T. cryptophlebiae* released, and Stern *et al.* (1965) only 147 of 1.5 million *T. semifumatum* released.

Indirect measurement of dispersal, by estimating dispersal based on parasitism, is possible both with naturally laid eggs of the host (Kanour and Burbutis, 1984; Van Heiningen *et al.*, 1985; Hawlitzky *et al.*, 1994) or with artificially placed eggs (Hase, 1925; Kot, 1964; Neuffer, 1987; Chernyshev *et al.*, 1988; Bigler *et al.*, 1990 Maini *et al.*, 1991). The disadvantage of this method is that parasitism is not only a result of dispersal, but also of host recognition, host acceptance and host suitability. Artificially placed eggs on cards might be hard to find for the wasps and thus underestimate dispersal (Chernyshev *et al.*, 1988). However, Maini *et al.* (1991) found no difference in parasitism by *T. maidis* (=*brassicae*) of natural and artificially applied egg masses in maize.

Factors influencing dispersal

Dispersal is determined by intrinsic and extrinsic factors (Lidicker and Stenseth, 1992). Intrinsic factors include the physiological state of the wasp and its genetic make-up. Extrinsic factors are interactions within the wasp population, the host plant, the host, and many abiotic factors. Flight is the main mechanism for dispersal in *Trichogramma*. Flight can be active or passive (wind-borne).

Intrinsic factors
Flight propensity is partly genetic. Forsse *et al.* (1992), for example, found that within one population of freshly emerged *T. minutum* two groups could be distinguished. One group was arrested by the presence of unparasitized host eggs at the emergence site, the other did fly away immediately.

     The physiological state of *Trichogramma* will influence its activity. Suboptimal diapause storage has a detrimental effect on the quality of emerging *T. brassicae*, and flight propensity of these adults is not as good as that of well-reared individuals (Dutton and Bigler, 1995). Suboptimal conditions during the development of the larvae and pupae will also cause a decline in flight activity (Salmanova, 1991). On the other hand, availability of food in the adult stage will increase dispersal (Ording and Schieferdecker, 1968). Females become more active after having mated (Pompanon *et al.*, 1995). It has been suggested that flight propensity is higher as a result of a stronger reaction to light during the first 48 hours of adult life in *T. brassicae* (Smits, 1982) compared to older adults. This would stimulate the wasps to colonize new habitats, if the old habitat has no hosts.

Extrinsic factors

Temperature
In daylight, temperature is the abiotic factor with the strongest influence on dispersal. At low temperatures, *Trichogramma* does not fly. Minimum flight temperatures at daylight are 18°C for *T. brassicae* (Bigler *et al.*, 1982), between 15 and 20°C for *T. minutum* (Forsse *et al.*, 1992) and 20°C for *T. cacoeciae* (Quednau, 1957). So the threshold temperature for flight of *Trichogramma* species in temperate regions is approximately 18°C (Shchepetilnikova, 1974). Low average temperatures will strongly inhibit dispersal (Van Heiningen *et al.*, 1985). Dutton *et al.* (1996) found a positive correlation between field parasitism in maize by *T. brassicae* and the degree-hours above 18°C. Parasitism however is not the best method to measure the effect of temperature on dispersal, since temperature also influences searching efficiency on the plant.

Light

During the night, *Trichogramma* do not fly (Ashley *et al.*, 1973). Other activity of *Trichogramma* is also very limited during the night (Pompanon *et al.*, 1994). *Trichogramma* is phototactic (Steenburgh, 1934; Noldus *et al.*, 1991a). Within trees, *Trichogramma* sp. (Kot, 1964), *T. pallida* and *T. embryophagum* disperse in the direction of sunlight (Kolmakova and Molchanova, 1981). Sudden sunlight can cause an immediate flight response in *T. brassicae* (Smits, 1982) and *T. minutum* (Steenburgh, 1934).

The flight threshold temperature is influenced by light. At high light intensities, flight of *T. embryophagum* could be observed at temperatures as low as 12°C, but at low light, the flight threshold was 20.5°C (Kadlubowski, 1962).

Wind and other weather factors

Wind can strongly influence dispersal. Trichogramma cannot fly against winds that are stronger than two meter per second (Steenburgh, 1934), so if they cross open spaces or fly above a crop, the wind can move the wasps over long distances (Stern *et al.*, 1965; Van den Berg *et al.*, 1987; Bigler *et al.*, 1990).

When flying between trees, the direction of the dispersal of *T. minutum*, *T. pretiosum* and *T. dendrolimi* is influenced by wind (Steenburgh, 1934; Yu *et al.*, 1984; Smith, 1988), although Schread (1932) found no effect of wind on between-tree dispersal direction of *T. minutum* in peach orchards. Strong winds will inhibit flight (Kadlubowski 1962; Kot, 1964). Bigler *et al.* (1990) showed that transportation by wind negatively affected parasitism in release fields in a mountainous area, where strong bidirectional winds prevail. Based on parasitism measurements inside and outside the release fields, it was concluded that more than 60% of the released *T. brassicae* population left the maize fields. Both, dispersal speed and the strong correlation between dispersal and wind direction indicate that wind borne flight was the main mechanism involved. Either after active emigration or after being transported passively by wind outside the release fields, *T. brassicae* parasitized over 25% of egg masses in fields where no parasitoids were released or occurred naturally. Greatti and Zandigiacomo (1995) also showed a clear effect of wind on dispersal direction. Much slower dispersal was found by Karadjov and Keita (1988): One day after release in maize, *T. maidis* (=*brassicae*) parasitized egg masses at a maximum distance of 10 meters. After five days, egg masses were parasitized at a distance of 20 meters. Single release point experiments in maize also showed that the parasitism of *O. nubilalis* egg masses by *T. maidis* (= *brassicae*) declined within eight meter of the release point (Bigler *et al.*, 1988; Maini *et al.*, 1991; Greatti and Zandigiacomo, 1995). If wasps are released from a central point, their density becomes at some distance so low that parasitism will be negligible. This means that the maximum distance from a release point at which parasitism is measurable depends on the number of wasps released.

Steenburgh (1934) observed that another abiotic factor, rain, will cause *T. minutum* to shelter. Dew can also inhibit movement, although *T. brassicae* is capable to some extent to walk on the water surface (Agami and Voegelé, 1988).

Crop
The crop will influence dispersal by influencing the microclimate. If a crop is dense enough, there will be little wind inside the canopy to affect the wasps. For example, inside a mature maize crop wind speeds do not exceed one meter per second, even if the speed is four meter per second at 75 centimeters above the canopy (Castro *et al.*, 1991). Temperatures are also more constant inside a dense canopy. Keller and Lewis (1985) showed that *T. pretiosum* did not disperse very far if released inside a cotton crop. If released above the crop, dispersal was stronger and influenced by wind. This implicates that *T. pretiosum* flies and walks mostly within the crop if they are released inside it. In the dense foliage of trees, dispersal is often very limited. *T. cacoeciae pallida* (Zhilyaeva *et al.*, 1981), *T. cryptophlebiae* (Newton, 1988), *T. embryophagum* (Kolmakova and Molchanova, 1981), *T. minutum* (Zhilyaeva *et al.*, 1981; Smith, 1988) and *T. pallida* (Kolmakova and Molchanova, 1981) all showed very little dispersal in tree habitats, mostly staying in the tree where they emerged. *Trichogramma* species often have a preference for certain host plants. If they are artificially released in unpreferred habitats, they will disperse fast, as, for example, *T. evanescens* and *T. semblidis* (which are field and swamp dwelling species) do from an orchard (Steenburgh, 1934). This makes it difficult to say whether the low dispersal found in trees is a result of low innate flight propensity of the species involved or of dense foliage and microclimatic effects, or of a preference for that habitat. A plant providing food for *Trichogramma* would on the one hand result in higher activity including increased flight by the wasps, but on the other hand it might induce the wasps to stay as a result of arrestment behaviour.

Host
The presence of hosts will decrease dispersal, because of egg laying activity and encounters with host-cues which induce arrestment responses. The presence of fresh host eggs lowered flight propensity in *T. minutum* (Forsse *et al.*, 1992) and *T. pretiosum* (Gross *et al.*, 1981). Kot (1964) found that at low host densities parasitism by *T. evanescens* was not correlated to distance, but at high densities, parasitism was higher near the release point, which indicates that dispersal was slowed down by the presence of hosts.

Interaction within the population
Not much is known about the last biotic factor, interaction within the population. Crowding at the point of emergence may increase flight propensity (Dutton and Bigler, 1995). Once females have left the emergence point, meeting other females is unlikely unless at egg patches of the host. During oviposition, females are not disturbed by contacts with other conspecifics, but contacts might increase dispersal afterwards (Bertschy, 1993). *Trichogramma* can also detect whether other females have already been present on eggs (Salt, 1937; Klomp *et al.*, 1980). Encountering many parasitized eggs might stimulate dispersal.

Conclusions

All the factors discussed above influence the dispersal of *T. brassicae* in maize. Dispersal is highest in sparse field crops, with sunny, warm, and windy weather, and a low host density, where relatively large numbers of females emerge from single host egg clusters. Indirect measurement is not an optimal instrument to study dispersal, because other behaviours (host-finding, host acceptance distances) have a strong influence on parasitism. There has so far been no direct measurement of *T. brassicae* dispersal in maize.

HOST-SEARCHING BEHAVIOUR

Host-habitat location

Searching behaviour of parasitoids is divided in three successive stages, host-habitat location, host location and host selection (Doutt, 1964). *Trichogramma* species are not confined to a single host-plant species, but there is some specialization in the habitats in which *Trichogramma* occur. According to Flanders (1937), *T. evanescens, T. embryophagum and T. semblidis* prefer field, arboreal and swamp habitats, respectively. Lopez *et al.* (1982) showed that *T. pretiosum* preferred a cotton habitat and *T. exiguum* a grain sorghum habitat. Clear habitat preferences of different species were also found by Hirose *et al.* (1988) and Smith and Hubbes (1986). Several *Trichogramma* species parasitize a host on certain plants, but not on others (Rabb and Bradley, 1968; Bar *et al.*, 1979; Martin *et al.*, 1981). On the other hand, intercropping can sometimes increase parasitism on non-preferred host plants (Duffield, 1994). How signals of the host-habitat are used depends on the host and host-habitat specificity of the parasitoid. If the host species of the parasitoid is found on a single plant only, the response to synomones (and other signals) emitted by this plant is likely to be strong and fixed. If the parasitoid is more polyphagic (i.e. attacks many host species) or attacks hosts on different plants, the innate response to plant signals is expected to be low, but can be reinforced by associative learning. If the host occurs on many plant species simultaneously, there might be no effect of plant signals (Vet and Dicke, 1992). It is thus expected that *Trichogramma* will have some innate responses to synomones of certain plant species, but since hosts will not always be present on the plants, the response cannot be very strong.

Placing cotton wicks with hexane extracts of *Amaranthus* and maize in soybean increased parasitism by *Trichogramma* sp. (Altieri *et al.*, 1981; 1982), and extracts of tomato placed in cotton increased parasitism by *T. pretiosum* (Nordlund *et al.*, 1984), which shows that plant synomones do influence host-finding. In a petri dish experiment, parasitism of *T. pretiosum* could be increased by tomato extracts, but not by maize extracts (Nordlund *et al.*, 1985), which supports the view that *Trichogramma* has innate preferences for some plant species.

The mechanism involved in host-habitat location by *Trichogramma* has not been studied. It is unlikely that *Trichogramma* uses true plume-following (Baker, 1986) or rest-interrupted upwind flight (Prokopy *et al.*, 1983; Harris and Miller, 1984). For a small insect like *Trichogramma*, which can not fly against strong winds (Steenburgh, 1934), following wind-borne odours is disadvantageous if it is physically unable to reach the odour source (Chapman, 1982). Random flight, landing when appropriate plant odours are sensed, as employed by weak fliers like aphids (Kennedy and Stroyan, 1959) and lacewings (Duelli, 1984) is a possible mechanism, since it does not require upwind flight (Nordlund, 1994). Even simpler, plant synomones might lower flight propensity after landing by *Trichogramma*.

T. *brassicae* does not react to maize odours if the female has no previous oviposition experience(Kaiser *et al.*, 1989a). However, after oviposition in the presence of maize odour, the wasp shows an arrestment response to this odour in an olfactometer. This response, caused by a single oviposition, had disappeared after five minutes . This experiment indicates that T. *brassicae* may use associative learning to modify its responses to plant synomones.

Movement on the plant

T. *brassicae* moves over flat surfaces such as leaves in relatively straight lines, interrupted by sharp turns (Gardner and van Lenteren, 1986). If linear structures such as edges or veins are encountered, the wasps shows a thigmotactic response, following the structure. This was observed for T. *evanescens* on Brussels spouts and artificial platforms (Noldus *et al.*, 1991a), and for T. *brassicae* on maize (Gass, 1988). It means that movement on the leaves is not truly random. Whether thigmotactic responses do guide *Trichogramma* to eggs is strongly dependent upon the host. If host eggs or host-cues are concentrated on these linear structures, following them may be profitable. However, thigmotaxis is very common in insects (Bell, 1990), and we can assume that it has not evolved as a reaction to host behaviour. The fraction of a surface that is searched within a certain time depends on activity (fraction of total time that is spent searching), walking speed, and, if the wasp is not following a linear structure, tortuosity. Bigler *et al.* (1988) showed that walking speeds of different T. *brassicae* strains directly relates to parasitism in maize fields. Walking activity and walking speed are both influenced by temperature and light. In general, *Trichogramma* are active between 9 and 36°C (Eidmann, 1934; Kot, 1979). Both walking speed and activity increase with rising temperature, and decrease sharply near the maximum temperature. At low temperatures, activity is very limited, and it rises most between 15 and 25°C. At high temperatures, *Trichogramma* are active most of the time (Biever, 1972; Pak and van Heiningen, 1985; Pompanon *et al.*, 1994). Walking speeds increase almost linearly with temperature until a maximum is reached at 35°C. At higher temperatures, the wasps die (Biever, 1972; Boldt, 1974; Gass, 1988). Humidity has an effect on walking speed only when it is very low (RH< 20%) (Boldt, 1974). *Trichogramma* are strictly diurnal, and their activity follows a daily rhythm. Activity starts shortly before dawn. Within less than an hour, most of the wasps are active. T. *brassicae* shows a marked decline in activity in the late afternoon, so activity has

nearly ceased by sunset (Fleury *et al.*, 1991; Pompanon *et al.*, 1994). There is a large inter- and intraspecific variation in walking speeds and activity (Pavlik, 1992; Pompanon *et al.*, 1994). It is unknown whether *Trichogramma* moves between leaves and plants for searching by walking, by hopping from leaf to leaf or by flying longer distances. It is assumed that walking and short jumps are the main movements when searching for host eggs (Pak *et al.*, 1985).

Effect of host habitat on searching

Once the host-habitat is reached, the host plant will continue to influence the searching process in several ways (Bergman and Tingey, 1979), both direct and indirect. Direct influences are effect of host plant volatiles, plant growth, toxic and nutritional values of the host plant and plant morphology. Indirect factors are the way in which the host plant influences the insect hosts' behaviour and positive, nutritional or negative, toxic properties.

Attractive or arrestant effect of synomones
The attractive or arrestant effect of host plant synomones has been discussed under host-habitat location. Host induced plant synomones will be discussed under the effect of the host on searching.

Plant size
Plant growth and plant size do definitely influence parasitoid searching. A larger plant is easier to find, both by olfactory and visual means, but larger plants also have a larger surface area to be searched for hosts (Kot, 1979). On the other hand, larger plants may provide a more stable microclimate, protecting the wasps from extreme temperatures, wind and humidity. For *Trichogramma*, like for most parasitoids, searching inside a crop in the absence of host-cues is assumed to be random, moving from plant to plant until arrested by encounters with host-cues or the host itself (Knipling and McGuire, 1968; Waage, 1979; Vinson, 1984; Bigler *et al.*, 1988; van Roermund and van Lenteren, 1995). This means that a larger surface would lead, at a constant number of hosts, to lower parasitism. Indeed, many experiments showed that parasitism was lower on larger plants, like for *T. pretiosum* on tobacco (Ables *et al.*, 1980) and for *T. nubilale* on sweet pepper (Burbutis and Koepke, 1981) and maize (Need and Burbutis, 1979; Kanour and Burbutis, 1984). In maize, the plants are still small when *O. nubilalis* first occurs, and the leaf area may increase fivefold or more later in the season. If the same number of *T. maidis (=brassicae)* is released later in the season, parasitism is lower than with early release (Maini *et al.*, 1991). Even a 10% increase in maize leaf surface area reduced parasitism from 34% to 22% (Van den Heuvel, 1986). It is clear that differences in maize size between years and locations can cause a large variation in parasitism.

Nutritional and toxic values of the host plant
Trichogramma in the laboratory will live longer and produce more offspring when able to feed on honey (Ashley and Gonzalez, 1974; Stinner *et al.*, 1974; Bourarach and Hawlitzky, 1989). A host plant that would have ready accessible nectar could in this way increase

Trichogramma effectiveness. Indeed, nectaried cotton has a higher natural parasitism by *Trichogramma* than nectariless cotton (Treacy *et al.*, 1987). It is unknown however whether this is a result of increased fecundity and longevity of the wasps, or decreased dispersal from the crop. Plants can also emit substances that are toxic to parasitoids and thus prevent parasitism (Campbell and Duffey, 1979), but this has not yet been found for *Trichogramma*. Maize does neither produce nectar nor substances harmful to *Trichogramma*.

Plant morphology
Two components of plant morphology, apart from size, influence parasitoid searching: structural heterogeneity, which is the variation among plant parts and plant surfaces, and structural complexity, which describes how the plant surface is divided into units and how these are connected. (Andow and Prokrym, 1990). Leaf surfaces, where most of the searching by *Trichogramma* takes place, can differ in their wax layers and hairiness. The wax layer, which covers the cuticula of plants (Jeffree, 1986), can absorb substances form outside, and release them slowly (Southwood, 1986). Noldus *et al.* (1991b) showed that sex pheromones of *Mamestra brassicae* are absorbed and emitted by Brussels sprouts leaves, causing an arrestment response in *T. evanescens*. It has not been studied whether different plant species and cultivars have different characteristics for absorbing chemicals.

Effects of leaf hairiness has been studied for several parasitoids, as it is a characteristic that can relatively easy be changed by selective breeding (Obrycki *et al.*, 1983; van Lenteren *et al.*, 1995). Hairs can on the one hand hinder walking, or even trap and kill parasitoids, but they can also provide a more suitable microclimate on the leaf (van Lenteren, 1990). Parasitism of *Trichogramma* sp. on tomato declined as hairiness increased (Kauffman and Kennedy, 1989). The same was found for *T. pretiosum* on cotton varieties (Treacy *et al.*, 1986) and for *T. chilonis* on soybean (Hirai, 1988). Sticky hairs can completely prevent *T. minutum* searching on tobacco (Rabb and Bradley, 1968). Maize plants have large trichomes that are spaced far enough apart for *Trichogramma* to walk between. *T. exiguum* walked faster on maize than on other plants with denser hairs (Keller, 1987). *T. maidis* (=*brassicae*) seemed to walk less straight on the upper leaf side of maize, because it collided with the hairs, which caused a change in direction (Gass, 1988). Structural complexity can have an effect on searching. Andow and Prokrym (1990) studied the searching efficiency of *T. nubilale* on artificial surfaces. They found that on a simple surface (square paper) the wasps found their host 2.4 times faster than on a complex surface (four strips connected at the base) with the same surface area. In fact, the giving-up time was also influenced by the structural complexity, because the wasps left the complex surface faster than the simple surface. One explanation for the shorter giving-up time is that the edge is much more often encountered on the complex structure, which might induce flight. *T. minutum* had a lower searching efficiency on artificial plants compared to real ones (Fye and Larsen, 1969), which may be a result of crucial differences in plant architecture between artificial and real plants. On the other hand, Kauffman and Kennedy (1989) found no effect of structural complexity (number of stem terminals per plot) on parasitism by *Trichogramma* spp. on tomato. Maize varieties used in Switzerland are quite uniform in both leaf hairiness and number of leaves, so different varieties are unlikely to result in different percentage parasitism as a result of structural differences (Suverkropp, unpubl.).

Indirect influence of the plant through the host
The host plant can influence the host and in this way, indirectly also affect the parasitoid. The nutritional and toxic proprieties of the host depend on its host plant, and the hosts behaviour can be altered by the host plant. However, the life stage attacked by *Trichogramma* does not feed on the plant, so its nutritious value will not be dependent on the host plant on which it is laid. The plant microclimate can probably affect egg quality. The position of the host eggs will also be influenced by plant size and structure and crop microclimate. Again, because of the uniformity of maize varieties in Switzerland, plant size and developmental stage will be the main plant factors influencing *O. nubilalis*.

Effect of the host on searching

Searching within the host habitat for hosts in the absence of host-cues is assumed to be random for most parasitoids (Knipling and McGuire 1968, Waage 1979, Vinson 1984). The host egg is small compared to the total plant surface, and there is evolutionary pressure on eggs to be inconspicuous. So, detectable cues of the host are lacking, and general host-plant odours are not a very reliable cue to host presence, especially not for a generalist like *Trichogramma*. Vet and Dicke (1992) list three ways how this problem can be overcome: 1. the wasp can use specific, detectable cues from other host stages like pheromones (infochemical detour), 2. the wasp can use cues created by the interaction of plant and host (host-induced synomones), or 3. the wasp can learn to link easy to detect but unreliable cues with reliable but hard to detect cues. These three solutions can also be used in combination. *Trichogramma* may use the infochemical detour by employing scales and sex pheromones produced by adult hosts as host-cues. It has been shown that *Trichogramma* is also capable of linking cues from the egg with cues from other host stages or the host plant (Kaiser *et al.*, 1989a). The host egg itself has only minimal interaction with the host plant, so it is unlikely that host-induced synomones are produced as a result of oviposition. The larval host stages of lepidopterans do cause host plants to produce chemicals which are employed by parasitoids (Turlings *et al.*, 1990). Whether *Trichogramma* reacts to larval-induced plant synomones has not been studied yet, so it is unknown if *Trichogramma* combines an infochemical detour with host-induced synomones.

Sex pheromones
Sex pheromones of lepidopteran hosts are volatile cues. It was shown that several species of *Trichogramma* react to host sex pheromones (Table 1.).

Table 1. *Trichogramma* species that show a reaction to host sex pheromones

Trichogramma species	host	source
T. brassicae	*Ostrinia nubilalis*	Frenoy *et al.*, 1991
T. evanescens	*Mamestra brassicae*	Noldus and van Lenteren, 1985a
T. evanescens	*Pieris brassicae*	Noldus and van Lenteren, 1985a
T. evanescens	*Pectinophora gossypiella*	Zaki, 1985
T. evanescens	*Erias insulana*	Zaki, 1985
T. evanescens	*Spodoptera littoralis*	Zaki, 1985
T. pretiosum	*Heliothis zea*	Lewis *et al.*, 1982
T. pretiosum	*Heliothis zea*	Noldus, 1988
T. cordubensis	*Heliothis armigera*	Cabello and Vargas, 1985
T. cordubensis	*Erias insulana*	Cabello and Vargas, 1985
T. sp.p.*buesi*	*Ephestia kuehniella*	Cabello and Vargas, 1985

The responses observed are: increased upwind walking in linear olfactometers (Frenoy *et al.*, 1991; Frenoy *et al.*, 1992), increased residence in the arms with pheromone of four-arm olfactometers (Noldus and van Lenteren 1985a; Zaki, 1985), inverse orthokinesis (decreased walking speed) (Noldus *et al.*, 1991a) and increased parasitism in olfactometer, petri dish and greenhouse situations (Zaki 1985; Frenoy *et al.*, 1991; Lewis *et al.*, 1982). In a wind tunnel experiments, *T. evanescens* and *T. pretiosum* showed no upwind flight in the presence of odours from calling *M. brassicae* and *Heliothis zea* females. On the contrary, the odours inhibited flight. *T. pretiosum* forced to fly made much shorter flights in the presence of the odours (Noldus *et al.*, 1991a). The strength of the responses is not uniform; *T. evanescens* reacts different to different hosts (Zaki, 1985). Sex pheromones emitted by calling lepidopteran females are usually a mixture of several compounds. Interestingly, the active compounds (those to which the lepidopteran male reacts) are not necessarily the kairomones to which *Trichogramma* has some innate response. *T. brassicae* does not react to Z-(11)-tetradecenyl acetate (Kaiser *et al.*, 1989a; Frenoy *et al.*, 1992), which is the active substance of *O. nubilalis* sex pheromone (Klun and Brindley, 1970), but to cis-9-octadecenoic acid (Frenoy *et al.*, 1991). Neither did *T. evanescens* react to (Z)-11-hexadecen-1-yl acetate, the

main active component of *Mamestra brassicae* sex pheromone (Noldus and van Lenteren, 1985a). Only *T. pretiosum* showed responses to an artificial sex pheromone (Lewis *et al.*, 1972). These volatile compounds are certainly detectable cues, but they are not always reliable as indicators of the presence of host eggs. Whether a female moth oviposits on or close to the spot where it mates depends on the species and the type of vegetation. This limited reliability has two consequences. First, it is unlikely that *Trichogramma* use volatile cues for long distance orientation, because this could cause them to spend a lot of energy to move to a place where no eggs are present. Second, their reaction to compounds from calling females may be modified by experience. Indeed, odours from calling moth females do not cause upwind flight (Noldus *et al.*, 1991a) and wasps can learn to associate them with eggs (Kaiser *et al.*, 1989a). Instead of being used as cues for long-distance orientation, it seems that sex pheromones function as arrestment kairomones, keeping *Trichogramma* in areas with a higher expectancy of host eggs. How important sex pheromones are as host-cues in a field situation is unclear. *O. nubilalis* has only one generation in central Europe, and strict crop rotation means that the next year, the moths usually emerge and mate outside maize fields (Cordillot, 1987; Bigler *et al.*, 1988). In this situation, sex pheromones probably have little impact on field parasitism. In areas with more than one *O. nubilalis* generation per year, sex pheromones could be important host-cues.

Scales
Scales that lepidopterans lose when they land on a plant or brush against it can function as contact cues, i.e. *Trichogramma* must encounter the scale before it can perceive it. Zaborski *et al.* (1987) observed that behavioural responses to the kairomone of host scales only started after the wasp had touched the object containing the kairomone with its antennae. Extracts of the scale kairomone on paper are just as effective as kairomone in the scales itself in eliciting responses; thus the physical form of the scales is not important for *Trichogramma* (Jones *et al.*, 1973; Beevers *et al.*, 1981). Response to kairomones in host scales or extracts of these is very common in *Trichogramma* species (Table 2), and all authors agree that the scales cause arrestment, which is congregation to an area by undirected, kinetic reactions (Kennedy, 1978; Shorey, 1977). Different mechanisms can result in arrestment.

First, increased klinokinesis (a higher rate of turning), which causes the wasp to walk in circular patterns, and often makes the parasitoid return to its starting point. This will result in arrestment and in a small area being searched intensively. The normal walking behaviour, with few turns, causes the wasp to cover long distances rapidly. Increased klinokinesis is the response most commonly mentioned for *Trichogramma* species (Laing, 1937; Beevers *et al.*, 1981; Smits,1982; Zaborski *et al.*, 1987; Gardner and van Lenteren, 1986; Shu and Jones, 1989; Bieri *et al.*, 1990; Schmidt and Carter, 1992).

Table 2. *Trichogramma* species that show a reaction to host scales

Trichogramma species	host	source
T. brassicae	*Ostrinia nubilalis*	Kaiser *et al.*, 1989a
T. brassicae	*Ostrinia nubilalis*	Bieri *et al.*, 1990
T. brassicae	*Mamestra brassicae*	Smits, 1982
T. brassicae	*Pieris rapae*	Noldus and van Lenteren, 1985b
T. brassicae	*Pieris brassicae*	Gardner and van Lenteren, 1986
T. evanescens	*Sitotroga cerealella*	Laing, 1937
T. evanescens	*Mamestra brassicae*	Laing, 1937
T. evanescens	*Cadra cuatella*	Lewis *et al.*, 1972
T. evanescens	*Plodia interpunctella*	Jones *et al.*, 1973
T. evanescens	*Choristoneura fumifera*	Schmidt and Carter, 1992
T. minutum	*Choristoneura fumifera*	Zaborski *et al.*, 1987
T. minutum	*Sitotroga cerealella*	Zaborski *et al.*, 1987
T. minutum	*Heliothis zea*	Thomson and Stinner, 1990
T. minutum	*Manduca sexta*	Thomson and Stinner, 1990
T. minutum	*Ostrinia nubilalis*	Thomson and Stinner, 1990
T. pretiosum	*Heliothis zea*	Beevers *et al.* 1981
T. achaeae	*Heliothis zea*	Lewis *et al.*, 1975
T. nubilale	*Ostrinia nubilalis*	Shu and Jones, 1989
T. exiguum	*Heliothis zea*	Thomson and Stinner, 1990
T. exiguum	*Manduca sexta*	Thomson and Stinner, 1990
T. exiguum	*Ostrinia nubilalis*	Thomson and Stinner, 1990
T. maltbyi	*Heliothis zea*	Thomson and Stinner, 1990
T. maltbyi	*Manduca sexta*	Thomson and Stinner, 1990
T. maltbyi	*Ostrinia nubilalis*	Thomson and Stinner, 1990
T. sp. nr *pretiosum*	*Heliothis zea*	Thomson and Stinner, 1990
T. sp. nr *pretiosum*	*Manduca sexta*	Thomson and Stinner, 1990
T. sp. nr *pretiosum*	*Ostrinia nubilalis*	Thomson and Stinner, 1990

Second, inverse orthokinesis, a reduction in movement speed results in arrestment. A reduction of movement speed will prevent the wasp from moving away, and it allows more careful examination of the surroundings. That searching is intensified is also shown by increased antennal movements (Beevers *et al.*, 1981; Zaborski *et al.*, 1987). Inverse orthokinesis is commonly observed in *Trichogramma* (Beevers *et al.*, 1981; Zaborski *et al.*, 1987; Gardner and van Lenteren, 1986; Shu and Jones, 1989; Bieri *et al.*, 1990; Schmidt and Carter, 1992).

Third, flight inhibition, which causes the wasp to stay longer on the plant, results in arrestment. Flight inhibition causes arrestment on the whole plant, instead of on a single leaf or part of a leaf like increased klinokinesis and inverse orthokinesis. Most observations on scale responses have been made in small laboratory set-ups with limited observation time, which are unsuitable for studying flight initiation or inhibition. This is probably the reason why this behaviour is mentioned less than increased klinokinesis and inverse orthokinesis (Beevers *et al.*, 1981; Gardner and van Lenteren, 1986). If no egg is found, the different responses will wane until the normal walking pattern is resumed (Zaborski *et al.*, 1987; Gardner and van Lenteren, 1986).

Several trials have been made to use artificially spread scale kairomones to increase *Trichogramma* parasitism in crops (Jones *et al.*, 1973, Lewis *et al.*, 1972), but success has been limited, because the distribution of the artificial kairomone has to have some relationship with the host distribution and density (Lewis *et al.*, 1979). For the highest level of parasitism, there has to be an optimal compromise between arrestment and efficient movement. Distributing the artificial kairomone as particles comes closer to this optimum than a uniform treatment with kairomone spray (Beevers *et al.*, 1981). Continuous exposure to the kairomone can lead to habituation. Oviposition experience does not seem to increase the response to scales (Gardner and van Lenteren, 1986). Tricosane, the active compound of scales of *Heliothis zea*, is not specific to this species (Jones *et al.*, 1973). Thomson and Stinner (1990) studied the responses of four different *Trichogramma* species to the scales of three moth species. Two species reacted equally to all scale types, two others reacted poorly to *O. nubilalis* scales. The level of host specificity in the scale response does not seem to be very high. In this experiment, conditioning with host scales actually lowered responses, which could indicate habituation.

Scales are a dependable cue to the presence of adult lepidopterans. How reliable they are as a cue to egg presence depends on the behaviour of the host. Vet and Dicke (1992) predict that it is worthwhile for a generalist to have innate, fixed responses to cues that are common to most of its hosts. Such a non-specific response allows the generalist to exploit the most abundant hosts in a habitat (Chiri and Legner, 1986).

Eggs
Eggs themselves emit some odours, are covered with contact chemicals, and may be a visual cue that can be detected from some distance. Table 3 shows the species for which reactions to host egg odours were observed.

Table 3. *Trichogramma* species that show a reaction to host egg odours

Trichogramma species	host	source
T. brassicae	*Ostrinia nubilalis*	Renou *et al.*, 1989
T. brassicae	*Ostrinia nubilalis*	Frenoy *et al.*, 1992
T. evanescens	*Ephestia kuehniella*	Ferreira *et al.* 1979
T. platneri	*Boarmia selenaria*	Wysoki and de Jong, 1989
T. platneri	*Cryptoblabes gnidiella*	Wysoki and de Jong, 1989
T. rhenana	*Spodoptera littoralis*	Bourarach and Hawlitzky, 1984
T. rhenana	*Helicoverpa armigera*	Bourarach and Hawlitzky, 1984

However, Kaiser *et al.* (1989a) found no reaction of *T. brassicae* to egg odours of *O. nubilalis*, and neither was *T. evanescens* able to find *Sitotroga cerealella* eggs by odour only (Laing, 1937). Responses to egg odours are upwind motion (Ferreira *et al.* 1979; Kaiser *et al.*, 1989a; Renou *et al.*, 1989; Frenoy *et al.*, 1992), increased walking activity (Renou *et al.*, 1989; Frenoy *et al.*, 1992), increased antennal drumming (Renou *et al.*, 1989), and increased parasitism (Bourarach and Hawlitzky, 1984). Egg odour functions as an attractant, that is, a chemical that causes the wasp to make an oriented movement towards its source (Shorey, 1977). It is not clear at what distance egg odours have an effect. Olfactometer studies, where egg odour is the only stimulus present apart from the air current, are not suitable to measure the effective distance for field situations. Frenoy *et al.* (1992) found that there is an optimum concentration of egg odour. Lower and higher concentrations did not cause upwind movement. An innate short range response to egg odours would be profitable, since it is a very reliable cue, but the importance of egg odours as attractants is still unclear.

Vision seems to be more important than olfaction in the last stage of host-location (Laing, 1937; Pak *et al.*; 1990). Eggs can be visually detected over short distances. *T. evanescens* detects eggs of *Sitotroga cerealella* at 1.8 mm (Laing, 1937) and *Pieris brassicae* and *Mamestra brassicae* at 2 mm (Glas *et al.*, 1981) or 3.2 mm (Pak *et al.*, 1990). *T. cacoeciae* detects eggs of *P. brassicae* and *M. brassicae* at 2 mm (Glas *et al.*, 1981). For *T. brassicae*, reactive distance for *M. brassicae* eggs was 4.0 mm, significantly higher than the 3.7 mm found for smaller *Ephestia kuehniella* eggs. Glass beads with the size of *M. brassicae* eggs were found at 3.7 mm. The shorter reactive distance for glass beads could be a result of the lack of egg odour (Wajnberg, 1994). The response to visual detection of eggs is a sudden change in movement (Glas *et al.*, 1981; Wajnberg, 1994). *O. nubilalis* egg masses, being flat and not brightly coloured, are probably detected from even shorter distances only.

When an egg is found it will be examined and oviposition may follow. Oviposition causes increased klinokinesis and inverse orthokinesis in a similar way as scales do (Laing, 1937; Gardner and van Lenteren, 1986), increasing the chance that other eggs will be found in the patch. When the parasitoid starts to find more parasitized eggs, additional searching is inhibited. This is true both for eggs the wasp parasitized itself and eggs parasitized by others (Morrison and Lewis, 1980).

Conclusions

The size of the maize plants is the main crop factor influencing searching of *T. brassicae* in maize. Of the host-cues involved in *T. brassicae* host-finding, sex pheromones can only play a role if *O. nubilalis* mates inside the maize fields where it will later oviposit. This is rarely the case in Switzerland and the other central European areas where *T. brassicae* is released against *O. nubilalis*. Scales are the most detectable cue, and the reaction of the parasitoids to scales, combined with scale distribution patterns, determines if this host-cue will increase searching efficiency. Bieri *et al.* (1990) made a simple model of the effect of scales on searching of *T. brassicae* at the leaf level. According to this model, if scales are spread too far from the egg-masses, increased klinokinetic and inverse orthokinetic responses are counter productive compared to random search, because they will arrest the wasp to far from the eggs. Egg cues, both visual and chemical, seem to have a very short-range effect.

HOST ACCEPTANCE

Trichogramma species usually parasitize lepidopteran eggs, but may also be found on eggs of Coleoptera, Diptera, Hemiptera, Homoptera, Hymenoptera and Neuroptera (Pinto and Stouthamer, 1994). In general, *Trichogramma* are quite polyphagous (Fulmek, 1955; Wäckers *et al.*, 1987; Yin and Chang, 1987; Thomson and Stinner, 1989; Hirai, 1991), even though in some cases the number of hosts species attacked by one *Trichogramma* species may be exaggerated (Noyes, 1994). All *Trichogramma* species where parasitism has been studied are capable of parasitising more than one host species (Pintureau, 1990).

After having encountered an egg or an egg mass, the wasp enters into the host acceptance phase. The egg is inspected to determine size, quality, and parasitization status. In principle, any egg-like object between 0.22-4.64 mm will be inspected by *Trichogramma* (Salt, 1940; de Jong and Pak, 1984). Size, shape, colour and surface chemicals affect host-recognition (de Jong and Pak, 1984). In a situation where more than one type of egg or object is present, the largest is preferred (Salt, 1935). An egg is examined first with the antennae, and if accepted, with the ovipositor (Schmidt, 1994). Only then will oviposition occur. Host eggs are also measured by *Trichogramma,* and the size determines the number of eggs that the parasitoid oviposits into the host egg (Schmidt, 1994). Not all suitable eggs are accepted. In host-acceptance tests, the acceptance is rarely 100%, even when good quality eggs are offered in which the specific strain can develop well. The acceptance is partly genetic, and within species there can be large differences (Pak, 1988). Host acceptance is also

influenced by age, egg depletion and host density effects (Klomp *et al.*, 1980; Waage, 1986; Schmidt and Smith, 1987).

If *Trichogramma* has oviposition experience on a certain host, the acceptance of that particular host species is increased (Kaiser *et al.*, 1989b; Bjorksten and Hoffmann, 1995). There is also an effect of larval experience with the host in which the *Trichogramma* hatched, but this is less pronounced than that of adult experience (Bjorksten and Hoffmann, 1995). *Trichogramma* can detect if an egg has already been parasitized (Salt, 1934), but superparasitism may occur, especially under crowded situations. In superparasitized hosts, the percentage males is higher. The chorion of eggs parasitised by *Trichogramma* become black. This is caused by the release of a secretion of the labial glands by the third instar larva, which forms a cocoon-like covering around the body of the host larva (Saakyan-Baranova, 1990). However, if superparasitism leads to overcrowding inside the egg, the larvae may die before reaching third instar, and parasitism does not show. This is important to realize in experiments where high densities of *Trichogramma* can occur, because parasitism may remain unnoticed. After oviposition, many species of *Trichogramma* feed on the host egg exudate, which increases the wasps longevity and activity (Lee *et al.*, 1986). The host egg does not die because of host-feeding, as is the case in many other parasitoid species that show host-feeding.

GENERAL CONCLUSIONS

In maize, dispersal of *T. brassicae* has only been studied with indirect methods. The fact that both very short and quite long dispersal distances were measured for *T. brassicae* may be a result of different environmental circumstances, but also of differences in experimental methods. The dispersal within the maize crop over time and space is unclear as yet and the main mechanism for movement inside maize crops is also unknown. Dispersal should, therefore, be studied using direct trapping methods to establish dispersal speed, distance and mechanism.

Landing behaviour of *T. brassicae* has not been studied, apart from the experiments by Smits (1982). Only direct observation or trapping on the plant will show where *T. brassicae* starts searching. It must also be established whether host-cues influence landing behaviour.

Movement of *T. brassicae* on maize plants has not been studied, nor is it known how much time the wasps spent on plants. Detailed observation of movement on the plant is therefore necessary. The reaction of *T. brassicae* to host-cues on leaves or in petri dishes has been studied in detail. How host-cues affect the wasps on a whole plant is unknown. Measurements of the effect of temperature on walking speed and walking activity are available in the literature, but walking activity has only been studied indirectly, over short periods of time or at one specific temperature. Walking speed is very variable between strains. Therefore, walking activity and walking speed has to be measured for the *T. brassicae* strain used in this study.

Dispersal or searching behaviour experiments based on parasitism should be approached with caution, because the host-acceptance phase can obscure effects of host-habitat location, host location and dispersal. Effects of non-acceptance, non-suitability, and superparasitism may all lead to lower parasitism, which means that the number of wasps finding eggs in an experiment or part of an experiment will be underestimated. In this thesis, host acceptance and other behaviour that occurs after *T. brassicae* has encountered an host egg mass is not the topic of further study.

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Chapter 4

Short range dispersal of *Trichogramma brassicae* in maize fields

ABSTRACT
Glue-sprayed maize plants were used to study dispersal behaviour of *Trichogramma brassicae*. To estimate the distance covered during first flight, *T. brassicae* was studied in a field cage with 73 glue-sprayed plants. Most of the females were found close to the release point, but several reached plants at distances up to 180 cm showing that walking and jumping are not the only mechanisms involved in initial dispersal. Mean distance of recapture from the release point was 60 cm. In a second experiment, glue-sprayed plants were placed in a maize field in a cross-pattern with 1.5 m distance between plants. On plants neighbouring the glue plants, egg masses were fastened to measure parasitism. Only 0.7 to 3 percent of the wasps released were recaptured. During the first day there was a sharp gradient with distance in the numbers recaptured, but during the second and third day there was no significant effect. The numbers captured on the second and third day were much lower than on the first day. Apparently 75% of the wasps had left the area within 7.5 meter of the release point at the end of the first day and 95% at the end of the second day. *T. brassicae* disperses in maize fields by short flights. There is little correlation between the number of wasps landing on a plant and parasitism.

INTRODUCTION

The egg-parasitoid *Trichogramma brassicae* Bezdenko is being used worldwide for the control of Lepidopterous pests. An estimated area of 32 million hectares was treated in 1993 with *T. brassicae* and other parasitoids of the family *Trichogramma* (Li, 1994). Although extensive applied research has been carried out on *Trichogramma*, much information on the behaviour of the individual wasp in the field is lacking. One of the factors which has a clear influence on the efficiency of biocontrol release systems is the dispersal and host-location behaviour of the released wasps (Bigler, 1994).

Which behaviour, walking or flying, is most important for dispersal, is still a matter of controversy (Flanders , 1937; Smits, 1982; Pak *et al.*, 1985). After mating at the emergence site, which is the first priority for a non-parthenogenetic *Trichogramma*, the female will start searching for hosts. Normally, it has to move away from the place of emergence and locate a host patch. After the females have mated and left, the males will also have to disperse if they are to mate again (Keller *et al.*, 1985).

Dispersal speed is influenced by many factors, such as crop type and architecture, wind and total leaf area. For example, in biocontrol releases of *T. brassicae* in Switzerland, 50 release points per hectare are used, which assumes that the wasps will be able to parasitize effectively within an eight metre radius (Bigler, 1983). The distance from the release point at which a sufficient level of parasitism is found varies from 5 to 50 metres, depending on the *Trichogramma* species and the crop (Franz and Voegelé, 1974). Low rates of dispersal were reported for several *Trichogramma* sp. (3 m, Torgovetski *et al.*, 1988), for *T. minutum* in cotton (4-8 m in several days, Fye and Larsen, 1969), for *T. pallida* and *T. embriophagum* in apple trees (3 m in one day, Kolmakova and Molchanova,

1981) and *Trichogramma* spp. in orchards and cabbage (50 m in one week, Kot, 1964). High rates of dispersal were found for *T. brassicae* in maize (400 m in one week, Bigler *et al.*, 1990), *T. semifumatum* in alfalfa (maximum 1000 m in 62 hours, Stern *et al.*, 1965) and *Trichogramma* spp. in cotton (130 m in two days, Stinner *et al.*, 1974). Apparently, dense crops, especially trees, inhibit dispersal (Meyer, 1941; Kot, 1964; Kolmakova and Molchanova, 1981). Strong winds in a fixed direction can increase dispersal of *T. brassicae* (Bigler *et al.*, 1990).

Many dispersal studies of *Trichogramma* have been made (see e.g. Keller *et al.*, 1985). The usual method to determine dispersion consists of using parasitism data from natural eggs or artificial egg cards, and has a clear flaw. Parasitized eggs indicate that wasps have been present but not how many. Unparasitized eggs do not guarantee that wasps have not been present, because generally not all eggs are accepted, even under optimal circumstances (Cerutti and Bigler, 1991). Some authors used sticky traps, which show where wasps have been present (Kolmakova and Molchanova, 1981; van den Berg *et al.*, 1987). Keller and Lewis (1985) used a combination of egg cards and sticky traps in cotton to study behaviour of *T. pretiosum*. They did not use a point release, so their results only indicate density of *Trichogramma* present in the field. It is unknown how *Trichogramma* reacts to colours and therefore it is unclear whether the number of wasps caught on sticky cards represents the number normally landing on plants. Sweep nets and vacuum suction samplers may give a better picture of the number of wasps present. Stern *et al.* (1965) could show high maximum rates of dispersal in alfalfa fields using these devices in combination with radio-actively marked wasps. Such methods are labour-intensive to use and cannot collect wasps continuously over a longer period. Using glue-sprayed plants, on which wasps have shown to land normally (Chapter 5), will give a clear indication of the number of wasps that visit a plant. This technique is used in the experiments described in this paper.

The objectives of this paper are: 1. to establish the distance covered during the first flight from the release point by *T. brassicae* and 2. to see how spatial and quantitative aspects of landing correlate with parasitism.

MATERIALS AND METHODS

Materials

Plants
The experiments were carried out in maize fields (cultivar Atlet) with normal planting density (75 cm between rows, 15 cm between plants). The fields used for the field dispersal experiments were normal agricultural fields with a size of approximately 0.6 and 0.2 ha. The experiments always took place at least 10 m from the field edge.

Wasps
Trichogramma brassicae Bezdenko was used in all experiments. The stock material was obtained in 1975 from Antibes (strain 16), which was imported from the former Soviet Republic of Moldavia, and has been maintained on *Ostrinia nubilalis* Hübner eggs since. The rearing system is described in Bigler (1994). To obtain the numbers of egg masses necessary for mass releases, the wasps were reared for two or three generations on *Ephestia kuehniella* Zeller, creating so-called F2 and F3 material.

Moth egg masses
Egg masses of *Ostrinia nubilalis* Hübner, the European corn borer were used in the field dispersal experiment. Egg masses were deposited by the moths on wet filter paper placed on top of the rearing cages. For use in the field, cut strips (3 cm long and 1 cm wide) with one egg mass were used.

Glue
Soveurode® aerosol glue for sticky insect traps sold in pressurized spray cans was used on the plants. This had neither an attractive nor a repellant effect on *T. brassicae* (Chapter 5).

Methods

Initial flight distance
In a full-grown maize field, a nine m^2 area containing four rows (a total of 73 plants) was enclosed in a wooden frame cage with fine netting (Scrynel® 110 HC). Plants and netting were sprayed with glue. In the middle of the cage a plant without glue was placed, and a release container with parasitized *Ephestia kuehniella* eggs producing about 5000 *T. brassicae* females was fastened with tape to this plant about 60 cm from the ground. These eggs were timed to emerge on the first day of the experiment. Outside the cage a similar container, closed with netting, was fastened to a plant to check the emergence rate and the sex ratio of the emerged wasps. The wasps were allowed to emerge and fly for three days, then the cage was opened, the release container and the control container were stored in a freezer and all plants were potted and inspected for the presence of parasitoids in the laboratory. The location and sex of each *T. brassicae* captured on the plants was recorded. The release container was checked, all wasps that had not left were counted, and three samples of 100 black eggs were randomly taken to determine the emergence rate. The experiment was run on July 22-24 and August 16-18, 1993. In the first replicate, the first day was relatively cool with only eight hours above 18°C and 5.4 mm of rain. The second and third day were warm and dry. In the second replicate all days were warm with at least eleven hours above 18°C.

Dispersal in the field

Twenty maize plants were potted and sprayed with glue. The plants were placed in a cross pattern in a maize field at 1.5, 3, 4.5, 6 and 7.5 m from a central release point. On a plant next to each glue plant, an egg mass on filter paper was clipped to one of the middle leaves. At the central plant, a release container with about 10000 female and 7000 male one day old or emerging *T. brassicae* was placed on the ground. The glue-sprayed plants and egg masses were left in the field overnight. The next two mornings, the glue-sprayed plants and egg masses were replaced by fresh ones. After the experiment, the egg masses were incubated at 25°C until the parasitized eggs turned black. To measure parasitism, white and black eggs in each egg mass were counted. The glue-sprayed plants were checked under the microscope and the number, sex and position of the wasps captured on the plants were recorded. The experiment was run three times, on June 8-10, June 19-21 and June 30-July 2, 1993. In the first replicate, the experiment was stopped after only two days. During all days there were at least ten daylight hours with temperatures above 18°C. During the second day of the second replicate, there was a short rainstorm with 2.9 mm of rain. The glue-sprayed plants had an average of 4.4 leaves in the first replicate (mean height 40 cm) and 6.6 leaves in the second (mean height 100 cm) and third (mean height 120 cm) replicate. There were no other releases close to the fields and no natural occurring *Trichogramma* spp.

Statistics

The number of wasp landing per plant were compared using ANOVA and the Duncan test. Data were square-root transformed to meet the assumptions of ANOVA. The total number of plants on which the wasps can land are expected to be proportional to the distance covered. If the wasps move out from the centre in a random diffusion process the numbers landing on the glued plants at 1.5, 3, 4.5, 6 and 7.5 m would be 5:4:3:2:1. To test if this assumption was correct for in the field, we divided the numbers landing by these proportions to correct for the area. An ANOVA test was run for the corrected data to see if there were significant differences in numbers landing. For the analysis of parasitism, the data (which represent a rate) were arcsine transformed and tested with an ANOVA and Duncan test.

RESULTS

Initial flight distance

Of the 73 plants in the cage, 51 were checked for *T. brassicae* in the first replicate. The actual number of females which had emerged was about 4000, of which 575 were found on the checked glue plants, a recapture of 14.3 percent. In the second replicate, 53 of the 73 plants were checked and 838 females of 3900 were recaptured, which is 21.1 percent. The number of females that landed on the plants can be seen in Figure 1. Most females were recaptured on the two middle rows, and the number of landings decreases sharply with increasing distance from the release point. However, some managed to reach the outer plants.
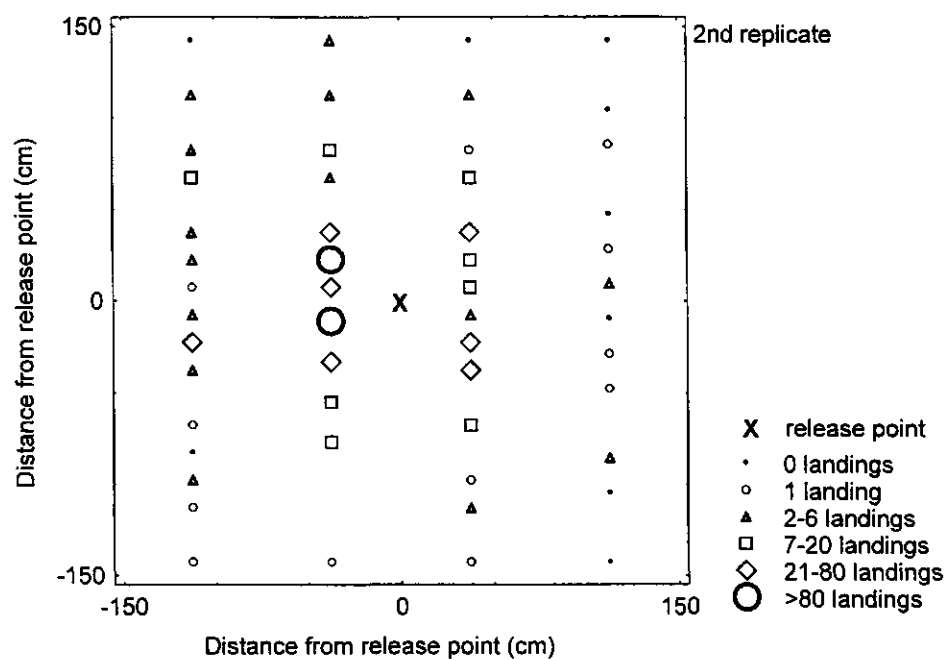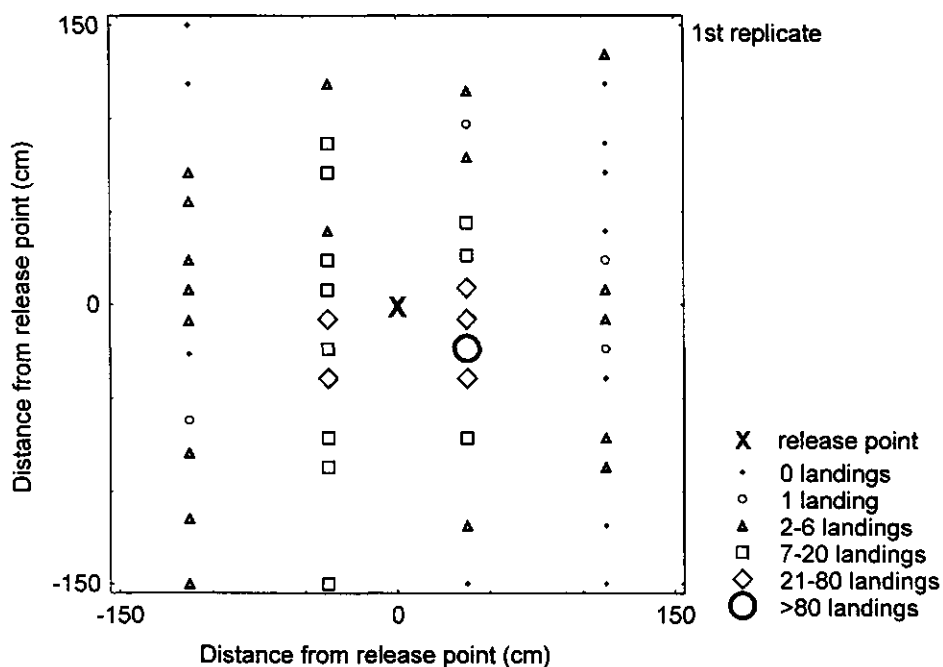
Figure 1. Number of female *T. brassicae* landing on glue-sprayed plants in a field cage.

Since the netting touched the tops of the plants, the wasps were forced to fly between the plants and not over them, which might have reduced mean flight distance. The mean flight distance was 59.4 ± 0.6 cm, the median 43.7 cm.

Part of the netting was checked for *T. brassicae*, especially the part directly above the release point, but none could be found. The netting was found to be not very sticky, so wasps that landed on the netting might have left again. The ground under the release plants was not checked.

Dispersal in the field

Landing
Of 17000 wasps released, 92 (0.54%), 381 (2.24%) and 333 (1.96%) were recaptured in the first, second and third replicates, respectively. This equals 0.54, 2.24 and 1.96 percent of the number released. Of the females, 0.67, 3.07 and 2.89 percent were recaptured. The actual numbers of females landing on the first, second and third days are shown in Figure 2. It is clear that the number of females landing on the first day was always much higher than on the second and third days. On the first day, there was a clear gradient with significantly more females landing on the plants closest to the release point. The shape of the first-day landing curve was similar for the three replicates. On the second and third day, there was no relation between distance and numbers landing. If we compare the number of actual landings with the expected number of landings based on diffusion dispersal, the mean number of wasps landing on the plants closer to the release point is significantly higher than expected (Figure 3) (ANOVA, P<0.0089). This indicates that the mean flight distance was lower than 1.5 m, and that the assumption of random diffusion is incorrect.
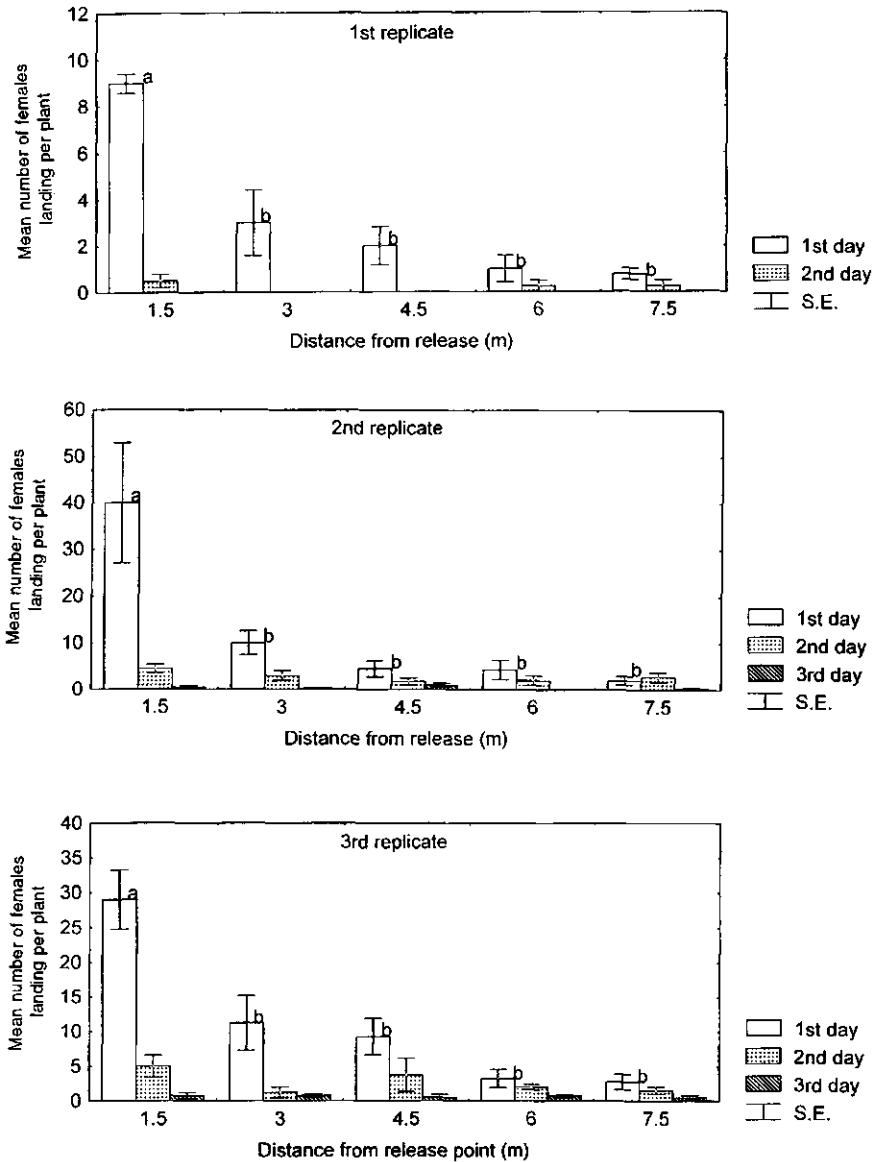
Figure 2. Mean number of female *T. brassicae* landing on glue-sprayed plants at different distances from the release point one, two and three days after release. Different letters indicate significant differences (P<0.05) in a Duncan test of square-root transformed data. No significant differences on second and third days.
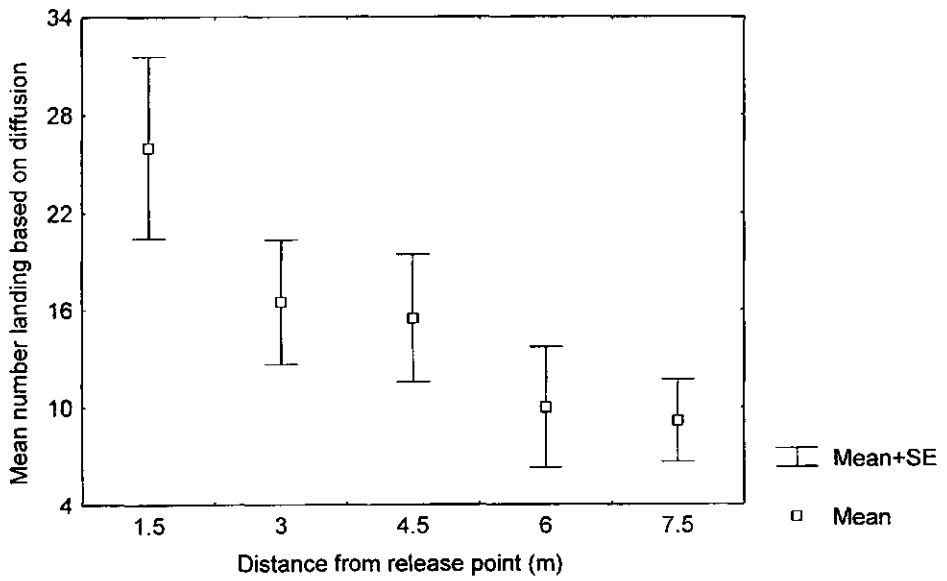
Figure 3. Mean number of female *T. brassicae* landing on glue-sprayed plants on the first day, corrected for distance based on random diffusion (data from three runs)

The number of males on the plants was lower on the first days than what would be expected from the sex-ratio of the release material (Table 1). On the second and third days, the proportion of males increased and came close to the expected 0.6 sex-ratio of the released material. Vertical landing distributions over the leaf levels of both males and females were similar to those found in the experiments of Chapter 5.

Table 1. Number and percentage of females of total number of released *T. brassicae* landed on glue-sprayed plants one, two and three days after release.

replicate	day	N	percentage females
1	1	86	0.73
	2	6	0.66
2	1	267	0.91
	2	100	0.56
	3	14	0.57
3	1	241	0.92
	2	71	0.76
	3	21	0.61

Parasitism
The parasitism of the egg masses placed in the field is shown in Figure 4. In the first two replicates, mean parasitism was 37 and 40% on the first day. On the second day, mean parasitism was 24 and 4%, respectively. On the third day of the second replicate, 6% of the eggs were parasitized. In the third replicate, there was only 3% mean parasitism on the first day and even less or none (2% and 0%) on the second and third day. There was no correlation between parasitism and distance from the release point in any of the replicates (ANOVA of arcsine transformed percentages). Most of the egg masses were only partly parasitized, on average 52.2% of the eggs in an egg mass were parasitized, and this was markedly lower on the second and third day.

There was no correlation between the number of wasps on the glue-sprayed plants and the parasitism of the egg masses at the same location.

DISCUSSION

The experiment for estimating initial flight shows that in most cases this initial flight distance is very small. Most of the wasps were found on plants close to the release plant. Since plants within rows were only 15 cm apart, and the plants had leaves that were up to 95 cm, short jumps (<5cm) from the release plant to the glued plants could account for some of the dispersal measured. This would mean that the wasps would be found at those spots where the leaves of the glued plants touched the release plant. This was not found in the experiment, indicating that walking and short jumps were not, as suggested by Pak *et al.* (1985), the main mechanisms of dispersal. A small part of the wasps reached plants that were either too far away for jumping or in the two outer rows, which shows that *T. brassicae* does fly between plants without intermittent landings and that not all movement is by flights of only a few centimetres. A problem in these experiments is the low percentage of wasps recaptured. Since the plants were all sprayed with glue, all the wasps that landed on the plants were captured. No wasps were found on the parts of the netting that were checked. Since the netting was checked thoroughly directly above the release point, it is unlikely that many wasps flew straight upwards. Apparently, most wasps fly between the plants if the crop is this high. Keller and Lewis (1985) found that most flight activity of *Trichogramma pretiosum* also takes place within the cotton crop and not above it. In an empty cage of the same size with a release container hanging free in the centre up to 24 to 40 percent of the wasps were found on the ground directly under the release point, and only 2 percent or less on the top (Dutton and Bigler, 1995).

The experiment on landing and oviposition indicated a fast dispersal behaviour. On the first day there was a clear gradient between the release point and the outermost glued plant, after that the wasps seem to be spread fairly even over the experimental area. The high number of wasps on the closest plants is expected to be the result of many wasps moving away from the release points with short flights. This confirms our observations from the initial flight experiment. If the mean flight distance was longer than 1.5 m, there would not be such a steep gradient, because many wasps would fly over the glue-sprayed plants.
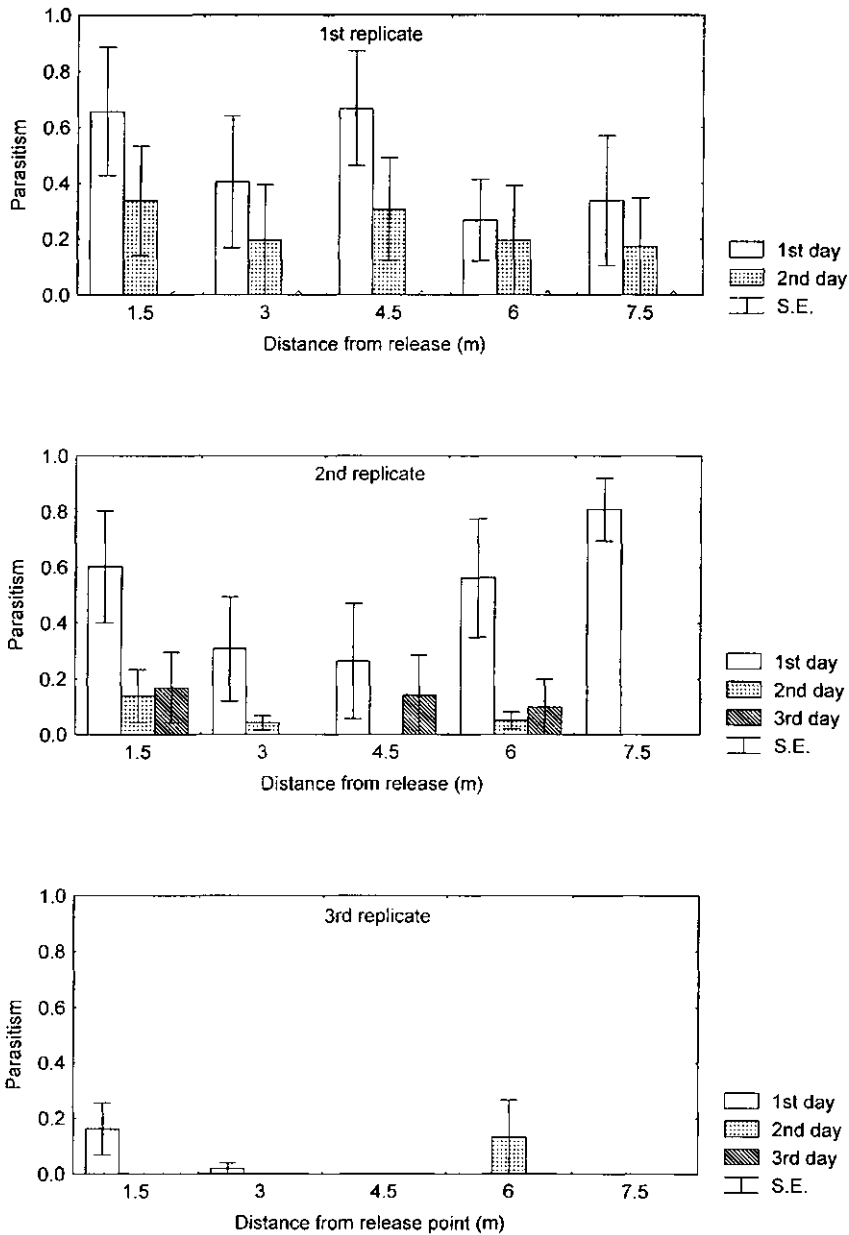
Figure 4. Mean percentage of parasitism of *O. nubilalis* egg masses at different distances from the release point one, two and three days after release. No significant differences (P<0.05) in a Duncan test of arcsine transformed data.

The minimum temperature for *T. brassicae* flight is 18°C (own observation) and *T. brassicae* is only active during daylight (Pompanon *et al.*, 1994). This means that the wasps had at least ten hours each day to disperse by flight in the experiments. The mean time spent between flights is about 20 minutes (Chapter 6), so about thirty flights could be made each day. If the mean distance of the first flight (60 cm) is a good indication for the distance of flights in general, this should cause most wasps to leave the experimental area in three days, even if flight was undirected. We assume that the number of wasps caught on the glued plants were proportional to the number of wasps present in the experimental area. This means that only 25% of the wasps were still in the experimental area on the second day and only 5% on the third day. This disappearance could be caused by dispersal or mortality. The mean life-span in the lab of the *T. brassicae* material used was at least ten days (Bigler *et al.*, 1987) and weather conditions in the field were not extreme, so the disappearance must be a result of dispersal. This high rate of dispersal would explain the high emigration rate found for *T. evanescens* (=*brassicae*) in small maize fields (Bigler *et al.*, 1990).

In the first replicate where the plants were small, the number of *T. brassicae* landing was much lower than in the other replicates. This might be an effect of the plant landing area available, but it is also possible that the dispersal speed is influenced by plant size. Residence time of *T. brassicae* is lower on smaller plants (Chapter 9), so the number of flights per day is higher. The open space between the plants also means that the wind is stronger inside the crop, which might influence speed of passive movement out of the experimental area.

Males dispersed slower than females. *Trichogramma* males emerge earlier on average than females (Waage and Ng, 1984; Lee *et al.*, 1986; Forsse *et al.*, 1992). Apparently, the males spend a longer time close to the area where they emerged than the females, before they start dispersing. Slow dispersal of *Trichogramma* males was also found by Stern *et al.* (1965) for *T. semifumatum*.

Parasitism was low throughout the experiment. The very low parasitism on the second and third days is the result of the very low numbers of *T. brassicae* still present. The numbers landing on the adjacent glue-sprayed plants on the first day suggest that substantial numbers of wasps must have landed on the plants with egg masses closest to the release point. The fact that not all egg masses were parasitized at this distance means that the chances of an individual female to find an egg mass on a plant on which it landed are not very high. However, the fact that the egg masses were on cards clipped to the leaves makes it more difficult for the females to find the egg masses (Chernyshev *et al.*, 1988). A gradient in parasitism around the release point was found in maize (Bigler *et al.*, 1988; Maini *et al.*, 1991), but not in cabbage (van der Schaaf *et al.*, 1984). Van den Heuvel (1986) measured a parasitism gradient in low maize crops but not in high ones. The absence of correlation between landing and oviposition in this experiment shows that parasitism is not a good indicator of dispersal as such.

Parasitism was just as high in the first as in the second replicate, although the number landing was much higher in the second replicate. This is most likely a result of the smaller leaf area of the plants in the first replicate. In theory, searching success of *Trichogramma* is directly correlated with leaf area, because *Trichogramma* searching is assumed to be mostly random (Kanour and Burbutis, 1984; Knipling and McGuire, 1968). In many field studies, this was also observed (Ables *et al.*, 1980; Burbutis and Koepke, 1981; van den Heuvel,

1986; Kot, 1979; Maini *et al.*, 1991; Need and Burbutis, 1979). However, this assumes that dispersal and disappearance are the same in crops with plants of different size. In a point release, there will be no such clear correlation, because dispersal has a much stronger effect on the number of wasps actually available for parasitization in the testing area. Parasitism by *T. evanescens* was also higher after plants became larger (and the leaf canopy closed) in small cabbage plots (van Heiningen *et al.*, 1985). The reason for the very low parasitism in the third replicate is unclear, since the difference in size of the plants with the second replicate was not large.

In conclusion, both experiments show that flight, and not walking or jumping, is the most important mechanism in between-plant movement for *T. brassicae*. This is supported by the fact that there is little effect of planting distance (van Alebeek *et al.*, 1986; Neuffer, 1987; Chapter 5) on dispersal. The second experiment clearly shows that parasitism is not a very good measure of dispersion. Host location, host recognition, host acceptance and host availability are all involved in determining the level of parasitism. If the object of study is purely the dispersal behaviour and not the performance of *T. brassicae* as a biocontrol agent, the use of trap plants will yield clear and unambiguous information.

ACKNOWLEDGEMENTS

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Chapter 5

Landing of *Trichogramma brassicae* on maize plants[1]

ABSTRACT
Landing behaviour of *Trichogramma brassicae* Bezdenko was studied in the greenhouse and in the field. Using insect glue to trap parasitoids on plants is shown to be a useful technique to record landing. Landing distributions over the leaf levels of the plant are the same in the greenhouse and the field. Most wasps land on the middle and largest leaves. There is a preference for the upper part of the plant, where the number of wasps landing per unit area is higher than on the lower leaves. Most of the wasps land on the middle part of individual leaves, or the part close to the stem. The upper side of the leaves is preferred over the lower side for landing. Release height of *T. brassicae*, position of the plant in the field (in or between rows) and kairomones released by scales and egg masses or sex pheromones of *Ostrinia nubilalis* have no effect on landing of *T. brassicae*.

INTRODUCTION

The parasitoid wasp *Trichogramma brassicae* Bezdenko is being used worldwide for biological control of Lepidopteran pests. Although the species has been intensively researched, very limited information is available on the behaviour of individual females in the field. Most studies concern the effect of mass releases on parasitism or the behaviour of parasitoids under laboratory circumstances. Detailed knowledge of field behaviour is important to identify the traits which have the greatest impact on host-finding. This is essential both for *Trichogramma* strain selection and for quality control in biological control projects.

    Trichogramma wasps have three ways of reaching a new plant to search for host eggs: by walking, hopping or flying. In case of walking, neighbouring plants need to touch each other. This is not uncommon in full-grown maize crops. The limited visual range of *Trichogramma* (Laing, 1937; Pak *et al.*, 1990; Wajnberg, 1993) means that places where leaves touch will only be randomly encountered. When leaves are close, *Trichogramma* can jump to the other plant. In other circumstances, flight will be necessary to move to other plants. In the case of flight, *Trichogramma* is free to choose its landing spot on the maize plant. It is presently unknown where *T. brassicae* lands on the plants, though this landing distribution is essential for understanding and evaluating host-finding behaviour. The small size of *T. brassicae* makes its impossible to follow the wasp in flight. Spotting them on a plant is possible, but requires that the observer can move around the whole plant to scan the leaves without disturbing them. Because of this, direct observation of landing in a crop situation is not possible, and an indirect method had to be found. Plants were sprayed with

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glue, causing all landing *T. brassicae* to stick to the plant on the spot where they had landed. To check whether this method gave results similar to direct observation, first a comparison was made in the greenhouse. Ables *et al.* (1980) found a correlation between release height and parasitism in different leaf levels on tobacco for *Trichogramma pretiosum*. To see whether this might be found in maize too, the effect of release height was studied in the greenhouse. It is known that several parasitoids use host-cues to locate or select plants to land on (Weseloh, 1981). Two experiments were conducted to see if landing by *T. brassicae* was influenced by host-cues. In one experiment the host-cues were concentrated in one patch, in the other the host-cues were spread all over the plant.

MATERIALS AND METHODS

Materials

Plants
Greenhouse reared maize plants (*Zea mays* L.) of cultivar LG11 were used for all experiments except the last one, where the plants (cultivar Atlet) were taken from the field. Field-grown maize has a thicker stem and larger leaves. In the last experiment, plants were in the full-grown stage with ripening grains. In the field, the male flowers had been removed to keep the plants clean from pollen.

Wasps
Trichogramma brassicae Bezdenko was used for all experiments. The basic rearing material used was obtained from Antibes (strain 16), which was imported from the former Soviet Republic of Moldavia, and has been maintained on *Ostrinia nubilalis* Hübner eggs since then. (for details of rearing system see Bigler (1994)). To rear the numbers of wasps necessary for mass releases, the wasps were reared for two or three generations on eggs of *Ephestia kuehniella* Zeller, creating so-called F2 and F3 material. *E. kuehniella* eggs were exposed to *T. brassicae* for 24 hours. Part of the wasps emerged the day before the experiments, while the rest emerged on the day of the experiment itself.

Moths
Ostrinia nubilalis Hübner, the European Corn Borer was used in the host-cue experiments. The stock colony was taken from a Swiss field population in 1990. Individuals used in the experiment were 1-2 days old. Egg masses used in the experiments were collected from wet filtration paper on top of the rearing cages.

Glue
Glue for sticky insect traps sold in pressurized spray cans was used to make the maize plants sticky.

Methods

Observation of T. brassicae landing in the greenhouse

Maize plants of 120 cm (9-10 leaves) were used for the experiment. With a marker each leaf of two maize plants was divided in three parts of equal length: close to the stem, middle and point. part. To calculate the leaf area, length and width of the leaves were measured and multiplied by 0.764 (Boot, 1985). One plant was sprayed with glue (Stoeckler Insekten-Leim-Spray or Neudorff's Kirschfliegenfalle). Both plants were placed in a greenhouse compartment, 1.5 meter apart from each other. Two meters from the plants, a cylinder with approximately 2000 emerging and one-day old *T. brassicae* (F2) was placed on a stand at 60 cm from the floor. After opening the container, the plant without glue was scanned continuously and systematically from top to bottom by an observer, using a mirror to check the lower leaf sides. As soon as a *T. brassicae* was detected, the location was marked on a list and the wasp was removed with a wet fingertip. After two hours the plant with glue was removed from the compartment and the number of *T. brassicae* stuck to the plant counted.

During the experiments all ventilation openings were closed to avoid air currents. The temperatures varied between 25 and 35°C. The experiment was replicated ten times.

Effect of height of release of T. brassicae on landing in the greenhouse
The leaves of two potted maize plants (190 cm, 12 leaves) were both divided into sectors as described for the first experiment. Both plants were sprayed with glue (Stoeckler Insekten-Leim-Spray). The plants were placed in a greenhouse compartment. One plant was placed on the floor, the other on a one meter pedestal. The plants were 1.5 meter apart. Two meters from both plants, a release container with 4000 one day old and emerging *T. brassicae* (F2) was placed at 1 meter height. Two hours after opening the release container, the plants were removed from the greenhouse and the number of wasps stuck to the leaves counted. The temperatures varied between 25 and 35°C. The experiment was replicated four times.

Landing of T. brassicae in the field
The fields used had normally developed maize crops, with no naturally occurring *T. brassicae*. The maize plants were sown 15 cm apart from each other within rows and 75 cm between rows. The site for the experiment was at least 7 meters from the nearest field edge. Eight potted maize plants were sprayed with insect glue (Soveurode) the day before release, so the strongest fumes from the glue could disappear. The plants were then placed in a maize field, in a circle with a 2 meter radius. Four plants were placed within rows, and four plants between the rows. In the middle of the circle, a cylinder with about 10000 one day old and emerging *T. brassicae* (F3) was fastened to a plant at 40 cm above the ground. The cylinder in the morning between 9 and 12 a.m. After 24 hours, the plants were taken out of the field to count the number and position of wasps stuck on the plant. The sex of the recaptured wasps was also determined. The experiment was replicated three times.

Mean size of maize plants was 100 cm (11 leaves) in the first two replicates and 140 cm (11 leaves) in the last. The experiments were conducted in July on sunny days with temperatures between 20 and 30 °C. In the evening of the first replication, there was a thunderstorm and some rain.

Landing of T. brassicae on leaves with localized host-cues
To get a localized patch of host-cues, a small cardboard cylinder (length 5 cm, diameter 3 cm) was fastened with one opening against the leaf of a maize plant. The other end was closed with netting. Two *O. nubilalis* females were enclosed in the tube and left inside for one night so they could move over a small area of the leaf. The next morning, the tube with the moths was removed. A 10 cm piece of the leaf was cut out, with the artificial patch in the middle. An *O. nubilalis* egg mass was placed in the middle of the artificial patch. One end of the leaf piece was placed in a clamp with wet paper to keep the leaf piece moistened. A clean piece of leaf of the same size was also placed in a clamp with wet paper. Both clamps were fastened to a stand with the leaf pieces about 10 cm apart. This stand was placed in the middle of a 80 x 80 x 80 cm box of plexiglass covered with white paper. One side of the box, where the observer was positioned, was left open. A release container with 1000 one day old and emerging *T. brassicae* (F3) was placed in the box, against the back. The container was opened and for 2 hours an observer noted the time and position of the wasps landing on the leaf pieces and removed each wasp after landing. The experiment was replicated eight times.

Landing of T. brassicae on plants with non-localized host-cues
Six plants were used. Three plants were exposed to ten *O. nubilalis* females and five males in the greenhouse. Contact between *O. nubilalis* and maize plants was not desired in this experiment since it was aimed to test volatile chemical kairomone cues and there should be no *O. nubilalis* scales on the plant. To prevent *O. nubilalis* from contacting the maize plants, plants were caged with thin netting (Monofiles Nylon netting 1/10 mm hole size, Scrynel). Previous work (Noldus, 1989) had shown that a mesh size of 340 µm was able to intercept 90% of scales, so we assumed 100 µm netting was adequate for this work. Netting was kept as close to the plant as possible and a second layer of netting was put over the thin one to keep *O. nubilalis* between the two layers of netting as close to the plants as possible. *O. nubilalis* females and males were introduced between the two layers of netting and left 24 hours. The plants that were not exposed to *O. nubilalis* were placed in another greenhouse in order to avoid any contamination with host volatiles. Afterwards, the group of treated plants and the group of untreated controls were placed 2 meters apart in a greenhouse. On each plant, three *O. nubilalis* egg masses were stuck to the leaves. Approximately 10.000 one day old and emerging wasps (F2) were released in the middle between the two plant groups. Two observers continuously checked one group of plants each for two hours and counted every *T. brassicae* that landed, and removed it after landing. The experiment was replicated four times.

RESULTS

Landing of *T. brassicae* on plants in the greenhouse

A total of 846 wasps were observed to land on the plants without glue, and 967 wasps were found on the plants with glue. Wasps started landing within seconds after opening the container. The number of wasps that land decreases with time. The distribution over the leaf levels is shown in Figure 1. As can be seen, most wasps land on the middle leaves. The average leaf level was 5.9 for the observed plants and 6.1 for the plants with glue. We were interested whether the number of landings is related to the surface area.
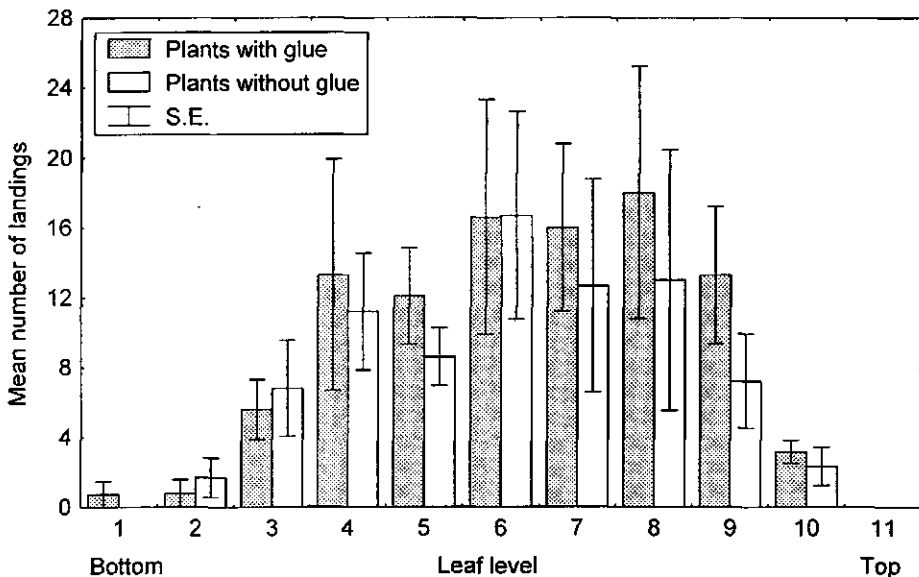
Figure 1. Distribution of landings of *T. brassicae* over different leaf levels of maize plants, determined by direct observation of landing and by using glue-sprayed plants in greenhouse experiments. The lowest leaf is level one.
(Mean±S.E. of ten replicates)

Figure 2 shows that for both plants without and with glue the number of wasps per unit of leaf area is clearly higher for the upper leaves.
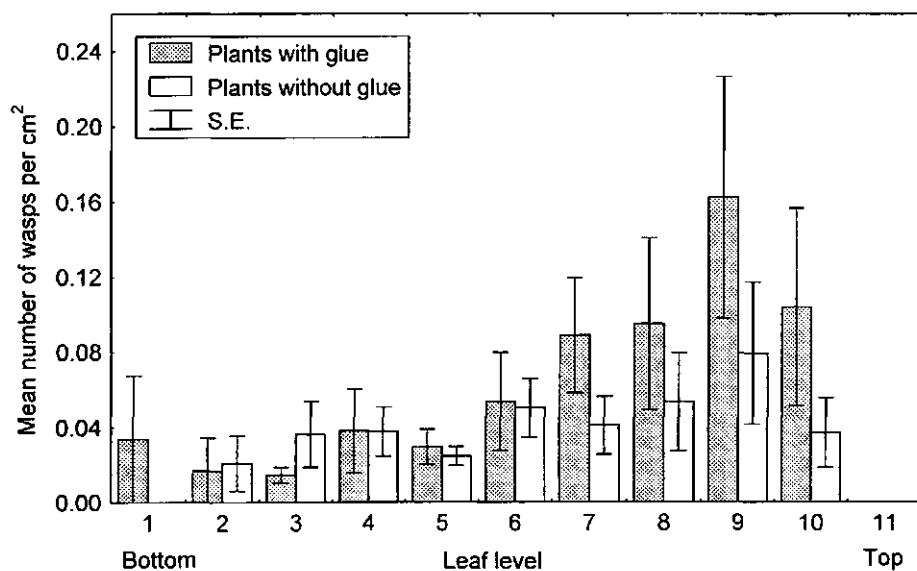

Figure 2. Number of *T. brassicae* landing per cm² on maize plants, determined by direct observation of landing and by using glue-sprayed plants in greenhouse experiments. The lowest leaf is level one.
(Mean±S.E. of ten replicates)

The distribution of landings over parts of the leaf was also studied (Table 1).

Table 1. Distribution of landing of *T. brassicae* over parts of the leaf.

	plants without glue	plants with glue	p[1]
Total number landing on leaves in ten repetitions	814	906	
Number of landings	74.0 ± 25.2[2]	90.6 ± 30.8	*[3]
Landing			
Part of leaf close to the stem	29.4 ± 9.8 (39.7%)	37.7 ± 13.7 (41.7%)	n.s.[4]
Middle part of leaf	32.1 ± 11.4 (43.4%)	37.0 ± 13.0 (40.8%)	n.s.
Point of leaf	12.5 ± 4.3 (17.0%)	15.9 ± 4.2 (17.6%)	n.s.
Landing			
Upper side of leaf	48.1±18.4 (65.0%)	51.2±16.8 (56.5%)	***[5]
Lower side of leaf	25.9±7.4 (35.0%)	39.4±14.8 (43.5%)	***

1 n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$
2 Mean ± s.e.
3 Wilcoxon Matched Pairs Test (CSS:Statistica, 1991)
4 Chi-square test (Siegel, 1956)
5 Chi-square test (Siegel, 1956)

A small part (3% on the plants without glue, 6% on the plants with glue) of the wasps did not land on the leaves but landed on the stem. There was a slight preference for the upper leaf side, which was somewhat more pronounced in the plants without glue. Most wasps land on the part of the leaf close to the stem or on the middle part of the leaf, and only 17% on the point of the leaf.

The correlation between the numbers that landed on the plants with glue and the numbers that landed on the clean plants each day was very high: 0.979 (Pearson product-moment correlation).

Effect of height of release of *T. brassicae*

3626 wasps were found on the plants where the wasps were released at 100 cm, and 4429 on the plants where the wasps were released at ground level. The results are shown in Table 2.

Table 2. Effect of height of release on landing of *T. brassicae* on maize plants.

	release at plant base	release at plant middle	p[1]
Total number of wasps	4429	3626	
Number landing	$1107,3 \pm 191,7$[2]	$906,5 \pm 210,9$	n.s.
Mean leaf level of landing	$5,15 \pm 0,29$	$5,56 \pm 0,28$	n.s.

1 Wilcoxon Matched Pairs Test (CSS:Statistica, 1991) n.s.: P > 0,05
2 Mean ± s.e.

The distribution over the leaf levels was very much like that of the first experiment. When wasps are released at ground level the mean level of landing is only 0.4 leaf lower (which is equivalent to about 8 cm) than when the wasps are released at 100 cm. The landing patterns on the low and the high plants were quite similar. There was no difference in the numbers of wasps landing in both treatments. Again, more wasps landed on higher leaf levels than can be explained just by surface area.

Landing in the field of *T. brassicae*

The distributions of landing in the field were about the same as in the greenhouse (Figure 3). Of the 10000 wasps released, 193 (1.93%), 357 (3.57%) and 252 (2.52%) were recaptured on the 8 plants. Of these, 73 % were females, while the sex ratio of the release material was 60% females, which means that less males land then expected (Chi-square test, P<0.0001). Although the wind was quite strong in two of the replicates, this was not apparent from the distribution over the plants.

Of the eight plants, four were placed in the maize rows and four between the rows (Table 3). Although less wasps land on the plants between the rows, the difference is not significant.
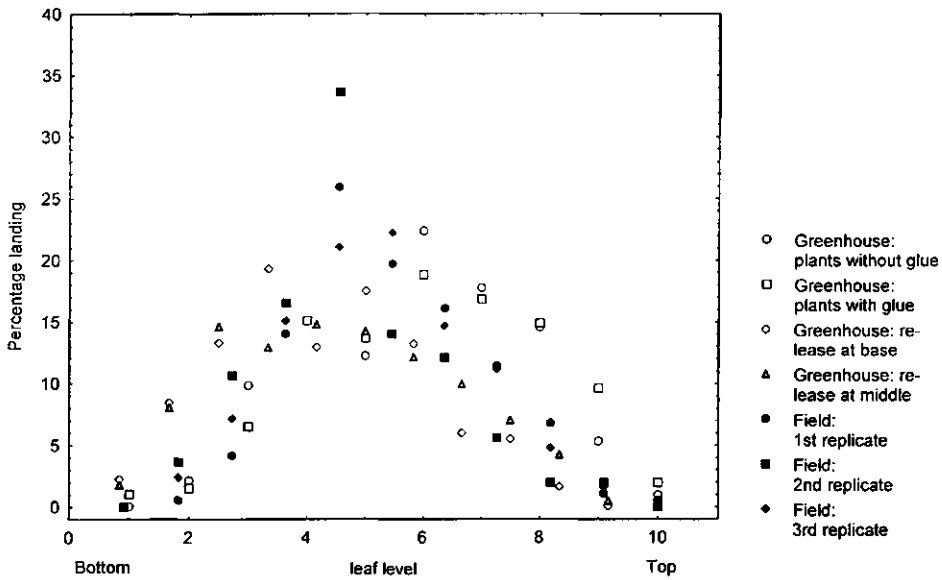
Figure 3. Relative distribution of landings of *T. brassicae* over leaf levels of maize plants. All distributions were reduced to the same x-length in order to make the distribution shapes easier to compare.

Table 3. Landing of *T. brassicae* on maize plants in and between crop rows.

	Between rows	In rows	p[1]
Number of plants	12	12	
Total number landed	334	259	
Number landing per plant	21.6 ± 4,1[2]	27,8 ± 4.6	n.s.

1 two-factor ANOVA. n.s.: P > 0.05
2 Mean ± s.e.

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Landing of *T. brassicae* on leaves with localized host-cues

The landing of 101 wasps was observed. The number of wasps that landed on the leaf pieces with and without *O. nubilalis* kairomones was almost the same (Table 4). The landing positions of the wasps on the leaf pieces were random and the number that landed within the patch with scales was no higher than that which could be expected from a random distribution.

Table 4. Landing of *T. brassicae* on pieces of maize leaves with and without kairomones and egg masses.

	clean leaves	leaves with kairomone and egg masses	p
Number landing	6,1 ± 0,3[1]	6,5 ± 0,6	n.s.[2]
Landing in host patch		0,36 ± 0,26	n.s.[3]

1 Mean ± s.e.

2 Wilcoxon Matched Pairs Test (CSS:Statistica, 1991) n.s.: P > 0.05

3 Chi-square test comparing the number of landings in the patch with the values expected from the area

Landing of *T. brassicae* on leaves with non-localized host-cues

1684 wasps were observed to land on the plants with host-cues, and 2015 on the clean plants. Again, there was no effect of host-cues (Table 5). In fact, the number of wasps that landed on the plants with host-cues was lower than that on the clean plants, although not significantly so. Again, landing started immediately after release and decreased with time.

Table 5. Landing of *T. brassicae* on maize plants with and without host kairomones.

	clean plants	plants with kairomone	p
Number landing	503.75 ± 149.17[1]	421.00 ± 124.12	n.s.[2]

1 Mean ± s.e.

2 Wilcoxon Matched Pairs Test (CSS: Statistica, 1991) n.s.: P > 0.05

CONCLUSIONS

Landing of *T. brassicae* on plants with and without glue was similar. The distribution of landings as found indicates that all parts of the plant are visited by *T. brassicae*, although landing occurred proportionally more in the upper leaf levels. This might be a result of the positive phototaxis of *T. brassicae*.

The effect of release of height on landing positions was relatively small. When the release point is lowered one meter, the mean landing level lowers by just eight centimeter. Apparently, the wasps do not fly in a straight line to the plants. In biocontrol, *T. brassicae* are either released from cards hanging on the plants or from capsules lying on the ground. According to the results of these experiments, the different height of release in both methods will have little effect on the number or position of landing *T. brassicae*.

In the greenhouse experiments, it is assumed that all wasps reach the plants directly. In field situations, we estimated, based on earlier observations (Chapter 4; Pak *et al.*, 1985), that less than one percent reaches plants at two meters distance directly, while the other wasps make one or more landings in between. Still, the landing distributions are similar to those in the greenhouse. Apparently the plant structure itself, combined with phototaxis, influence landing strongest. Landing and flying off repeatedly will not affect the distribution over the leaf levels. The fact that relatively more wasps land on the higher leaves can explain the results of Neuffer (1987) who found that parasitism of *O. nubilalis* was higher above and at the release level and above than below the release level. Whether a plant is situated within or between a row does not play an important role in the number of landings.

No effect of host-cues on landing was found. In the experiment with the leaf pieces, the host-cues were concentrated in a small area. The patches could contain scales, excrements, sex pheromones and eggs of *O. nubilalis*. Noldus and van Lenteren (1985) could not show any effect of *Mamestra brassicae* kairomones on landing, but Smits (1982) found a clear effect of *Mamestra brassicae* host-cues (produced by moths on the leaf) on landing of *T. brassicae*. In his experiment, the number of wasps landing on leaves with kairomones was twice that of clean leaves. In olfactometers, *T. brassicae* often shows some attraction to eggs (Ferreira *et al.*, 1979; Kaiser *et al.*, 1989; Renou *et al.*, 1989; Renou *et al.*, 1992) or sex pheromones (Kaiser *et al.*, 1989; Zaki, 1985). In olfactometer experiments, the odour is the only stimulus available and movement is mostly by walking, so flight and landing behaviour is not well simulated. In our experiment with whole plants, only volatile host-cues could reach the plants. Fresh host egg masses were distributed on all plants, so that their odour was present as well.

Although the number of landings was not influenced by the presence of host-cues, after landing they had a clear effect on behaviour. Both experiments were repeated with a similar setup to test the effect of host-cues on searching efficiency. In both cases searching efficiency was significantly increased by the presence of kairomones (Chapter 6). Most authors (e.g. Lewis *et al.*, 1975; Noldus *et al.*, 1991) are convinced that the main effect of kairomones in *Trichogramma* searching is arrestment. The results found in these experiments confirm this view. As *T. brassicae* attacks different hosts and as the position of these hosts is not fixed in a certain zone, the pattern of landing of *T. brassicae* ensures an efficient sampling of the plants in search of other host-cues.

ACKNOWLEDGEMENTS

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Chapter 6

Movement and host-finding of *Trichogramma brassicae* on maize plants

ABSTRACT
Direct observation of searching patterns and residence times of *Trichogramma brassicae* on maize plants were made at 18°C and 25°C. Temperature had a strong effect on the residence times. Wasps spent an average of 44.9 and 20.8 minutes on the plant at 18°C and 25°C, respectively. Observations on single plants showed that wasps mainly walked from one leaf to another. The leaf part closest to the stem was the most visited and searched leaf area. At 18°C, many wasps went to the lower leaf side and stopped searching. Leaf level, leaf side or size of the leaf where the wasp landed had no effect on residence time. Although 18 to 24% of total searching time was spent in following the leaf edge and mid-rib, *Ostrinia nubilalis* host egg masses touching the mid-rib were not found more often than those away from the mid-rib. Neither was host-finding influenced by the position of egg masses relative to the stem or by the leaf level. Naturally laid egg masses were found twice as often as artificially placed egg masses. The scales that *O. nubilalis* left in small patches on the plant seemed to be as effective a cue as host-cues artificially confined to a small area around the host egg mass. Maize plants were covered with fine netting and a number of *Ostrinia nubilalis* kept close to the plant for one night, in such a way that the moths were unable to touch the plants. On the next day, host-finding and residence times of *T. brassicae* were significantly higher on these plants than on untreated plants. This means that volatile cues left by *Ostrinia nubilalis* had an arresting effect and were used in host-finding by *T. brassicae*.

INTRODUCTION

Trichogramma brassicae Bezdenko is used in inundative releases for biological control of the European corn borer, *Ostrinia nubilalis*, in maize fields (Li, 1994). The wasps of the genus *Trichogramma* are generalist egg parasitoids of moths and butterflies. Thus it can be expected that *Trichogramma* parasitoids will be adapted to the behaviour of Lepidopterans in general, but not necessarily to the behaviour of specific host species.

There are a number of specific mechanisms that could increase searching efficiency for *T. brassicae*. In the first place, the wasp has to allocate an optimal amount of time to search on a patch that could contain hosts (Morrison and Lewis, 1981). After this time, behaviour that will make the parasitoid leave the patch (flight, walking straight instead of turning at the edge of the patch) is no longer inhibited. It depends on search behaviour over the plant whether a leaf or a whole plant is defined as patch. If flight is the main mechanism for movement between leaves, and the wasp is just as likely to land on a neighbouring plant after leaving, then each leaf is a separate patch. If the wasp walks from leaf to leaf while searching, then the whole plant must be considered one patch. The flight propensity of *T. brassicae* depends on the temperature. Below 18°C, there will be no flight (Bigler *et al.*, 1982). It is thus likely that residence times will increase as temperatures decrease. The structure of the plant or leaf can also influence residence times. Residence time of *T. nubilale*

is higher on a single flat surface than on narrow ribbons with the same surface area (Andow and Prokrym, 1990).

In the second place, a search pattern can make the parasitoid search in those parts of the host-habitat where hosts are most likely to be present. On the leaf, *T. brassicae* walks in relatively straight lines in the absence of host cues (Gardner and van Lenteren, 1986). *T. pretiosum* follows the edge of surfaces (Noldus et al., 1991a), but *T. nubilale* does not (Andow and Prokrym, 1990). Information on movement patterns of *Trichogramma* on whole plants is lacking. In the field, there are no leaf positions where *O. nubilalis* egg masses are not parasitized (Milani *et al.*, 1988). There was no effect of leaf level on parasitism of *O. nubilalis* in maize by *T. brassicae* in a French field experiment (Hawlitzky *et al.*, 1994). In Italian experiments in maize clear differences were found between two *T. maidis* (=*brassicae*) strains, one mainly parasitizing above 140 cm, the other below 100 cm (Milani *et al.*, 1988). German experiments showed higher parasitism in the middle and upper leaf levels of maize plants for *T. evanescens* (Neuffer, 1987). *T. nubilale* shows a preference for *O. nubilalis* egg masses in the lower and middle third of maize plants (Burbutis *et al.*, 1977). But field parasitism does not necessarily reflect movement on the plant, since it is also a result of landing, host recognition, host acceptance and host suitability.

In the third place, the parasitoid can react to host-cues while searching for hosts. *T. brassicae* reacts to several cues of *O. nubilalis*, such as scales (Kaiser *et al.*, 1989; Bieri *et al.*, 1990)., egg odour (Renou *et al.*, 1989; 1992) and sex pheromone (Frenoy *et al.*, 1992). The reaction to scales is increased klinokinesis (searching path becomes more tortuous), reversed orthokinesis (walking speed decreases) and flight inhibition (Gardner and van Lenteren, 1986; Bieri *et al.*, 1990). The result of these responses is arrestment. The klinokinetic and orthokinetic responses will arrest the parasitoid in a very small area, while flight inhibition will arrest the wasp on the plant. Scales of *O. nubilalis* are found in small patches on the plant, but not necessarily within two or three centimetres of an egg mass (Chapter 2). Bieri *et al.* (1990) predict in a model study that a area with scales around an egg mass has an optimum size: if the scale area becomes too large, the scale responses will arrest the wasp in a place where the eggs are not present. The same could be predicted for scale patches away from the egg mass. Scales have no attractive effect on *T. brassicae* (Noldus and van Lenteren, 1985b; Chapter 5). Sex pheromones are volatile cues that are not concentrated on one place of the plant, for example near egg masses. *Trichogramma* reacts to moth sex pheromones with upwind walking in olfactometers (Frenoy *et al.*, 1992) and reversed orthokinesis (Noldus *et al.*, 1991a). Volatiles of calling females of *Heliothis zea* inhibited flight of *T. pretiosum*. If the wasps were forced to fly, they made much shorter flights in the presence of the odours (Noldus *et al.*, 1991a). Levels of parasitism in petri-dish and olfactometer set-ups are increased in the presence of moth sex pheromones (Zaki 1985; Frenoy *et al.*, 1991). Egg mass odour (Kaiser *et al.*, 1989; Renou *et al.*, 1992) and visual recognition (Laing, 1937; Pak *et al.*, 1990) of egg masses plays a role at very short range (2-3mm) only.

Information on movement and searching behaviour of *T. brassicae* on the plant is lacking. We, therefore, made direct observations of *T. brassicae* when searching on maize with the objectives 1. to determine residence times and how they are influenced by temperature, 2. to determine whether the position of host egg masses on the plant has an effect on host-finding by *T. brassicae* and 3. to quantify the responses of *T. brassicae* to host-cues in terms of searching success and residence time at the plant level.

MATERIALS AND METHODS

Materials

Plants
Maize plants (*Zea mays* L.) grown under greenhouse (cultivar LG11) and field conditions (cultivar Atlet) were used in the experiments. Field-grown plants were potted one day before use.

Wasps
Trichogramma brassicae Bezdenko was used for all experiments. The stock colony was obtained in 1975 from INRA, Antibes, France (strain 16). The strain originates from the former Soviet Republic of Moldavia. The stock colony is maintained on *Ostrinia nubilalis* eggs. The rearing system is described in Bigler (1994). The wasps used for the experiments were reared for one or two generations on *Ephestia kuehniella* eggs. Females used were one to three days old, had no oviposition experience and had not been exposed to leaves or leaf odours prior to the experiments. In experiments with individual wasps, each female was placed in a glass tube containing a droplet of water and honey one to eight hours before it was used in experiments.

Moths
The European corn borer, *Ostrinia nubilalis* Hübner, was used in the experiments. The rearing was established every year with overwintering larvae collected in the field in Switzerland.

Event recording
For detailed behavioural observations portable computers (Tandy 102® or Psion Organiser®) were used with an event recording program (The Observer®).

Methods

Movement on plants without host-cues
A single greenhouse grown maize plant with ten leaves (height: 85 cm, leaf area 800 cm^2) was used for all observations. At night, the plant was placed at 4°C to inhibit further growth. During the experiments the maize plant was placed in a climate room under fluorescent lamps (40 W) covered with filtration paper.

To start an observation, a female wasp was placed on the upper side of the fifth leaf, about five centimetres from the stem, by gently knocking the tube and its behaviour was recorded.

During the experiment three types of behaviour were distinguished. *Walking* was defined as all movement on the plant surface. *Standing still* was defined as the time spent on the plant surface when the wasp was not moving. This included resting, grooming and feeding. *Flight* was defined as any movement in the air.

The locations recorded were leaf one to ten, ear and stem. Leaf one was the lowest leaf. Leaf length was divided in three equal parts, close to the stem, middle and point. Location in these leaf sectors and leaf side were recorded. Encounters of the wasp with the mid-rib or the leaf edge were also recorded. Following the vein was defined as an direction chance after encountering the vein in combination with walking along the vein. Following the edge was defined as walking along the edge while staying within one millimetre of the leaf edge.

An observation was terminated when the wasp left the plant by flight without landing on another leaf, when it did not move for more than 20 minutes, or when it spent more than 150 minutes on the plant. The experiment was done at 18°C and 25°C. For each temperature, 55 observations were made, each observation with a new parasitoid.

Residence times on plants without host-cues
Wasps were observed on greenhouse grown plants with six to seven leaves and an average leaf area of 825 cm^2 in a climate room at 20°C and 25°C. Half of the wasps were introduced on the upper leaf side, the other half on the lower side. Wasps were also introduced on different leaf levels. An observation was terminated when the wasp left the plant or when it did not move for 20 minutes. For each temperature, 50 observations were made, each observation with a new parasitoid.

Effect of egg mass position on host-finding
Seven series of observations were made.The first three involved plants exposed to *O. nubilalis* females in the field cage, with naturally laid egg masses. In the last four series, the egg masses were placed artificially on the plant. A total of 99 egg masses were used in the experiment: 47 touching the mid-rib and 52 away (< 1mm) from the mid-rib. In each series of observations, three plants were used.

These plants were placed on a row ten centimetre apart and 2000 female *T. brassicae* were released five centimetre away from the middle plant. During two hours all *O. nubilalis* egg masses were continuously checked for *T. brassicae* females. Wasps found on an egg mass were recorded and removed from the plant using a aspirator. After two hours of observation,

plants were left unobserved for one hour and a last count of *T. brassicae* on the egg masses was made.

Effect of oviposition host-cues on host-finding
Six field-grown maize plants were used. Three plants were exposed to *O. nubilalis* females in a 2x2x2 m field cage in order to allow them to oviposit. The other three plants were not exposed to *O. nubilalis*. Egg masses were artificially placed on these plants in the same position as in the plant with naturally laid egg masses. Each group of plants had approximately 16 egg masses. In the greenhouse, the plants were placed in two groups. The groups were two meters apart. A container with 2000 *T. brassicae* females was placed in the middle (one meter from each group) and opened. For two hours, all *T. brassicae* females that reached the egg masses were counted and removed from the egg masses. This experiment was done twice.

Effect of localized host-cues on host-finding
A cage of three centimetre diameter with one *O. nubilalis* female was fastened over night to the centre lower side of the fourth or fifth leaf of a small maize plant (six leaves). The next day, one *O. nubilalis* egg mass was placed in the middle of the potential "kairomone" area. Another egg mass was placed in the same position on the next higher or lower leaf. The plant was placed in a greenhouse compartment and a container with 2000 *T. brassicae* females was opened at 140 cm from the plant. During 80 minutes, the females that found the egg masses were recorded. Eighteen replicates were made.

Effect of localized host-cues on residence time
A cage of three centimetre diameter with one *O. nubilalis* female was fastened overnight to the centre lower side of a full sized maize leaf. The next day, this leaf was fastened to a stand and an egg mass was stuck to the centre of the potential "kairomone" area. Three *T. brassicae* females were allowed to fly to the leaf. Their behaviour was recorded using a portable computer and event recording software. Moving, standing still, following edge and mid-rib were defined as in the first experiment. Locations recorded were leaf upper side, leaf lower side, and inside or outside kairomone area. Observations were ended if the wasp left the leaf by flight or walking, found the egg mass, or after two hours. As a control, the same observations were made on a clean (kairomone free) leaf with an egg mass placed in the same position as on the treated leaves. On the leaves with kairomones, 54 observations were made, and 36 on the control leaves.

Effect of volatile host-cues on host-finding
Six field-grown plants were used. Three of the plants were exposed to ten *O. nubilalis* females (one to four days old) and five males in the greenhouse. Contact between *O. nubilalis* and maize plants was prevented in this experiment and contamination by *O. nubilalis* scales was avoided. Only volatile cues were allowed to reach the plants. This was done by caging the maize plants with netting (Monofiles Nylon netting 100 µm hole size, Scrynel). Previous work (Noldus, 1989) had shown that a mesh size of 340 µm was able to

intercept 90% of scales so we assumed 100 μm netting was adequate for this work. The netting was kept as close to the plant as possible. *O. nubilalis* were introduced in the cage and left for 48 and 24 hours in the first and the second replicate, respectively. The other plants (non-exposed to *O. nubilalis*) were placed in another greenhouse in order to avoid any volatile contamination. Fifteen and 12 egg masses were placed on the plants of each group in the first and second replicate, respectively. Experimental procedure in the greenhouse was the same as for the previous experiment. Two replicates were made.

Effect of volatile host-cues on residence time

Field-grown maize plants (height: 1.60 m, 9-11 leaves) were used in this experiment. Plants were treated in three ways. First, plants with natural host-cues were treated as described for the previous experiment: ten female and five male *O. nubilalis* were released in the cage, where they could move close to the plant but not contact it. Second, to obtain plants with artificial host-cues, a rubber stopper with synthetic *O. nubilalis* sex pheromone (97% (Z)-11-Tetradecenylacetate, 3% (*E*)-11-Tetradecenylacetate) was placed on top of the middle leaf of the plant overnight. In both set-ups, moth sex pheromones could adhere to leaves during a 24-hour period (Noldus, 1989). Third, control plants were used which were kept in a separate compartment to avoid contamination by *O. nubilalis* volatiles.

Two plants from two different treatments were placed two meters apart in a 25°C greenhouse compartment. A release container with 1500 females was placed between the plants and opened for three minutes. During two hours, the number of wasps on each of the plants was counted every five minutes. Six replicates were made for each combination of treatments (natural vs. artificial host-cues, natural host-cues vs. control, artificial host-cues vs. control).

RESULTS

Movement on plants without host-cues

The behaviour on plants without host-cues is summarized in Table 1.

Table 1. Behaviour of *T. brassicae* on maize plants without host cues

	18°C	25°C	p[1]
Number of observations	55	55	
Residence time (minutes)	44.9 ± 6.4[2]	20.8 ± 3.3	****[3]
Observations ended by			
-Standing still	37	7	****[4]
(no movement > 20 min)	(67.3%)	(12.7%)	
-Flying away	11	48	
	(20.0%)	(87.3%)	
-Broken off	7	0	
(observation >150 min)	(12.7%)		
Mean number of	1.8 ± 0.2[2]	1.2 ± 0.1	***[5]
leaf visits			
Number of wasps that	23	7	**[6]
visited more than one leaf	(42%)	(13%)	
Walks between leaves	50	7	
Flights between leaves	2	3	
Upward movement	45	3	
Downward movement	7	7	

[1] n.s.: $P>0.05$; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ****: $P<0.0001$

[2] Mean$\pm$s.e.

[3] Mann-Withney U-test

[4] Chi-square test

[5] Mann-Withney U-test

[6] Chi-square test

Movement is strongly influenced by temperature. Sixty-seven percent of the observations at 18°C were terminated because wasps stopped moving for more than 20 minutes. Seven of the wasps were still searching after 150 minutes at 18°C. Flight is infrequent at low temperatures, only 20% of the wasps left leaves by flight. At 25°C residence times were significantly

lower, approximately half as long as at 18°C. At 25°C 87% of the wasps left leaves by flight. At both temperatures, the distribution of the residence times was approximately a negative exponential, which means that most of the wasps stayed on the plant for a short period of time only.

Table 1 shows that the majority of the wasps visit only one leaf after landing, especially at 25°C. At 18°C, movement between leaves was mostly by walking. The number of moves between leaves at 25°C was too small to compare with that at 18°C, but it seems that walking is important for between-leaf movement even at high temperatures where *T. brassicae* tends to fly. At 18°C movement was mostly upwards. Walking paths on the stem were usually very straight. Sometimes a 180° turn was made, and the wasp returned to the leaf it had just left. This occurred only if the wasp had not yet reached the next leaf. Maize leaves encircle the stem. On the side of the stem opposite to the leaf, the wasp has to cross a barrier of hairs if it continues following the stem instead of walking on the leaf. On the leaf side, there is an almost seamless connection between the stem and the mid-rib on the leaf underside. Wasps that walked upward at this side of the stem automatically walk on the leaf.

At 25°C, 61.7% of the residence time was spent on the upper side of the leaf (Figure 1). If the total time is split into walking and standing still, it is clear that the bias towards the upper leaf side is a result of the walking behaviour, because 71.8% of the walking took place on the upper side of the leaf. Time spent standing still was the same on the upper and lower leaf side. Wasps were released on the upper side, which might be the reason that more time was spent on the upper side. In fact, of the 55 wasps observed at 25°C only 22 wasps visited the lower side. For these 22 wasps, the behaviour was more equally distributed over both sides of the leaf. Still, more time was spend walking on the upper side. At 18°C the results were very different (Figure 1). Of the total residence time, only 43.5% was spent on the upper side of the leaf. The average walking time was also higher on the lower side. Of 55 wasps, 48 visited both leaf sides. Again, wasps with a short residence time spent more time walking on the upper side. Standing still was mostly observed (87.9%) on the lower side. Most of the observations at 18°C were terminated because the wasp did not move for 20 minutes. In most cases, this took place on the lower side. Even when this final period of standing still was omitted, more time was spend standing still on the lower than on the upper side.

To determine if an equal amount of time was spent on each section of the leaf, the leaf was divided in three sections, close to the stem, middle of the leaf and point of the leaf. At both temperatures, most time was spent close to the stem and least at the point (Figure 1). Since wasps were released in the sector close to the stem, many wasps only visited the subsector close to the stem. At 25°C, the wasps that visited all three subsectors spent more time in the middle part of the leaf than in the part close to the stem. Very little time is spent in the point subsector at either temperature.
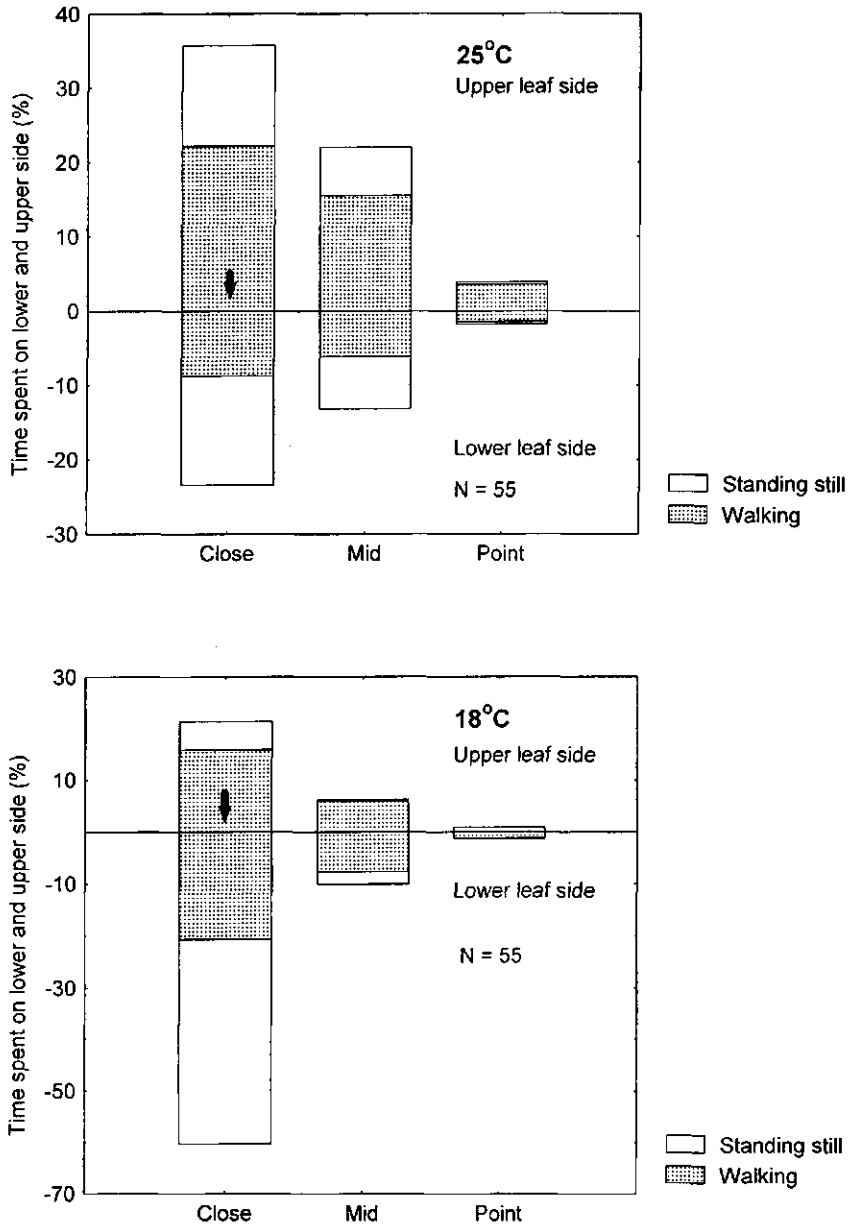
Figure 1. Time spent by *T. brassicae* on maize leaf sides and subsectors at two temperatures. Arrows indicate the release position of the wasps. Leaf length was divided in three sectors: close to the stem, middle, and close to the leaf point.

If a wasp encounters the leaf edge, four behaviours are possible. It can either follow the edge, turn away from the edge, move to the other leaf side or follow the edge and move to the other leaf side. Table 2 shows that the most usual behaviour was following the edge without changing sides.

Table 2. Behaviour of *T. brassicae* on maize leaf edges.

	18°C	25°C	p[1]
Number of observations	55	55	
Total number of edge encounters	584	416	
Edge followed after encounter	273	230	
without changing side	(46.8%)	(55.2%)	
Edge followed and side changed	93	56	
	(15.9%)	(13.5%)	
Walked back on the leaf	127	78	
	(21.7%)	(18.8%)	
Side changed	91	52	
without following	(15.6%)	(12.5%)	
Total number of edge encounters	377	289	
Mean duration of following (seconds)	35.8 ± 5.1[2]	25.6 ± 3.2	***[3]
Median duration (seconds)	15.5	10.2	
Percentage of total walking time spent following the edge	12.5%	14.7%	
Edge as percentage of leaf area	5.0%	5.0%	

[1] ***: $P<0.001$

[2] Mean$\pm$s.e.

[3] Mann-Withney U-test

Changing sides was less common after the edge had been followed. The duration of edge following was usually about 10 to 15 seconds, although in some instances the edge was followed for 10 minutes or more. Standing still on the edge does occur but is rare. The distribution of the edge following durations fits a log-normal distribution. The edge area of the leaf (<1 millimetre from leaf edge) constitutes of 5% of the total leaf area. If the percentage of time spent following the edge is compared with this, it is clear that the wasps spent more time following the edge than what would be expected from random movement.

When the mid-rib is encountered, it is followed in about half of the cases (Table 3). Close to the leaf point the mid-rib is hardly recognisable and rarely followed.

Table 3. Behaviour of *T. brassicae* on maize leaf mid-ribs.

	18°C	25°C	p[1]
Number of observations	55	55	
Total number of mid-rib encounters	541	436	
Mid-rib followed after encounter	289	246	
	(55.8%)	(56.4%)	
Mid-rib encountered without following	252	190	
	(44.2%)	(43.6%)	
Mean duration of following mid-rib (seconds)	15.6 ± 1.0[2]	17.4 ± 1.9	n.s.[3]
Median duration (seconds)	8.7	7.9	
Mean duration of following mid-rib upper side	13.0 ± 1.4[2]		n.s.[4]
Mean duration of following mid-rib lower side	15.8 ± 1.3		
Mean duration of following mid-rib upper side		15.8 ± 2.3[2]	*[4]
Mean duration of following mid-rib lower side		16.9 ± 2.1	
Percentage of total walking time spent following mid-rib	5.6%	9.5%	
Mid-rib as percentage of leaf area	8.5%	8.5%	

[1] n.s.: $P > 0.05$; *: $P < 0.05$
[2] Mean$\pm$s.e.
[3] Mann-Withney U-test
[4] Mann-Withney U-test

On the upper side of the leaf the mid-rib is a shallow hairless depression, while in the lower side it forms a sharp crest which leads to very straight movement by the wasps. The duration of mid-rib following was usually short. At 25°C, the mid-rib was followed longer on the lower than on the upper leaf side, but at 18°C there was no significant difference. The time spent following the mid-rib is not higher than what would be expected from the mid-rib area.

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Residence times on plants without host-cues

In the previous experiment wasps were released at the same leaf level and on the upper side, close to the stem. To measure residence times in a more natural situation, females were released on all leaf levels and on the upper and lower leaf sides. There was a clear difference in residence times on plants without host-cues at 20°C and 25°C. At 20°C wasps stayed about twice as long as at 25°C (Table 4).

Table 4. Residence times of *T. brassicae* on maize plants without host cues at two temperatures

	20°C	25°C	p[1]
Number of observations	50	50	
Mean residence time (minutes)	37.9±5.5[2]	16.8±2.4	***[3]
Mean number of leaves visited	1.5±0.1[2]	1.2±0.1	n.s.[4]
Number of wasps			
that visited more than one leaf	12	6	n.s.[5]
	(24%)	(12%)	
Observations ended by			
Standing still	10	2	*[6]
(no movement > 20 minutes)	(20%)	(4%)	
Flying away	40	48	
	(80%)	(96%)	

[1] n.s.: P>0.05; *: P<0.05; ***: P<0.001
[2] Mean±s.e.
[3] Mann-Withney U-test
[4] Mann-Withney U-test
[5] Fisher's Exact Probability test
[6] Fisher's Exact Probability test

The average number of leaves visited and the number of wasps that visited more than one leaf was also higher at 20°C. At both temperatures, most observations were ended by the wasps flying away but significantly more did so at 25°C. No correlation was found between starting leaf level, leaf side, leaf length, leaf width or leaf area on residence times. Residence times had a descending distribution as shown in Figure 2. Both residence times and number of leaves visited at 25°C were quite similar in this and in the previous experiment (Figure 3). There is a considerable difference between residence times at 20 and 18°C. At 20°C, 80% of the wasps flew away, while at 18°C on 20% flies.
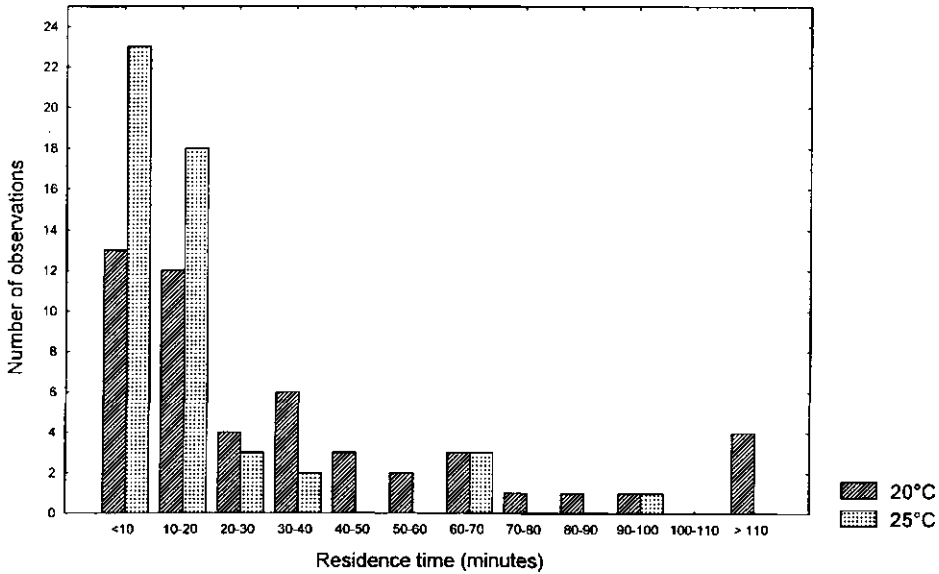
Figure 2. Residence times of *T. brassicae* on maize plants without host-cues at two temperatures.
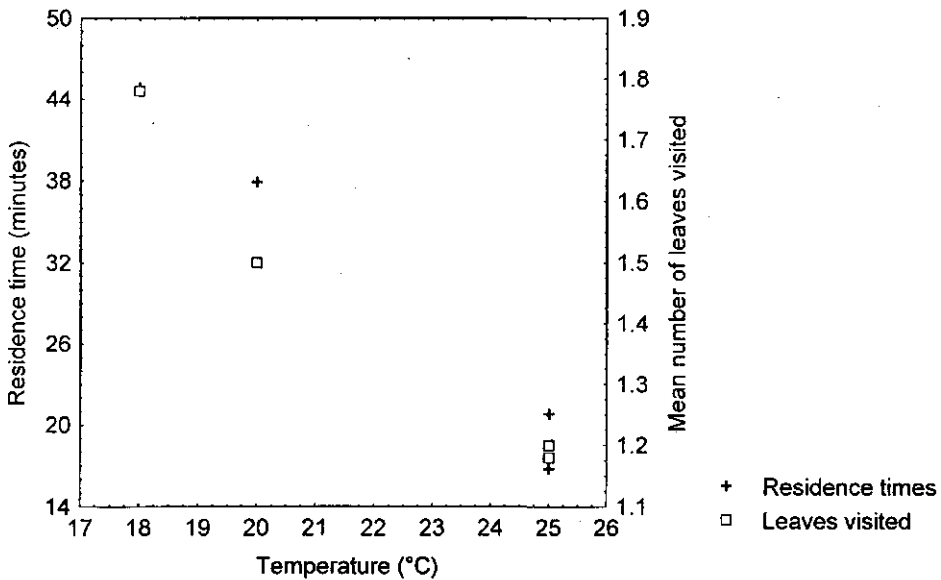

Figure 3. Residence times and mean number of leaves visited by *T. brassicae* on maize plants at different temperatures (data from first and second experiments combined).

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Effect of egg mass position on host-finding

No difference was found in the number of females that found egg masses touching the mid-rib and the number of wasps that found egg masses away from the mid-rib (Table 5).

Table 5. Effect of *O. nubilalis* egg mass position relative to the mid-rib on number of *T. brassicae* finding egg masses on maize plants.

	Egg masses away from mid-rib	Egg masses touching mid-rib	p[1]
Number of replicates	7	7	
Total number of egg masses	49	50	
Total number of females finding egg masses	440	344	
Average number of females per egg mass	8.9 ± 1.3[2]	7.4 ± 1.6	n.s.[3]

[1] n.s.: P>0.05

[2] Mean±s.e.

[3] Mann-Withney U-test

There were no significant differences either in the number of wasps finding egg masses in the leaf sub-sectors, close to the stem, middle leaf and close to the point (Table 6). There was no correlation either between leaf level of the egg mass and number of wasps that found the egg mass.

Table 6.
Effect of egg mass position relative to the stem on number of *T. brassicae* finding *O. nubilalis* egg masses on maize plants.

Position	Close	Mid	Point	p[1]
Number of replicates	7	7	7	
Total number of egg masses	30	44	25	
Average number of females per egg mass	8.7 ± 1.9[2]	6.1 ± 1.2	11.2 ± 2.5	n.s.[3]

[1] n.s.: P>0.05

[2] Mean±s.e.

[3] Kruskall-Wallis test

Effect of oviposition host-cues on host-finding

On clean plants, *T. brassicae* found more naturally laid host egg masses compared to the number artificially placed host egg masses found (Table 7). The naturally laid egg masses were found more then twice as often as the artificially placed egg masses.

Table 7. Total number of *T. brassicae* females found on *O. nubilalis* egg masses on maize plants exposed to *O. nubilalis* (eggs naturally laid) and non-exposed maize plants with artificially placed egg masses.

	Natural	Artificial	p[1]
Number of replicates	2	2	
Number of egg masses	33	32	
Total number of females finding egg masses	183	80	
Average number of females per egg mass	5.5 ± 0.8[2]	2.5 ± 0.3	**[3]

[1] **: P<0.01

[2] Mean±s.e.

[3] Mann-Withney U-test

Effect of localized host-cues on host-finding

A total of 376 wasps found the egg masses, but there was a large daily variation in the numbers. The results are shown in Table 8. Scales close to an egg mass double the number of wasps that find the egg mass. Egg masses with host-cues were not found faster or slower than those without.

Table 8. Effect of localized *O. nubilalis* host-cues on host-finding of *T. brassicae*

	host-cues	control	p[1]
Number of replicates	18	18	
Number of wasps that found the eggs	14.6 ± 2.7[2]	6.3 ± 0.6	**[3]
Average time to find the eggs (minutes)	24.3 ± 0.9[2]	22.9 ± 1.4	n.s.[4]

[1] n.s.: P>0.05; **: P<0.01

[2] Mean±s.e.

[3] Wilcoxon matched pairs test

[4] Mann-Withney U-test

Effect of localized host-cues on residence time

Confining an *O. nubilalis* female on a three centimetre spot on the leaf had no effect on either the residence time on this leaf or the activity of the wasp (Table 9). Neither was the way in which the wasp left the leaf influenced by the presence of host scales. Although egg masses on the treated leaves were found more often, the difference was not significant. If only the wasps which came within 1.5 cm of the egg mass and entered the kairomone area on the treated leaves are considered, there was an significant effect.

Table 9. Effect of localized host-cues on leaf residence times and walking activity (walking time/total time) of *T. brassicae* at 20°C.

	Treated	Control	p^1
Number of observations	54	36	
Mean residence time (minutes)	8.0 ± 1.2^2	8.9 ± 1.5	n.s.^{3}
Mean walking activity	0.78 ± 0.03^2	0.76 ± 0.04	n.s.^{4}
Observation ended by			
Standing still	2 (3.7%)	(11.1%)	
Flying away	37 (68.5%)	28 (77.8%)	
Walking away	6 (11.1%)	2 (5.6%)	
Finding egg	9 (16.7%)	2 (5.6%)	n.s.^{5}
Number that came within 1.5 cm of egg mass	23	26	
Percentage of wasps that came within 1.5 cm of egg mass that found egg mass	39.1%	7.6%	**^{6}

[1] n.s.: P>0.05; **: P<0.01
[2] Mean±s.e.
[3] Mann-Withney U-test
[4] Mann-Withney U-test
[5] Chi-square test
[6] Chi-square test

Effect of volatile host-cues on host-finding

A significantly larger number of *T. brassicae* females were found on the egg masses on plants exposed to *O. nubilalis* (Table 10).

Table 10. Total number of *T. brassicae* females that found artificially placed *O. nubilalis* egg masses on maize plants exposed to *O. nubilalis* (no contact between *O. nubilalis* and maize plants allowed) and on maize plants not exposed to *O. nubilalis*.

	Exposed to *Ostrinia*	Not exposed to *Ostrinia*	p[1]
Number of replicates	2	2	
Number of egg masses	27	27	
Total number of females finding egg masses	127	46	
Average number of females per egg mass	4.7 ± 0.7[2]	1.7 ± 0.4	**[3]

[1] **: P<0.01
[2] Mean±s.e.
[3] Wilcoxon Matched Pairs test

Effect of volatile host-cues on residence time

A total of 374 wasps landed on the plants. Residence times were longest on plants with natural *O. nubilalis* host-cues (Figure 4). Residence times on plants with artificial sex pheromone were not significantly higher than those on the control plants. After two hours, 18% of the wasps were still present on the plants with natural kairomones, against 7% on the plants with artificial sex pheromones and 4% on the control plants.
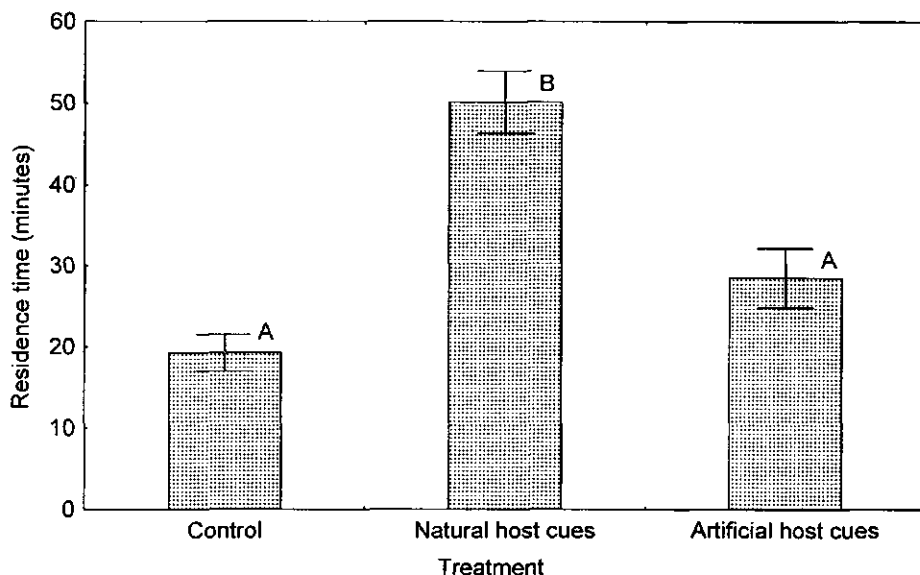
Figure 4. Effect of *O. nubilalis* volatile host-cues on average residence times of *T. brassicae* on maize. Bars represent mean±standard error. Different letters indicate significant differences of means (LSD test of log-transformed data, P<0.05)

DISCUSSION

Most visits of *T. brassicae* to plants without host-cues were short, so only a small part of the surface of each plant will be searched during a visit. Because most wasps stayed on the plant for a short period only, and did not move very far over the plant during that time, the location of the area searched was strongly influenced by the landing site. Based on landing and searching as observed, it would be expected that eggs on the middle and upper leaves, and close to the stem would be found most often. However, the experiment on the effect of egg mass position on host-finding did not show significant effects of leaf level or distance from the stem on host-finding.

The effect of the distribution of landing sites and walking patterns was apparently not strong enough to cause significant differences in the chance that an egg mass is found at different positions on the plant. The effect of leaf level on parasitism found by Milani *et al.* (1988) and Burbutis *et al.* (1977) might be a result of the circumstances in the field, where there can be differences in light intensity, temperature, humidity and wind speed between the upper and lower part of the plant, effects which are less strong or missing in the greenhouse where the experiments were conducted.

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Following the mid-rib and the edge was common behaviour of *T. brassicae*, and this was also noticed by other authors (Gass, 1988; Noldus, 1989; Blanché, 1990; Noldus *et al.*, 1991a). This behaviour is a thigmotactic response to linear structures encountered. Many insects are directed by such features if no host-cues are present (Bell, 1990). It has been proposed that following the edge of leaves is an efficient way for *Trichogramma* to scan the leaf for scales left by moths that hang from the leaf edge (Noldus et al., 1991b). *O. nubilalis* does not have any preference for the leaf edge when landing or ovipositing (Chapter 2), so it is unlikely that following the edge will be advantageous for *T. brassicae* when searching for *O. nubilalis* host-cues. Eggs of *O. nubilalis* are usually found touching the mid-rib (Beck, 1987; Chapter 2), so mid-rib following, especially on the lower leaf side, could increase searching efficiency. *T. maidis* (=*brassicae*) spent 20% of total time on the lower leaf side following the midrib, and 10% of total time following the mid-rib on the upper side (Gass, 1988). In this study, differences in mid-rib following between upper and lower side were not so pronounced. The experiment on the effect of the position of egg masses showed that following the mid-rib had no effect on host-finding: egg masses touching the mid-rib were not found more often than those further away.

Egg masses naturally deposited by a female on the plant were found twice as often as egg masses artificially placed on the plant. When *O. nubilalis* oviposits naturally on the plant, many of the egg masses have few or no scales in the vicinity. Further, there are many patches of scales on the plant that are far away from an egg mass (Chapter 2). The fact that many scale patches are not around an egg mass does not seem to have a detrimental effect on *T. brassicae* host-finding, indicates that it is well adapted to patchwise distributed scales. Beevers *et al.* (1981) found that flight initiation of *T. pretiosum* is inhibited by contact with scale extract, even when the kairomone area has been left. The result is that the wasp will make an intensive search of the kairomone area and then stay longer on the plant, but start covering more area again. Lewis *et al.* (1979) found that uniform treatment with kairomone extracts did increase parasitism by *Trichogramma* less than kairomones that were distributed as discrete patches. An uniform treatment may cause wasps adapted to patchwise distributed kairomones to be to strongly arrested and thus, result in spending much time in areas without hosts (Gardner and van Lenteren, 1986).

The last two experiments show the effect of volatile host cues. Although netting kept *O. nubilalis* away from the maize plants, more egg masses were found on the treated plants than on the controls. Since the moths could not touch the plants, the kairomones involved must be volatiles. In the experiments with volatile host cues, the kairomone is expected to be on the whole plant, not only where egg masses are present. In such a situation, a strong klinokinetic response would not lead to higher parasitism, since the cue is not connected with the egg mass positions. The mechanism involved seems to be flight inhibition, causing the wasp to spend more time on the plant where the volatile host-cues were encountered. This is confirmed by the last experiment, which showed that the wasps stayed longer on plants with *O. nubilalis* volatile cues. Based on other work with *Trichogramma* (Noldus and van Lenteren, 1985a; Frenoy *et al.*, 1991), we hypothesize the host volatiles to be the sex pheromone of *O. nubilalis*. *Trichogramma* sex pheromones can be absorbed by plants and released over at least 24 hours (Noldus *et al.*, 1991b). Synthetic sex pheromone did not increase residence times. This finding agrees with the results of Kaiser *et al.* (1989) and

Frenoy *et al.* (1992). The compound of the sex pheromone mixture that attracts male *O. nubilalis* does not function as a kairomone for *T. brassicae.*

If host-cues provide reliable information on the presence of host eggs, innate and fixed responses by the parasitoid are predicted (Vet and Dicke, 1992). Responses of *Trichogramma* species to host scales are indeed innate and fairly nonspecific (Thomson and Stinner, 1990). The response of *T. brassicae* to *Mamestra brassicae* scales is not reinforced by oviposition, but frequent contact with scales can lead to habituation (Gardner and van Lenteren, 1986). On the other hand, *T. brassicae* showed associative learning in its responses to sex pheromones of *O. nubilalis* (Kaiser *et al.*, 1989). Associative learning is a method by which unreliable host-cues can be used in searching (Vet and Dicke, 1992). In the field, *O. nubilalis* mate outside the crops where they oviposit (Buechi *et al.*, 1981; Derridj *et al.*, 1986; Cordillot, 1989). In that situation, *O. nubilalis* sex pheromone do not very well indicate host presence, and scales will be a more reliable host-cue to indicate egg mass presence for *T. brassicae.*

In conclusion, we found that residence times of *T. brassicae* on maize are short, and only a small part of the whole plant is searched per visit. There is some movement between leaves by walking. The position of *O. nubilalis* egg masses on the plant or on the leaf does not influence their chance of being found by *T. brassicae.* The presence of host-cues does increase host-finding. These results will be used to evaluate and quantify effects of the environment, the host, and parasitoid quality on encounter probability in an individual based simulation model we are presently developing.

ACKNOWLEDGEMENTS

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Chapter 7

Effect of temperature on *Trichogramma brassicae* walking speed and walking activity

ABSTRACT

Walking speeds and walking activities (walking time divided by total time) of *Trichogramma brassicae* were determined at 12, 16, 20 and 25°C. Walking speed was measured during a five-minute period, and walking activity over a four day period. Both walking speed and walking activity were strongly influenced by temperature. Walking speed increased linearly with the temperature and was twice as high at 20°C as at 12°C. At 25°C, walking activity was high during the whole day, at 20 and 16°C it decreased during the afternoon, while at 12°C the wasps became most active only at noon or later. At low temperatures, there was a strong individual variation in walking activity. At 25°C, *T. brassicae* was active most of the time, so area searched per time unit can only increase at temperatures above 25°C if walking speed increases. At temperatures below 20°C, searching was more restricted by low walking activity than by low walking speed. Even disregarding other effects of temperature, the reduction in walking speed and walking activity at sub-optimal temperatures means that *T. brassicae* can only search half of the area at 20°C, and only one-seventh at 15°C that it can search at 25°C.

INTRODUCTION

The egg parasitoid *Trichogramma brassicae* spends a large part of its adult life searching for host egg masses. Since *T. brassicae* is unable to detect the presence of host egg masses on plants before landing (Chapter 5), egg masses have to be found by walking over the leaves of plants.

To estimate the encounter probability of *T. brassicae* with host egg masses, detailed data on the factors that influence host encounter rate are necessary. The area searched per time unit depends on the width of the searching path, the walking speed and the walking activity (walking time divided by total time) of *T. brassicae*, similar as in the parasitoid *Encarsia formosa* (van Roermund *et al.*, 1996). In this paper, we will concentrate on factors that are influenced by temperature.

Insects are poikilotherm and thus their activity is strongly influenced by temperature. The lowest temperature at which *Trichogramma* species are still active is between 5°C (Eidmann, 1934) and 9°C (Kot, 1979). *T. maidis* (=*brassicae*) does not show any oviposition at 35°C (Russo and Voegelé, 1982) Gass (1988) measured a linear increase in walking speed of *T. brassicae* between 8 and 20°C, and at 20°C, walking speed was 0.34 cm/s. Biever (1972) showed that walking speed continued to rise linearly from 20 to 35°C for *T. evanescens*, where a maximum of 0.55 cm/s was reached at 35°C. At 40°C, all wasps died within a few minutes. Boldt (1974) also measured walking speeds of *T. evanescens* at 25, 30 and 35°C, and found a maximum walking speed of 0.50 cm/s at 35°C, similar to Biever (1972).

Walking activity (defined here as walking time divided by total time) of *T. brassicae* has been observed at 22°C by Pompanon *et al.* (1994) at LD 12:12. In this experiment, walking activity during a five second period was automatically recorded every ten minutes for 10 to 15 days. The wasps had access to food but not to host eggs. A walking activity of 0.8 was found for females during the light period. Measurements on *T. evanescens* at higher temperatures showed that walking activity rose from 0.5-0.7 at 20°C to 0.9 at 25°C (Biever, 1972; Boldt, 1974). At temperatures above 25°C, it remained the same.

Walking speeds of T. brassicae show considerable variation between strains (Cerutti and Bigler, 1991), so it is not clear whether the data by Gass (1988) are representative for the strain used at present. Walking activity has not been measured at temperatures below 20°C. To collect data for estimation of encounter probability, walking activity and walking speed of *T. brassicae* will therefore be measured at 12, 16, 20 and 25°C. The resulting data will later be used in a simulation model to estimate the encounter probability of *T. brassicae* with egg masses in the field.

MATERIAL AND METHODS

Material

Wasps
One day old mated *Trichogramma brassicae* Bezdenko females were used for the experiments. The stock colony was established with material from Antibes (strain 16), which was imported from the former Soviet Republic of Moldavia and has been maintained on *Ostrinia nubilalis* Hübner eggs since then. The wasps used in our experiments were reared for three generations on eggs of *Ephestia kühniella* Zeller.

Methods

Walking activity of T. brassicae
Walking activity was measured at 12, 16, 20 and 25°C. For each temperature, 30 one day old *T. brassicae* females were placed into glass tubes (73mm long, 14mm diameter) with a drop of water and honey. Three wasps died before the end of the experiment at 25°C and one wasp at 20°C. The tubes were laid out horizontally on a rack and this was placed in a climate room. Light went on at 6.00 and was turned off at 22.00. After the wasps were left 18 hours in the climate rooms to acclimatize, walking activity was observed for four days. From 8.00 to 17.30, a check was made every half hour to see whether each individual wasp was moving, standing still, or dead. Each check was taken as an equal period of time where the wasp either walked or stood still, to calculate walking activity (walking time divided by total time).

Walking speed

One day old *T. brassicae* females were isolated in glass vials with a droplet of water and honey, and put at temperatures of 12, 16, 20 and 25°C for at least twelve hours before testing. All wasps were from the same rearing batch. Each female was put in the arena (five centimetre diameter with water around) and walking speed was measured for five minutes, using automatic tracking equipment as described in Bigler *et al.* (1988). True walking speed was measured, which means that speed was only measured when the wasp was actually moving. For each temperature, 30 measurements were made. Each wasp was used only once.

RESULTS

Walking activity of *T. brassicae*

The mean walking activity is shown in Table 1. Walking activity was strongly influenced by temperature, and the differences between the four temperatures were highly significant.

Table 1. Walking activity of *T. brassicae* in glass tubes at four temperatures observed over four days from 8.00 to 17.30 (Light period 6.00-22.00).

Temperature (°C)	N	Walking activity	p^1
12	30	0.10 ± 0.02^2	a ***
16	30	0.25 ± 0.04	b ***
20	29	0.68 ± 0.03	c ***
25	27	0.90 ± 0.01	d ***

[1] ***: $P<0.001$; Different letters indicate significant differences in a LSD-test of arcsin transformed data.
[2] Mean $\pm$ S.E.

The daily walking activity patterns are shown in Figure 1. Wasps showed some activity during the whole observation period. At 25°C, walking activity was high throughout the whole day. At 20°C, walking activity decreased in the afternoon. On the first two days at 20°C, mean walking activity was lower than on the last two days. At 16°C, walking activity also decreased somewhat in the afternoon, but the pattern was not as clear as at 20°C. At 12°C, the wasps became active in the morning and reached their maximum walking activity at noon or later.
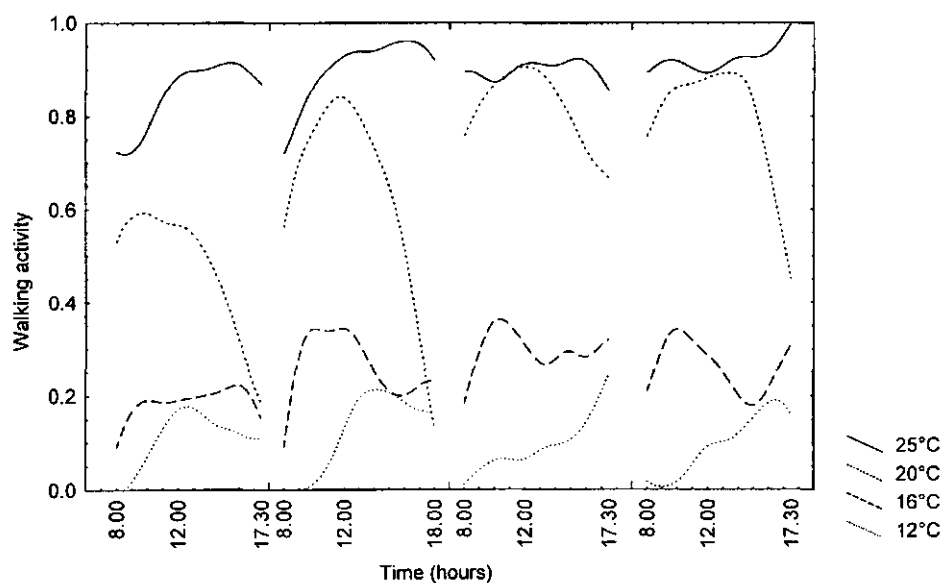

Figure 1. Mean walking activity (walking time divided by total time) of *T. brassicae* females during four days at four temperatures. Curves smoothed with least squares smoothing procedure (McLain, 1974).

A lot of individual variability in walking activity was found between the wasps (Figure 2). At 12°C most of the wasps were hardly active at all, but a few showed walking activities up to 0.4. At 16°C, 30% of the wasps had a walking activity of 0.1 or less, but three wasps had walking activities of 0.6 to 0.8. Individual variation was also high at 20°C, with walking activities from 0.3 to over 0.9. At 25°C, all wasps were very active, with a minimum walking activity of 0.7.
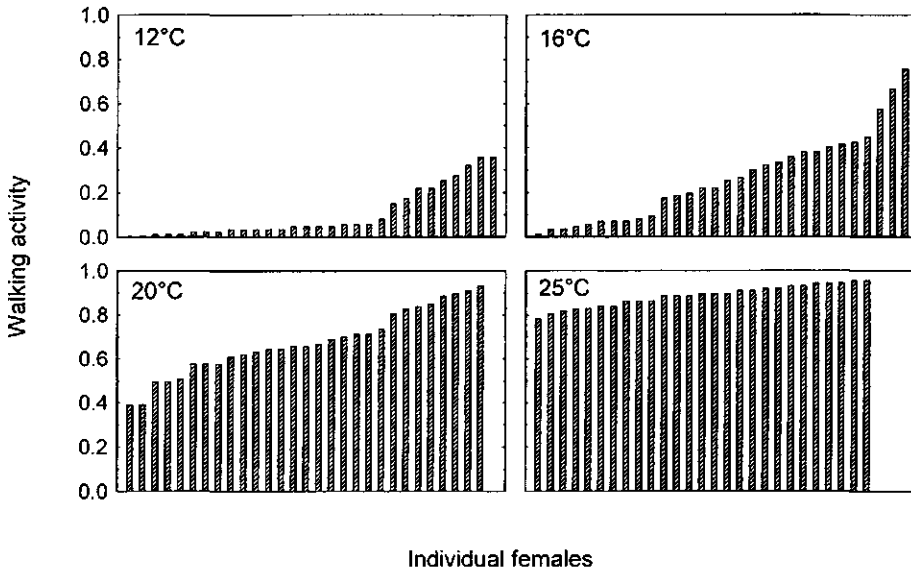
Figure 2. Distribution of walking activity of individual *T. brassicae* females during a four day period, ranked from lowest to highest walking activity.

Walking speed

At 25°C, the tracking equipment was unable to track the wasps. Many wasps flew away or made short jumps and flights during recording, but the system also lost track of many wasps during walking. Apparently, the wasps walked too fast for the image processing system, so it was not possible to collect accurate measurements at 25°C. The results for 12, 16 and 20°C are shown in Table 2. It is clear that temperature had a very significant effect on walking speed. At 20°C, mean walking speed was twice as high as at 12°C.

Table 2. Walking speed of *T. brassicae* at three temperatures measured during five minutes.

Temperature (°C)	N	Walking speed (cm/s)	p[1]
12	30	0.166 ± 0.004[2]	a *
16	29	0.265 ± 0.014	b *
20	32	0.311 ± 0.018	c *
25	30	-[3]	

[1] *: P<0.05, Different letters indicate significant differences in a LSD-test.
[2] Mean ± S.E.
[3] No usable results due to limitations of measuring equipment.

DISCUSSION

Daily walking activity curves at 20°C and 25°C are similar to those found by Pompanon *et al.* (1994). Visual checks at 30 minute intervals apparently give similar results as automated five second measurements every ten minutes. Pompanon *et al.* (1994) found a high correlation between walking activity and walking speed during the day. This means that walking speed is lower at the beginning and at the end of the day.

Because some activity is already found at low temperatures, and walking activity was already close to the maximum at 25°C, resulting in a sharp increase in between, a logistic curve was fitted to the walking activity data (Figure 3). Mean walking activities over four days which we determined are quite similar with the data found for *T. evanescens* by Biever (1972) and Boldt (1974), who determined rate of search which includes walking activity and walking speed. Pak and van Heiningen (1985) compared the parasitism of 20 *T. maidis* (=*brassicae*) strains at different temperatures in a small test area with host eggs. They defined activity as the proportion of females that parasitized at least one egg. With a two-hour test, activity varied from 0.15 at 12°C to 0.88 at 30°C. In a 24 hour test, activity was 0.18 at 12°C but 0.94 at 25°C and 0.92 at 30°C. Although this 'activity' is not the same as the walking activity we measured, it also rises sharply from 12°C and comes close to the maximum at 25°C.

A straight line was fitted to the walking speed data. As Figure 4 shows, walking speeds are very close to those found for *T. brassicae* by Gass (1988). For later calculations, we assumed that the walking speeds will further increase in a linear fashion at higher temperatures until a maximum is reached at about 35°C, similar to walking speeds found for *T. evanescens* by Biever (1972).

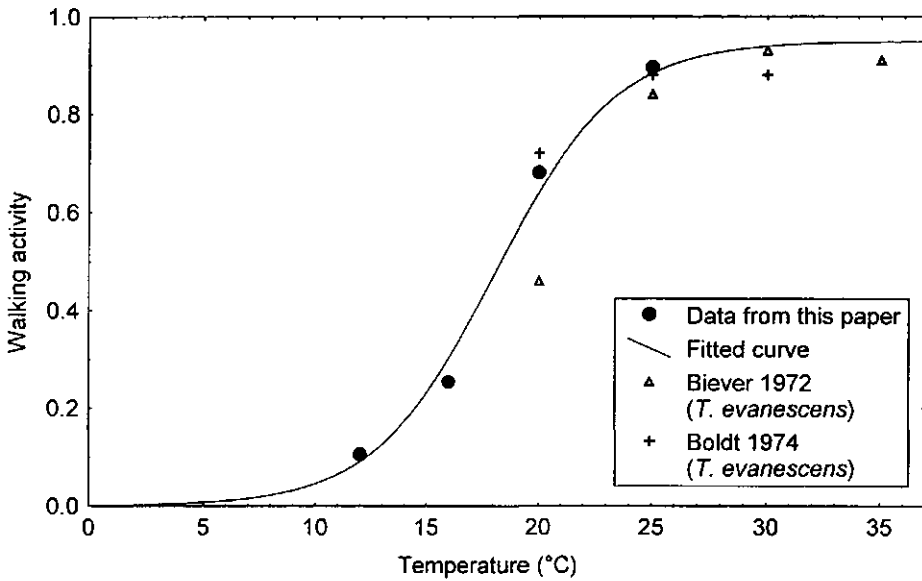Figure 3. Relation between temperature and walking activity of *T. brassicae*. Data from literature added for comparison.

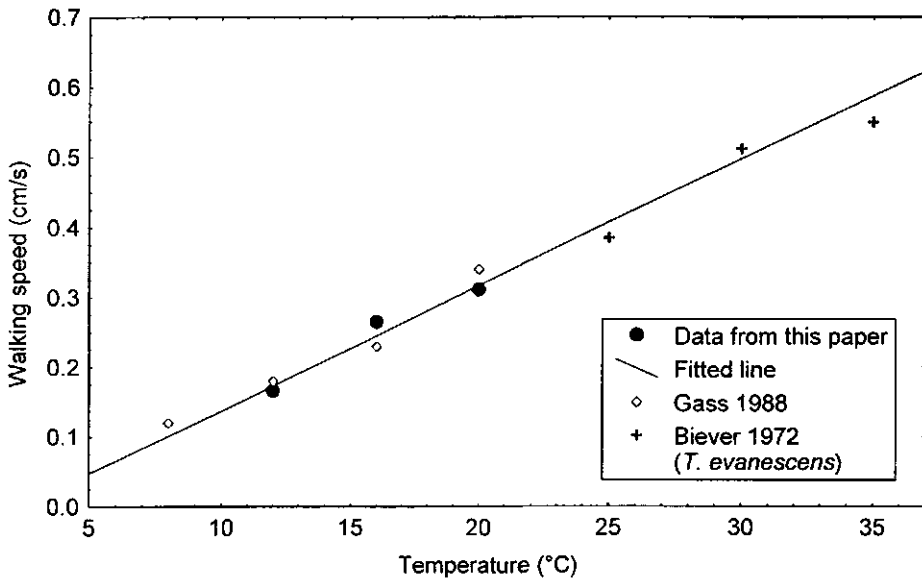Fitted curve: walking activity = 0.95 / (1 + 69 x 0.69$^{(temperature-6.64)}$).

Figure 4. Relation between temperature and walking speed of *T. brassicae*. Data from literature added for comparison.

Fitted curve: walking speed (cm/s) = -0.018 + 0.017 x temperature.

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Boldt (1974) calculated that 1.5 times as much *Trichogramma* are needed at 20°C compared to 35°C to reach the same level of parasitism. This is based on the distance travelled (walking speed multiplied by walking activity). If walking speed and walking activity predicted from our experiments are multiplied to find the distance travelled (Figure 5), it is found that wasps can search an area at 35°C that is 2.7 times as large as the area covered at 20°C. It can be seen from Figure 5 that walking activity is the limiting factor in searching at low temperature. At higher temperatures, the parasitoids are searching all the time, so the area searched, so only a higher walking speed can further increase the area searched. In the field, searching at temperatures below 18°C will even be more reduced, because *T. brassicae* does not fly below 18°C (Bigler *et al.*, 1982) and thus, cannot disperse effectively over the crop.
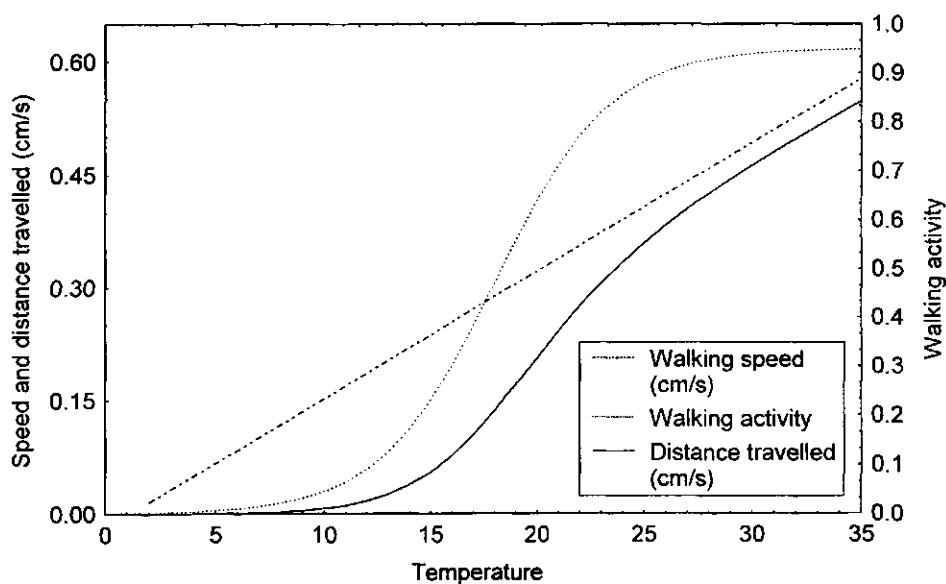

Figure 5. Distance travelled per time unit by *T. brassicae* based on predicted walking speed and walking activity.

Low walking activity at low temperatures can be a major constraint in the applicability of *T. brassicae* as a biological control agent in temperate countries, because cool weather leads to unacceptable low levels of field parasitism (Pak *et al.*, 1989). Walking activity and speed (Limburg and Pak,1991) and parasitism at low temperatures (Pak and van Heiningen, 1985) of *T. evanescens* have a genetic component. If walking activity of *T. brassicae* also has genetic components, which is very likely, it would be useful to select rearing strains that show relative high activity at low temperatures. Rearing systems should be designed to maintain this trait, and regular quality checks made similar to the test described in this article to establish if the population still has adequate walking activity at low temperatures. If higher walking activities at low temperatures can be sustained, the efficiency of *T. brassicae* as a biocontrol agent in temperate climates will be increased.

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Chapter 8

Simulation of the host-finding behaviour of *Trichogramma brassicae* on maize leaves and plants

ABSTRACT

To simulate the host-finding behaviour of *Trichogramma brassicae* parasitoids, stochastic Monte Carlo simulation models were developed. The models yielded encounter probabilities with *Ostrinia nubilalis* egg masses on maize leaves and plants. The model for searching on leaves emphasized the importance of leaf area. Spending proportionally more time for walking along the leaf edge by *T. brassicae* had little negative impact on the encounter probability. According to the model for plants, *T. brassicae* had a probability of 0.06 to 0.12 to encounter an egg mass on a maize plant during a single visit, depending on plant size. At 25°C, only walking speed of the parasitoid had a significant impact on encounter probability. At 18°C, the diameter of egg masses, the parasitoids walking activity, residence time on leaves and factors influencing between-leaf movement also had a significant effect on the encounter probability. The model showed that the distribution of landings over the plant leaf levels of *T. brassicae* should be similar to the oviposition distribution of *O. nubilalis* for maximum encounter probabilities. At higher temperatures, *T. brassicae* spends less time on the plant and visits fewer leaves, but the model showed that the encounter probability is higher, because of the higher walking speed and walking activity. Four reactions of the parasitoid to host-cues (scales) were incorporated in the model (increased residence time on leaf side and leaf, increased chance to visit more leaves, and an increased chance to find an egg mass if the host-cues are close to the egg mass). The presence of host-cues doubled the encounter probability. The model showed that scales which are close to an egg mass have the strongest effect on encounter probability, but also scales anywhere else on the lower side where an egg mass is present, increase the encounter probability. At 18°C, scales on the lower side of the leaf above the one with the egg mass did also significantly increase the encounter probability.

INTRODUCTION

Trichogramma brassicae Bezdenko is an egg parasitoid widely used in Europe biological control of *Ostrinia nubilalis* Hübner, the European corn borer (Li, 1994; Smith, 1996), using inundative methods. Because *Trichogramma* species show inter- and intraspecific variation in searching behaviour, host preference and response to environmental conditions, selecting an appropriate strain for the specific pest, crop and environment is very important for the success in biological control programmes (Hassan, 1994). Quality control of the mass produced material is necessary to make sure that *Trichogramma* reared under unnatural conditions for many generations will give an adequate performance in the field (Bigler, 1994). In both species and strain selection, and in quality control programmes, traits of the parasitoid are chosen that can be measured in the laboratory. These traits are supposed to influence the success of the parasitoid as a biological control agent. Examples of these traits are sex-ratio, longevity, host-acceptance, host-suitability, fecundity, walking speed and flight propensity. The actual contribution of these components to the success of the parasitoid as a biological

control agent is often unknown, because their effect depends on the behaviour of the parasitoid in the field. Field experiments to measure the effect of quality control components have been made (Dutton *et al.*, 1996), but testing for each single parameter is impractical due the immense amount of labour involved, and the variability in field conditions. An alternative approach to test the effect of single traits on biological control efficacy is to model the behaviour of the parasitoid. This model can be verified and validated with experimental results and then be used to evaluate the effect of changes in each behavioural trait on searching.

Simulation of *T. brassicae* host-finding: concepts and general description of the model

Van Roermund *et al.* (1996, 1997a, b, c) developed individual based models to simulate the foraging behaviour of *Encarsia formosa*, a parasitoid of the greenhouse whitefly *Trialeurodes vaporariorum*. With these models, the functional response of the parasitoid to different host densities was obtained at the leaf, plant and crop level. The models were developed to determine the effect of certain parasitoid and host traits on the searching efficiency of the parasitoid. The models described in this article are an adaptation of the stochastic Monte Carlo simulation model of van Roermund *et al.* (1997a) for leaves. That model simulates searching by a parasitoid during a single visit to a leaf. The residence time of *Encarsia formosa* on the leaf is influenced by host-cues and host encounters. In a Monte Carlo model, a large number of individual wasps are simulated. The behaviour of each wasp is determined by fixed input parameters, but also by stochastic processes. In a stochastic process, events do occur with a certain probability. The event itself cannot be predicted, but the chance for a certain outcome is known. The outcome of discrete events (for example, whether the parasitoid lands on the upper or lower leaf side) is found by comparing a random number with by a single, experimentally determined probability, while continuous stochastic variables (for example, the time that a parasitoid will search on a leaf) are found by randomly taking a value from an experimentally determined probability distribution, that expresses the mean and variation of the variable. The outcome of the model for a single wasp cannot be predicted. By taking the mean of many simulation runs, the probability that a wasp will encounter an egg mass can be determined.

The searching behaviour of *E. formosa* and *T. brassicae* is similar in many respects.
-The residence times of the parasitoids on uninfested leaves are exponentially distributed (van Roermund *et al.*, 1994; Chapter 6; van Roermund and van Lenteren, 1995).
-Searching for hosts on the leaf surface is random (van Lenteren *et al.*, 1976; Gardner and van Lenteren, 1986; van Roermund and van Lenteren, 1995).
-Landing on leaves is not influenced by host-cues (Noldus and van Lenteren, 1990; Sütterlin and van Lenteren, in press; Chapter 5).
-Contact with host-cues increases residence time (van Vianen and van der Veire, 1988; van Roermund *et al.*, 1994; van Roermund and van Lenteren, 1995; Chapter 6).

The hosts, *O. nubilalis* egg masses and *T. vaporariorum* immatures have three things in common:
-Hosts are normally only found on the lower leaf side.
-Hosts are immobile.
-Hosts are clustered (In the case of *O. nubilalis*, the egg masses are not clustered, but each egg mass is a cluster of hosts).

These similarities made the *E. formosa* model a good starting point for modelling the behaviour of *T. brassicae*. There are, however, a number of important differences between the two systems, which had to be accounted for.
The behaviour of the parasitoid is different in the following respects:
-The initial residence time of *E. formosa* on leaflets without hosts is not affected by temperatures above 18°C (van Roermund and van Lenteren, 1995), while *T. brassicae* spends less time on leaves at higher temperatures and more time on larger leaves (Chapter 6).
-*E. formosa* does not show thigmotaxis (i.e. the parasitoid does not follow linear structures)(van Lenteren *et al.*, 1976), while *T. brassicae* does so and will spends a significant part of its searching time following leaf edges. This means that searching on the leaf by *T. brassicae* is not random any more if the edge is encountered (Chapter 6).
-In *E. formosa*, the time that the parasitoid stays on a particular leaf side decreases when the parasitoid has visited both sides of the tomato leaflet (van Roermund *et al.*, 1994). This was not found for *T. brassicae* on maize leaves (Chapter 6).
-In the model for *E. formosa*, it is assumed that the parasitoid lands at random on leaves of different leaf levels, while landing of *T. brassicae* follows a specific pattern over the leaf levels (Chapter 4).
-*E. formosa* has to fly to reach a new leaf (van Roermund and van Lenteren, 1995) while *T. brassicae* can also walk to other leaves (Chapter 6).

Differences between the hosts also have an impact on parasitoid searching:
-Host related products: Honeydew of *T. vaporariorum* occurs mainly on the upper side of lower leaves. In the *Encarsia* model, it is assumed that, when honeydew is present, the whole upper leaf side is covered (van Roermund *et al.*, 1997c). However, scales of *O. nubilalis* can be found on both leaf sides. They are often concentrated in patches, and do not necessarily occur near egg masses (Chapter 2).
-Although *T. vaporariorum* occurs in clusters, individual hosts are some distance apart from each other. A single leaf may have many individuals (Eggenkamp-Rotteveel Mansveld *et al.*, 1982a,b). *O. nubilalis* produces egg masses of up to 64 eggs, with an mean of 17 eggs (Poos, 1927; Caffrey and Worthley, 1927). In the field more than one egg mass per leaf is very rare (Hawlitzki *et al.*, 1994).
-The host plants have very different shapes: a tomato plant has many small leaflets that have about the same size, while a maize plant has only a few large leaves of different size.

SIMULATION MODELS

The model for searching on leaves

Description of the model for searching on leaves

The model for leaves describes the searching behaviour of a female *T. brassicae* on a single maize leaf with one single egg mass on the lower leaf side, and without other host-cues (Figure 1). This mimics experimental situations where egg masses are artificially applied to leaves. After selecting a leaf side on which the parasitoid starts, the total times that the parasitoid will spend on the leaf (TGUP) and on the current leaf side (TSIDE) are calculated (for input data see appendix 1). The time is then set to zero and the simulation cycle starts. During each cycle, a number of possible events are checked. If the parasitoid spent all the time that was allocated for the current leaf side (TWALKS>TSIDE) it moves to the other leaf side and the model calculates again how long the parasitoid will walk on this new leaf side. If the parasitoid is on the lower leaf side, egg masses can be found. The encounter rate is calculated based on the formula by Skellam (1958), which includes leaf size, egg mass size, width of the parasitoids searching path, walking speed and walking activity:

RE = (WIDTH+EGGDM)*WS*ACT*DENS

RE: rate of encounter (encounters/minute)
WIDTH: width of *T. brassicae* search path (cm)
EGGDM: diameter of *O. nubilalis* egg mass (cm)
WS: *T. brassicae* walking speed (cm/minute)
ACT: walking activity (fraction of time on leaf spent on walking)
DENS: number of egg masses on leaf side divided by leaf area (number/cm^2)

If an egg mass is encountered during the time step, the simulation finishes. If not, the time is increased by the time step (1.2 seconds) and the next cycle starts.
If the time spent walking on the leaf is longer than the time allocated for the leaf (TWALK>TGUP), the parasitoid leaves the leaf and the simulation is finished. If the total time is longer than the maximum simulation time (two hours) the simulation is also finished.
Residence time on a leaf (giving up time, TGUP) and residence time on a particular leaf side (TSIDE) were randomly taken from the observed exponential distributions (Chapter 6). Mean residence times for leaves depend on leaf size and temperature.

Figure 1.
Flowchart of the model simulating host-finding of *Trichogramma brassicae* on a maize
leaf.

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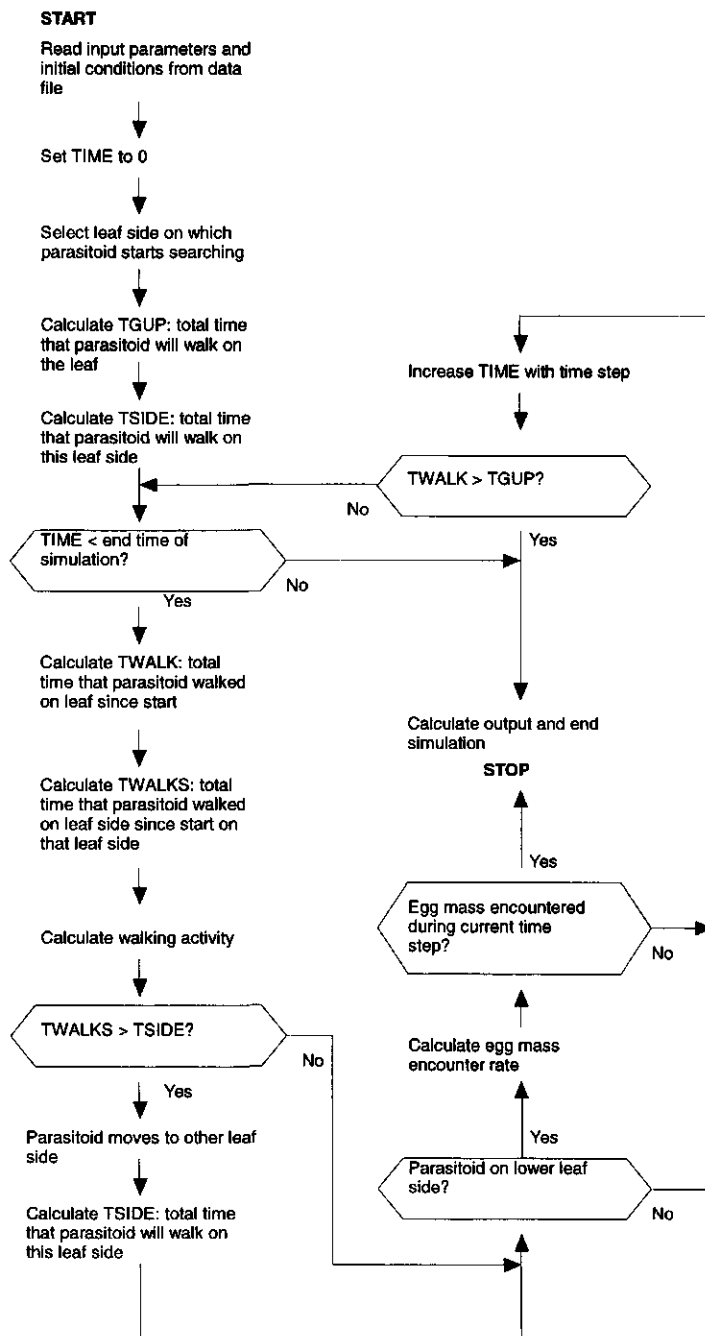
Validation of the model for searching on leaves

The observed leaf size, residence times on leaves and leaf sides, and side on which the parasitoid started searching were used as input for the model. Searching was simulated for 1000 parasitoids. This number of iterations was chosen because it was high enough to give a standard error/mean ratio of 0.05 for the expected outcome. Because the outcome for the simulation of a single wasp is either one (egg mass encountered) or zero (no egg mass encountered), the standard deviation for any outcome of the model can be calculated in advance. If the model would give the same outcome as the experiment, S.E divided by mean would be 0.05 for large, 0.04 for medium and 0.02 for small leaves at 1000 iterations.

Following the leaf edge

In the model, the parasitoid searches at random on the leaf. *T. brassicae* spends about 12-14% of its time (Chapter 6) in the zone within one millimeter of the leaf edge. In the field, *O. nubilalis* egg masses are rarely found on the leaf edge (Chapter 2), so the chance of finding an egg mass while following the leaf edge is negligible. To study the effect of edge following on the encounter probability, it was assumed that no egg masses would be found while following the edge, so during 13% of the time no eggs could be found. During the other 87%, the wasp is searching on the leaf minus the edge area (5% of the total leaf area). The simulation model was run for 1000 parasitoids with the reduced leaf area and residence time on the leaf to see if the encounter probability changed significantly.

THE MODEL FOR SEARCHING ON PLANTS

To simulate behaviour of the parasitoid on the plant, the following features were added to the model. The leaf level on which the parasitoid starts was simulated by using the observed distribution of parasitoid landings over the leaf levels (Chapter 5). The residence time on the leaf is depended on leaf size. After the wasp has spent the allocated residence time on the leaf without finding an egg mass, it can either stop searching on the plant by standing still or flying away or continue searching by walking to another leaf. This is influenced by temperature, but most parasitoids visit only one leaf before leaving or standing still for a longer period (Chapter 6).

    T. brassicae reacts to egg odour, scales and sex pheromones of *O. nubilalis* (Chapter 3). The effect of egg odour as a non-contact cue seems to be very limited (Laing, 1937; Pak *et al.*; 1990). Sex pheromones do have a clear effect, but are rarely present inside maize fields in the Swiss situation, because *O. nubilalis* mates outside the fields where they oviposit (Buechi *et al.*, 1981; Cordillot, 1989). Therefore, only the effect of encounters with host scales has been considered in the model.

O. nubilalis females make many landings on the upper and lower side of maize leaves. After landing, they do not move over the leaf, but probe the leaf with their abdomen. Afterwards, oviposition may take place. Both landings that result in oviposition and landings that do not, can result in patches of abdominal moth scales being left on the plant (Chapter 2). Encountering these scales will arrest the wasp on the leaf side and on the leaf where it encounters the scales, by a klinokinetic response, and on the plant by lowering the flight propensity (Chapter 3, Chapter 6). In the model, encountering patch with scales increases the residence time on the leaf side, the residence time on the leaf and the chance to visit another leaf on the plant. If the scales are directly around an egg mass, the diameter of the egg mass is increased in the formula for calculating the egg mass encounter rate.
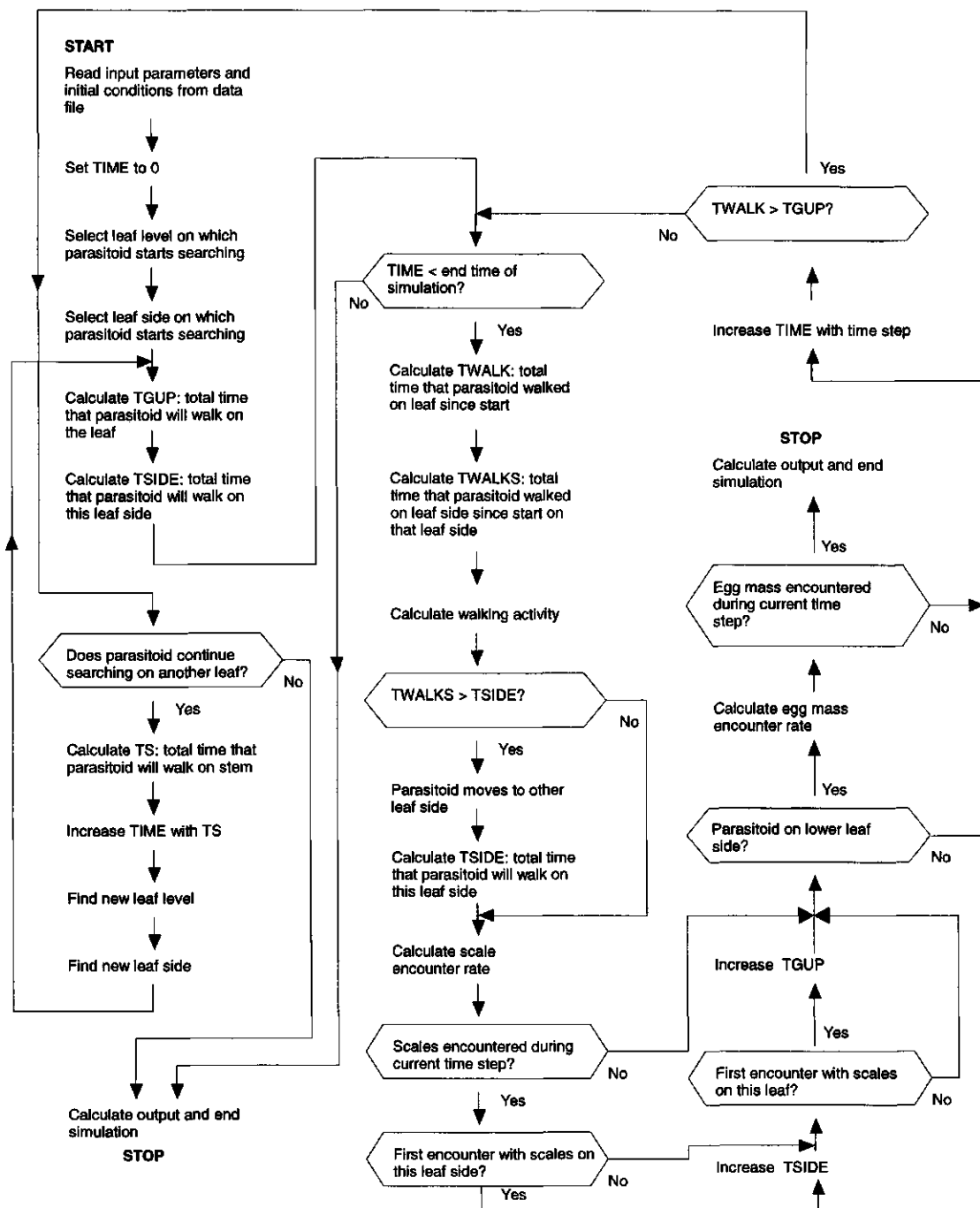
Figure 2.
Flowchart of the model for *Trichogramma brassicae* host-finding on a plant.

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Description of the model for searching on plants

The model describes the searching behaviour of a female *T. brassicae* on a maize plant with one or more egg masses on the lower side of the leaves and host-cues (scale patches) on both leaf sides (Figure 2). First, the leaf and leaf side on which the parasitoid starts searching are selected. The total time that the parasitoid will spend on the first leaf (TGUP) and on the current leaf side (TSIDE) are then calculated. Input data are given in appendix 2. The time is set to zero and the simulation cycle starts.

At the beginning of each cycle, it is calculated how long the wasp has been walking on the leaf (TWALK) and on the current leaf side (TWALKS).
-If the parasitoid has spent all the time that was allocated for the current leaf side (TWALKS>TSIDE) it moves to the other leaf side and the model calculates again how long the parasitoid will walk on this new leaf side.
-The scale patch encounter rate is calculated using the equation of Skellam (1958). If a scale patch is encountered for the first time, the time to be spent on the leaf side (TSIDE) is increased. Also the time to be spent on the leaf (TGUP) is increased, and the chance to visit another leaf after leaving the current leaf.

If the parasitoid is on the lower leaf side, egg masses can be found. The encounter rate is calculated by the equation of Skellam (1958) as well. If an egg mass is encountered during the time step, the simulation is finished. If no egg mass is found, the time is increased by a time step of 1.2 seconds.
-If the time spent walking on the leaf is longer than the time allocated for the leaf (TWALK>TGUP) the parasitoid leaves the current leaf. If the total time is longer than the maximum simulation time (two hours) the simulation is also finished.

When leaving a leaf, it is calculated the model determines whether the parasitoid will walk to another leaf. If it does, the time spent walking on the stem is calculated, as well as the new leaf level and leaf side. A new TGUP and TSIDE are determined and the simulation cycle starts again for the new leaf.

Because the parasitoid can encounter leaves of different sizes, an equation was developed that describes the relationship between leaf area and residence time on leaves. Data were used from the experiment described for validating the model. Residence time increased with increasing leaf size. An exponential curve was found using least-square fitting. The residence time was also temperature-dependent: it decreased with higher temperatures (Chapter 6). Combining these, the resulting equation is

$$RTL1 = (11.77*e^{(0.002405*AREA)})/(0.198+0.033*TEMP)$$

RTL1: Mean residence time on the first leaf visited (minutes)
AREA: Area of one side of the leaf (cm^2)
TEMP: temperature (°C)

The parasitoid spent less time on the second and subsequent leaves visited. This was more pronounced at lower temperatures. One reason might be that a parasitoid which arrived on a leaf by walking was generally closer to the stem, and thus had an increased chance of encountering the stem again. Based on data from Chapter 6, the following relationship was found:

$$RTL2 = (1.067-0.024*TEMP)*RTL1$$

RTL2: mean residence time on the second and subsequent leaves visited (minutes)
TEMP: temperature (°C)
RTL1: mean residence time on the first leaf visited (minutes)

Residence time on a leaf side is correlated with residence time on a leaf (and hence with leaf size and temperature), but temperature has an even stronger effect, because increased walking speed and activity cause the parasitoid to encounter the leaf edge more often. Based on date from Chapter 6, the following relationship was found:

$$RTUP = (1000*RTL1)/(TEMP^2*BUSI)$$
$$RTLOW = (1000*RTL1)/(TEMP^2*BLSI)$$

RTUP = mean residence time on upper leaf side (minutes)
TEMP = temperature (°C)
BUSI = temperature dependent index for residence time on upper side
RTL1 = residence time on first leaf visited (minutes)

RTLOW = mean residence time on lower leaf side (minutes)
BLSI = temperature dependent index for residence time on lower side

After searching on a leaf, the parasitoid can continue searching on another leaf or finish searching. The chance that the parasitoid will continue searching increases with the number of leaves visited (Chapter 6). This chance was expressed by the following equation using iterative least square fitting:

$$CWALK(N) = (1/(A+B*N))/(1/(A+B*(N-1)))$$
$$CWALK(1) = 1/(A+B)$$

CWALK(N): chance that parasitoid will continue searching on another leaf after visiting the Nth leaf.
A, B: temperature dependent variables
N: number of leaves visited during this plant visit

Validation of the model for searching on plants
The validation experiment was simulated with the model, using plant sizes (Small plants: mean leaf area 683 cm^2, seven leaves, large plants: mean leaf area 1392 cm^2, eleven leaves), and temperature (25°C) from the experiment. Searching by 8000 wasps was simulated. This number of iterations was chosen because it was high enough to give a good standard error/mean ratio for the expected outcome that was lower than 0.05. If the model would give the same outcome as the experiment, the S.E divided by the mean would be 0.03 for large and 0.02 for small plants at 8000 iterations.

Sensitivity analysis of the model without host-cues
To study the effect of single input parameters on encounter probability, a sensitivity analysis was performed. Simulations were run at 18 and 25°C, where each single input parameter was increased with 25%.

The following input parameters were tested: Leaf area of the plant (AREA), diameter of *O. nubilalis* egg masses (EGGDM), width of *T. brassicae* searching path (WIDTH), walking speed of *T. brassicae* (WS), Walking activity (ACT), chance to land on lower leaf side compared to upper leaf side (LSIDE), residence time on first leaf visited (RTL1), residence time on second and subsequent leaves visited (RTL2), residence time on lower leaf side (RTLOW), residence time on upper leaf side (RTUP), and the following between-leaf movement parameters: chance to move upward from upper leaf side (CWUU), chance to move upward from lower leaf side (CWLU), chance to arrive on upper leaf side when leaving from upper leaf side (CAUU), chance to arrive on upper leaf side when leaving from lower leaf side (CALU), chance to return to same leaf when leaving from upper leaf side (CRU), chance to return to same leaf when leaving from lower leaf side (CRL), chance to return to upper leaf side of the same leaf when leaving from upper leaf side (CRUSU), chance to return to upper leaf side of the same leaf when leaving from lower leaf side (CRLSU). A G-test (Sokal and Rohlf, 1995) was used to compare the encounter probabilities with the control.

The simulations were made for plants with 11 leaves with a total leaf area of 1386 cm², with a single egg mass on the lower side of the fifth leaf. For each parameter, 8000 iterations were done.

Effect of T. brassicae landing levels and O. nubilalis egg mass distribution on encounter probability.
To study the effect of the distribution of *T. brassicae* landings over the plant leaf levels and of the distribution of egg masses of *O. nubilalis*, the mean parasitoid landing level was shifted one level up, or the egg mass was shifted one leaf up or one leaf down. The simulation setup was the same as for the sensitivity analysis.

Effect of temperature on encounter probability and plant residence time
The effect of temperature on the encounter probability was simulated using the same setup as in the sensitivity analysis. Temperatures from 14 to 34°C were run with 1000 iterations each.

Effect of host-cues

Validation of effect of host scales on searching in the plant model
The validation experiment for the effect of scales was simulated by the model, using plant sizes (3800 cm², 10-12 leaves) and egg mass numbers and positions from the experiment as input data. The simulation was run with 8000 iterations. Because of the high density of egg masses on the plants, it was assumed that a large percentage of the wasps encountered scale patches.

Single factors of host cue effect
To study which scale effect caused the increase in encounter probability, the simulation model was run for each of the four scale effects separately: staying longer on a leaf where scales were encountered (IRTSIDE), staying longer on a leaf where scales were encountered (IRTL), an increased chance to visit another leaf after encountering scales (ICWALK) and an increased diameter for egg masses surrounded by scales (IEGGDM).

Effect of position of scale patches
In the previous simulation, egg mass and scale patch density were high. In the field, the number of egg masses and scale patches per plant is much lower. The model was used to study whether one single scale patch on a plant has an effect on the encounter probability. For these simulations the plant had nine leaves with one egg mass on the lower side of the fifth leaf. The position of the scale patch during the simulations were: around the egg mass, on lower side of fifth leaf but not around the egg mass, on upper side of fifth leaf and on upper or lower side of fourth or sixth leaf. For each simulation, 5000 iterations were run for 18 and 25°C.

RESULTS

The model for searching on leaves

Validation of the model for searching on leaves

Apparently, the model overestimates the encounter probability of the wasps (Figure 3). This was unexpected, since the values of the parameters 'width of searching path' and 'walking activity' were chosen on the low side. A reason for this overestimation might be that in reality the wasps do not always move over the entire leaf, and spend most of their time on a limited part of the leaf. In that case, wasps that land far away from the egg mass have a smaller chance to find it than the model would find.
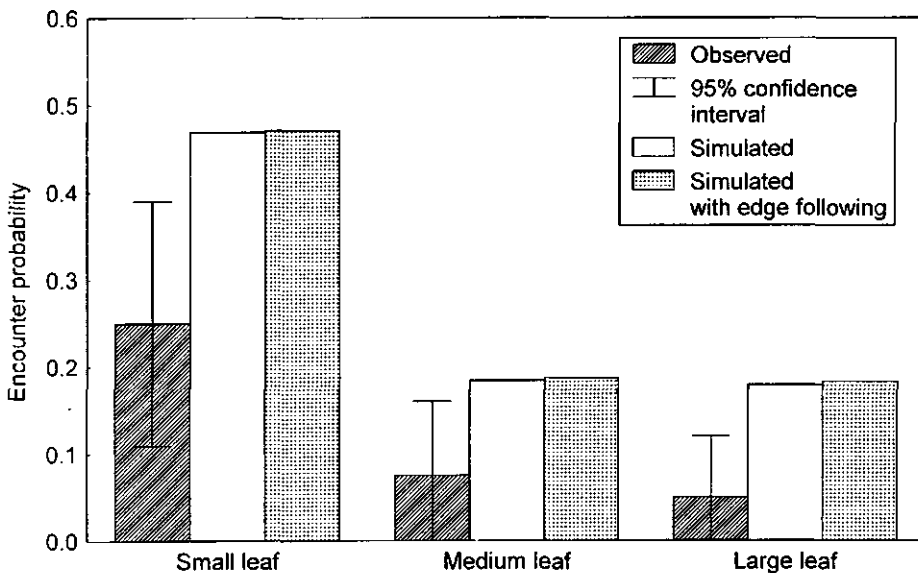

Figure 3.
Observed and simulated encounter probability of artificially placed *O. nubilalis* egg masses by *T. brassicae* on maize leaves of three sizes. Experiments: N = 40, simulations: 1000 iterations. Simulation of following of the leaf edge is described in text.

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The effect of the three leaf sizes on encounter probability was the same in both the experiment and the simulation. The five-fold increase in leaf area between the small and the medium leaves caused a much lower encounter probability on the medium-sized leaves. The four-fold increase in leaf area between the medium and large leaves, however, caused only a small further decrease of the encounter probability, both in the simulation and in the experiment.

Following the leaf edge

Following the leaf edge had very little effect on the encounter probability (Figure 3). For this reason, edge following was not incorporated in the plant and crop versions of the model.

The model for searching on plants

Validation of the model for searching on plants
On average, between 0.12 and 0.18 of the wasps that landed on the plant encountered the egg mass, depending on plant size (Figure 4). This means that the larger part of the wasps was unsuccessful in finding hosts. In this case, the model underestimated the encounter probability found in the experiment. The results from the experiment and the simulation are within the same order of magnitude, given the large standard deviations. The effect of plant size on encounter probability is simulated satisfactory.
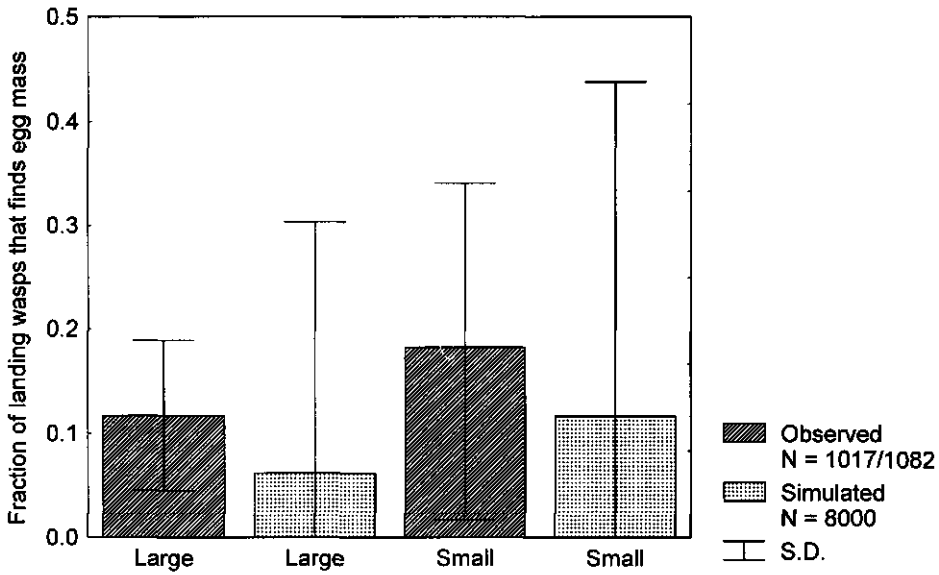
Figure 4.
Observed and simulated encounter probability of artificially placed *O. nubilalis* egg masses
by *T. brassicae* on maize plants of two sizes (one egg mass per plant). Experiments: N =
1017/1082, simulations: N = 8000.

Sensitivity analysis of the model without host-cues

At 25°C, only increasing the walking speed (WS) gave a significant increase in encounter probability (Figure 5). Increasing residence time on leaves (RTL1), diameter of the egg mass (EGGDM), chance to return to same leaf when leaving from upper leaf side (CRU) and chance to land on the lower side (LSIDE) also caused considerable increases in encounter probability, but these differences were not significant. The reason that increasing the activity at 25°C had a small effect is probably that walking activity was already very high (88%), and could not be increased by 25% because of the 100% upper limit. Between-leaf movement parameters were not very important at 25°C, because there was little between-leaf movement at higher temperatures.

Increasing the width of the searching path of the parasitoid (WIDTH) lowered the encounter probability, but the difference was not significant. If the width of the searching path is small compared to the diameter of the egg mass, it hardly affects the rate of encounter and thus the encounter probability, according to the equation of Skellam (1958). In the model, the mean diameter of an egg mass is ten times the width of the searching path.

At 18°C, the diameter of the host egg mass (EGGDM), the walking activity (ACT), the walking speed (WS) and residence time (RTL1), resulted in the strongest increase in encounter probability. Three of the between-leaf movement parameters also significantly increased the encounter probability at 18°C: CWUU, CALU, and CRU. It is interesting to see that a 25% increase in leaf area seems to have no effect at all on the encounter probability. This confirms the findings of the leaf experiments that if leaves already have a certain size (300-500 cm^2), a further increase in area will have a limited effect on the encounter probability. The change of parameters that describe movement between the upper and lower leaf sides (RTUP and RTLOW) also had little effect on the encounter probability.
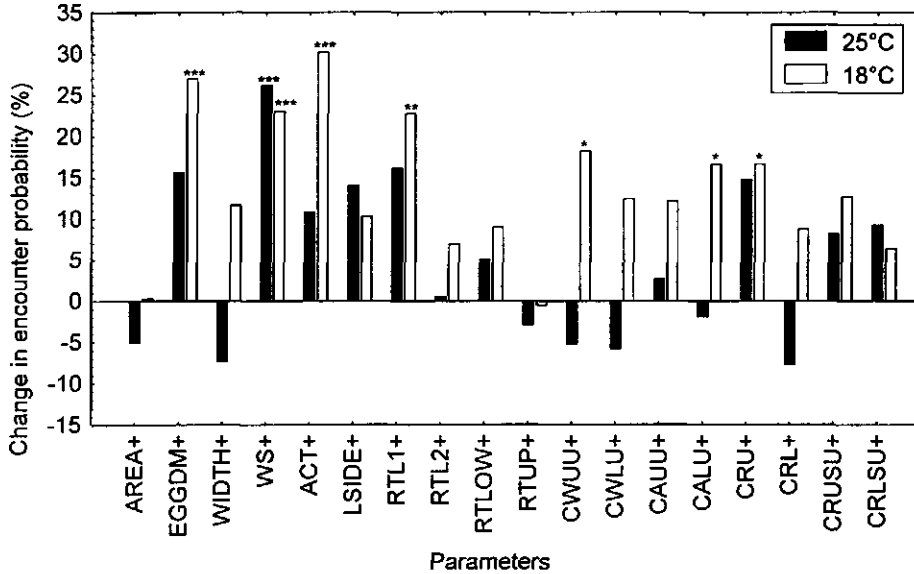
Figure 5.
Sensitivity analysis of the model for *T. brassicae* searching on plants. One egg mass on the fifth leaf of an plant with eleven leaves. Single input parameters were increased by 25%. Parameters tested: Leaf area of the plant (AREA), diameter of *O. nubilalis* egg masses (EGGDM), width of *T. brassicae* searching path (WIDTH), walking speed of *T. brassicae* (WS), Walking activity (ACT)(increased at 25°C by 11%), chance to land on lower leaf side (LSIDE), residence time on first leaf visited (RTL1), residence time on second and subsequent leaves visited (RTL2), residence time on lower leaf side (RTLOW), residence time on upper leaf side (RTUP), chance to move upward from upper leaf side (CWUU), chance to move upward from lower leaf side (CWLU), chance to arrive on upper leaf side when leaving from upper leaf side (CAUU), chance to arrive on upper leaf side when leaving from lower leaf side (CALU), chance to return to same leaf when leaving from upper leaf side (CRU), chance to return to same leaf when leaving from lower leaf side (CRL), chance to return to upper leaf side when leaving from upper leaf side (CRUSU), chance to return to upper leaf side when leaving from lower leaf side (CRLSU).
N = 8000. Asterisks over the columns indicate a significant increase in encounter probability compared to the control (G-test, *: $P<0.05$; **: $P<0.01$; ***: $P<0.005$).

Effect of T. brassicae landing levels and O. nubilalis egg mass distribution on encounter probability.

Moving an egg mass one leaf up (ODIS+) strongly increased the encounter probability, moving the egg mass one leaf down (ODIS-) decreased the encounter probability (Figure 6). This is caused by the higher number of landings of *T. brassicae* on higher leaf levels of the plant (Chapter 5). Shifting the distribution of landings of the parasitoids up (LL+) also decreased the encounter probability, for the same reason. Since most wasps visited only one or two leaves, it is very essential for encountering a host that they land on the right leaf.
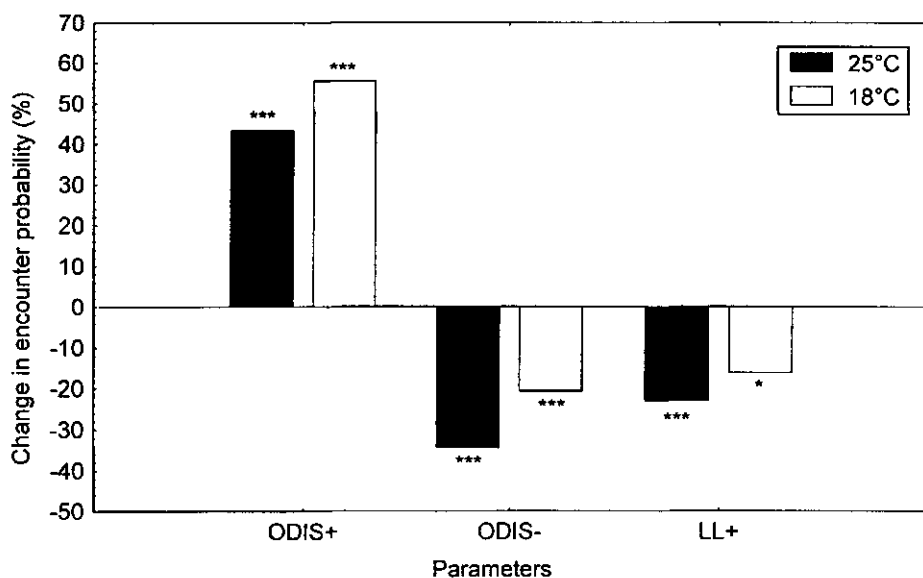
Figure 6.
Simulation of effect of higher (ODIS+) and lower (ODIS-) mean oviposition leaf level and of higher mean landing distribution (LL+) on encounter probability of *T. brassicae* with an host egg mass. N = 8000. Asterisks over the columns indicate a significant change in encounter probability compared to the control (T-test, *: P<0.05; **: P<0.01; ***: P<0.005).

Effect of temperature on encounter probability and plant residence time

The encounter probability rose with increasing temperature (Figure 7.) In the model, temperature influences residence time on leaves, walking speed, walking activity and between-leaf movement. The higher the temperature, the higher the chance to encounter an egg mass, even though more leaves were visited at lower temperatures and the parasitoid stays shorter on the leaf.

The residence time on the plant determines how many plants a parasitoid can visit during a day. The parasitoids stay much longer on the plant at lower temperatures, although their chance of encountering an egg mass during this period was smaller (Figure 8).
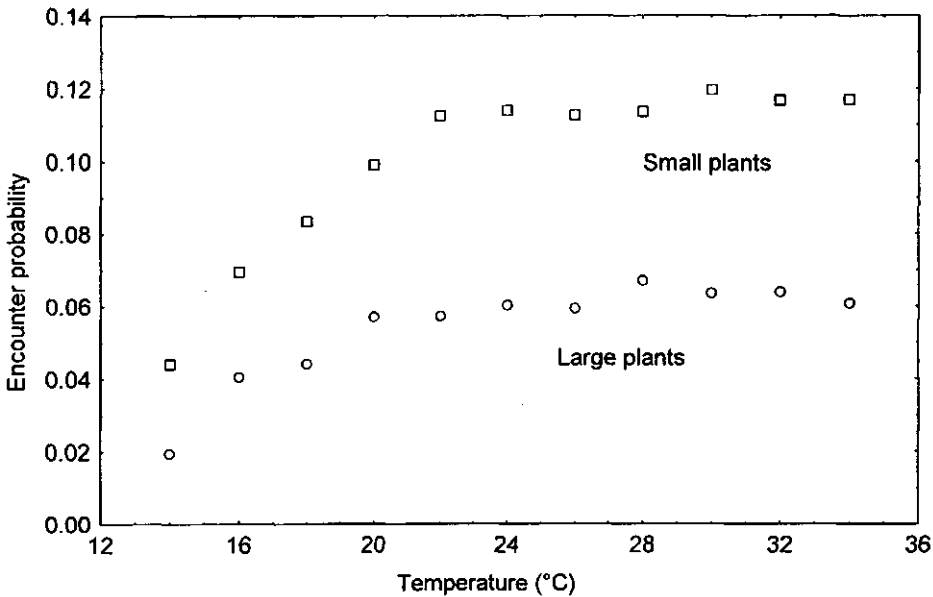
Figure 7.
Simulated effect of temperature on *T. brassicae* encounter probability with an artificially applied egg mass on a maize plant. N = 8000 for each data point.
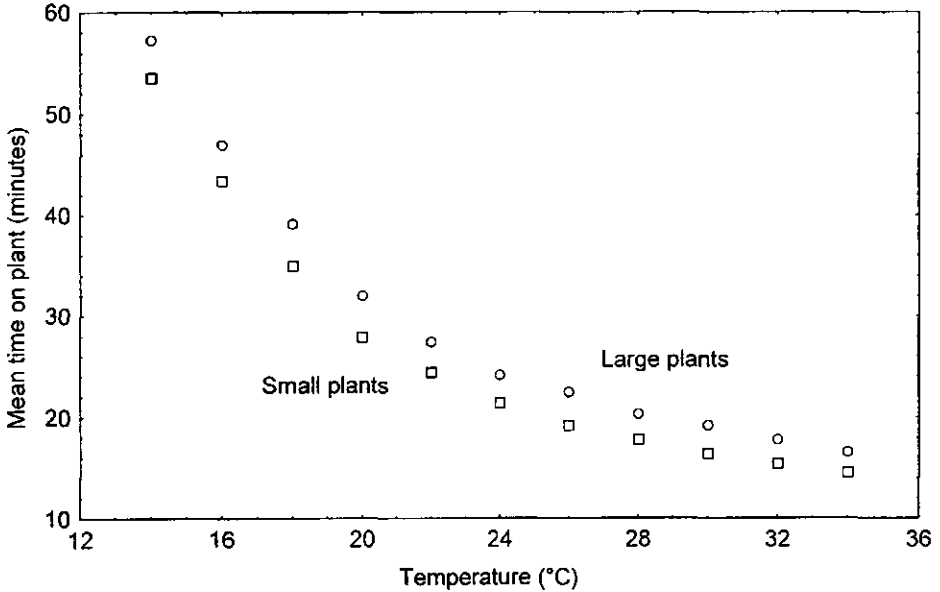
Figure 8.
Simulated effect of temperature on *T. brassicae* residence time on a maize plant. N = 8000
for each data point.

Effect of host-cues

Validation of effect of host scales on searching in the plant model
The experiment showed that 2.2 times as many parasitoids found an egg mass on plants with
host-cues compared to the control (Figure 9.). Based on the simulated encounter probability
for wasps without host-cues, the number of times that each egg mass would be encountered
in the simulation could be calculated. The results of the experiment and the simulation were
very close. Apparently, the model simulated the effect of host-cues (scale patches) on the
encounter probability very well.

Single factors of host cue effect
The effect of a longer residence time on leaves and leaf sides and of an increased egg mass
diameter was stronger than that of the increased number of leaf visits. It is clear that the
increase of the encounter probability as a result of the presence of scales is caused by a
combination of the four effects, and not by a single effect only (Figure 10).
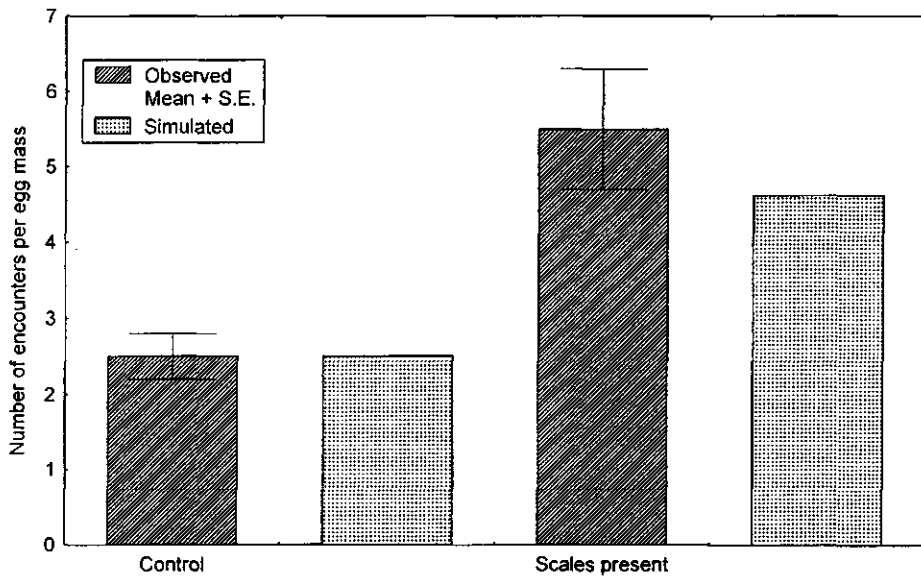
Figure 9.
Experimental and simulated effect of scale patches on the encounter probability of *T. brassicae*. Experiment: N = 183/80. Model: N = 8000.
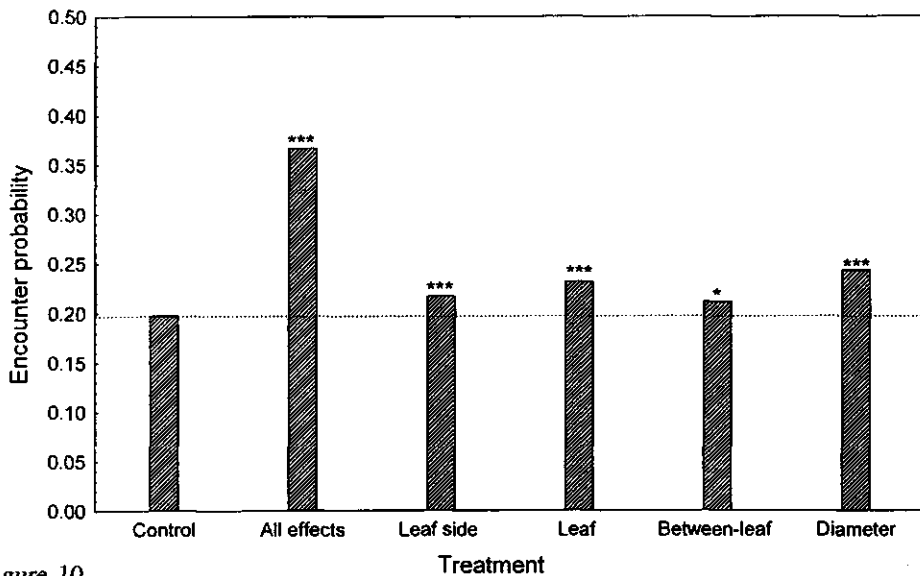
Figure 10.
Simulated effect of different reactions of *T. brassicae* to scale encounters on encounter probability with host egg masses. N = 8000. Asterisks over the columns indicate a significant increase in encounter probability compared to the control (G-test, *: P<0.05; ***: P<0.005).

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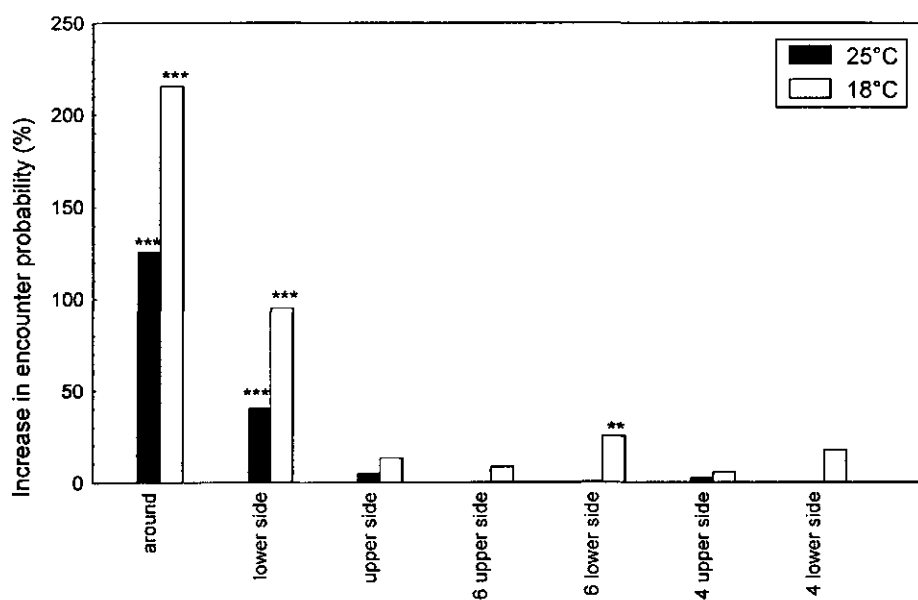
Figure 11.
Simulation of the effect of the position of a scale patch on the plant on encounter probability of *T. brassicae* with a host egg mass on the fifth leaf. The figure shows the increase in encounter probability compared to encounter probability on a plant without scales with egg mass on the fifth leaf. N = 5000.
Position of the scale patches:
Around: scale patch around the egg mass on the lower side of the fifth leaf. Lower side: scale patch on the lower side of the leaf with the egg mass, but not around it. Upper side: scale patch on the upper side of the fifth leaf (with the egg mass). Higher upper side: scale patch on the upper side of the sixth leaf. Higher lower side: scale patch on the lower side of the sixth leaf. Lower upper side: scale patch on the upper side of the fourth leaf. Lower lower side: scale patch on the lower side of the fourth leaf. Asterisks over the columns indicate a significant increase in encounter probability compared to encounter probability on a plant without scales (G-test, **: P<0.01; ***: P<0.005).

Effect of position of scale patches
A scale patch around the egg mass had a very significant effect on the encounter probability (Figure 11). This was caused by both arrestment of the parasitoid on the leaf side and an increase in the egg mass encounter rate. Scales on the lower leaf side of the leaf but not around the egg mass also had a significant effect on the encounter probability, because of arrestment. Scales on the upper leaf side of the fifth leaf had no significant effect, neither did scales on other leaves, except when scales were on the lower side of the sixth leaf at 18°C. The longer arrestment on the lower side of the sixth leaf will cause more wasps (of the 42% of the wasps that visited more than one leaf) to leave the leaf in a downward direction. The relative increase in encounter probability as a result of scales was much stronger at 18°C than at 25°C (encounter probability of control was 0.047 and 0.089, respectively).

DISCUSSION

The leaf model overestimated the probability of encountering egg masses. Reus (1988) already found that random search as simulated by Skellam (1958) overestimates encounter probability of *Trichogramma* on leaves. The most likely explanation is that *T. brassicae* does not move freely over the entire leaf, but instead stays more in the area where it started searching (Chapter 6), thus revisiting leaf areas it has already searched. The model showed that this difference could not be ascribed to edge-following behaviour.

Other models of individual behaviour of *Trichogramma* have been developed, but none of them describes behaviour at the plant level. Three models simulated the actual walking behaviour of *Trichogramma*. Blanché *et al.* (1996) simulated searching and oviposition in a rearing system at very high host densities. Yano (1978) simulated searching and oviposition of *T. dendrolimi* on flat surfaces. His model incorporates detailed walking behaviour, and the typical turning behaviour that *Trichogramma* shows after ovipositing, but effects of semiochemicals were not simulated. Blanché (1990) simulated walking behaviour on experimental arenas and leaves, based on a detailed analysis of walking patterns and reactions to kairomones. This model stresses the importance of edge and vein following behaviour, but the simulations were done for dicotyledon leaves, where the wasps encounter more veins than on monocotyledons like maize.

Bieri *et al.* (1990) simulated searching of *T. evanescens* (=*brassicae*) in an experimental arena using a formula developed by Hunter and Symonds (1970), which incorporates most of the input parameters used in the Skellam (1958) formula. It assumes that the parasitoid reacts with lower speed and increased turning to host-cues. Trails (5-15 cm) of *O. nubilalis* abdominal scales were used as host-cues. This model predicts that if the scale patch becomes too large, the encounter probability with the egg mass will be reduced, because the walking speed inside scale patches is much lower. However, direct observation of *O. nubilalis* behaviour on plants has since then shown that such trails rarely occur on maize plants, because the female usually oviposits on or very close to the landing spot on the plant. Although larger patches do occur, scale patches of *O. nubilalis* have an mean size of only 0.6 cm (Chapter 2).

The validation of the model for searching on plants was reasonable, although the model underestimates rather than overestimates the encounter probability. Because temperature influences walking speed, walking activity, residence time on leaves and on leaf sides, it is not surprising that it has a very strong influence on the encounter probability. The sensitivity of the encounter probability to the various input parameters is also temperature dependent: at 25°C fewer parameters have a significant impact than at 18°C. The model emphasizes the importance of the level on which the parasitoids land: if the parasitoids land too high or too low on the plant, the chance that they will encounter egg masses is very small.

The model simulates the effect of kairomones in the experiment very well. All the kairomone effects in the model (area restricted search, simulated by the increased host diameter, and retention of the parasitoid on the leaf side, leaf and plant where the kairomones were encountered) increase the encounter probability. The model indicates that patches of scales on the same leaf that are not close to an egg mass can also increase encounter probability.

The results of the sensitivity analysis of the plant model can be compared with those found by van Roermund *et al.* (1997b) for number of encounters on a plant by *E. formosa* at a low host density and clustered host distribution. For both parasitoids, the walking speed, walking activity, residence time on leaves and diameter of the host showed a strong effect on the encounter probability. At 25°C, walking activity of *T. brassicae* is over 0.9, so it could not be increased by 25% in the simulation. It is likely however that a decrease of walking activity at 25°C would result in a significant decrease in encounter probability.

Leaf area had the strongest effect on the number of *E. formosa* host encounters on tomato plants. The reason that this was not the case for *T. brassicae* is the difference in leaf sizes. Maize leaves are up to 25 times as large as tomato leaflets. As the leaf model shows, increasing the size of a small leaf has a stronger effect on the encounter probability than increasing the size of a large leaf. Width of the searching path was more important for *E. formosa* than for *T. brassicae.* This is a result of the relative size of the hosts compared to the width of the searching path. Since these are added in the Skellam formula, increasing the larger of the two by a percentage will have a stronger effect than increasing the smaller one. The searching path of *E. formosa* is about the same as the size of an average host, while that of *T. brassicae* is only one-tenth of the host's diameter.

Parameters that influence the encounter probability on a single plant may not have the same effect in the field. For example, longer residence times on leaves will increase the encounter probability on single plants, but they would also cause a parasitoid to visit less plants during a day in the field. For this reason, a crop model will be developed based on the plant model described here (Chapter 9).

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Chapter 9

Simulation of the host-finding behaviour of *Trichogramma brassicae* in a maize crop

ABSTRACT

A Monte Carlo simulation model was developed to simulate the host-finding behaviour of *Trichogramma brassicae* parasitoids. The model calculates encounter probabilities with *Ostrinia nubilalis* egg masses in a maize crop during one day. The chance for an individual parasitoid to encounter at least one egg mass during a day is only six percent according to the simulation. The model was not very sensitive to parasitoid parameters such as walking speed or walking activity. The encounter probability increased linearly with mean temperature and host density. The relationship with plant size was not linear, but decreases exponentially with increasing plant size. Although the simulated encounter rates found in the simulation are low, they are not inconsistent with parasitism rates found in the field.

INTRODUCTION

Trichogramma brassicae Bezdenko is an egg parasitoid that is widely used for biological control of *Ostrinia nubilalis* Hübner (European corn borer) in maize (Li, 1994; Smith, 1996). A first release of *T. brassicae* is made shortly before the first egg masses of *O. nubilalis* are found in the field. To keep the damage caused by the *O. nubilalis* larvae emerging from these egg masses below the economic injury level, the released parasitoids must parasitize a sufficiently high percentage of the eggs. A second release is made ten days after the first, to bridge the period when the parasitoids of the first release have either died, moved away, or depleted all their eggs. After that, a second generation of *T. brassicae* starts emerging from the parasitized egg masses in the field. As long as O. *nubilalis* oviposits in the maize field, *T. brassicae* will continue to emerge and be present in the field. It is clear that an accurate timing and a sufficient number of wasps released are essential for this biological control system to function properly. The number of released parasitoids (60.000 per hectare) in Switzerland is based on experience, and biological control results in an average reduction of *O. nubilalis* damage of 80% (Bigler, 1983; 1986).

Apart from the timing of the release and the number of parasitoids to be released, several other factors influence the level of parasitism achieved in the field. In the first place, the quality of the released parasitoids plays a role. The quality of the parasitoid is the whole of the traits that make the parasitoid an effective biological control agent, such as the parasitoid's sex-ratio, longevity, host-acceptance, host-suitability, fecundity, walking speed and flight propensity (Bigler, 1994). If and how much each trait actually contributes to successful biological control is unknown.

In the second place, parasitism may be influenced by the abundance and distribution of the host (Morrison *et al.*, 1980; Walde and Murdoch, 1988). The more host eggs available, the higher the chance that the parasitoid will encounter eggs, but due to egg depletion of the parasitoid the percentage parasitism may decrease with higher host density.

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Third, parasitism is influenced by the host plant. The most important effect of the host plant that influences parasitism is plant size. On larger plants, parasitism is expected to be lower because the parasitoid must search a much larger area (Ables *et al.*, 1980; Andow and Prokrym, 1991). Other host plant effects (structural complexity and structural heterogeneity) influencing parasitism (Andow and Prokrym, 1990) are not very relevant for maize as cultivars are very similar in this respect (Chapter 3).

Finally, there is an effect of environmental factors. Temperature has a crucial effect on walking speed, walking activity (Chapter 7) and flight activity (Dutton and Bigler, 1995), which results in a strong effect on parasitism.

The quantitative effect of each single factor on parasitism is still unknown. In the field it is usually not possible to study the effect of one factor and while keeping all other factors the same. One way in which the effect of separate factors on searching can be studied is by developing a simulation model of the searching behaviour of the parasitoid (e.g van Roermund *et al.*, 1996, 1997a, b). For assessing the effect of separate factors on encounter probability, individual-based models are indispensable. Searching can be split in several components, which can be measured in the laboratory studies. In models of *Trichogramma* that are based on the responses of entire populations instead of individual parasitoids (Knipling and McGuire, 1968; Kanour and Burbutis, 1984; Goodenough and Witz, 1985), a single parameter for searching efficiency is used which is difficult to determine.

Based on observations of *T. brassicae* searching behaviour, a model was developed for a parasitoid searching on a single maize plant (Chapter 8), which simulates the encounter probability of *T. brassicae* with *O. nubilalis* egg masses. In this chapter, the model is extended to simulate the searching of *T. brassicae* females in a maize crop during one day. This model is used to study how parasitoid behavioural parameters, host density and distribution, plant size and temperature affect the host encounter probability in the field.

THE MODEL FOR SEARCHING IN A CROP

Searching in the field by a parasitoid consists of a series of plant visits, which each consist of one or more leaf visits. The model for searching in a crop describes the searching behaviour of a female *T. brassicae* in a maize crop during one day from sunrise to sunset, and assumes that the parasitoid will not leave the field during this time. The model first draws the number of host egg masses and moth scale patches on the first plant from a Poisson distribution, based on an average distribution of hosts and scales found in the field (Chapter 2). The model for searching on a plant, as described in Chapter 8, is then used to simulate the visit (Figure 1), which consists of searching on one or more leaves of the plant. Visits to a plant can be terminated in three ways: 1. when the parasitoid encounters an egg mass (which also ends the simulation) 2. when the parasitoid flies away from the plant or 3. when the parasitoid is standing still for the rest of the plant visit. If no egg mass is encountered on the plant and the end of the simulation time is not reached, the parasitoid will make another plant visit. The parasitoid can fly away immediately from the first plant,

or it can stand still for a while. If it flies to another plant, the number of egg masses and scale patches on the leaves of the new plant are calculated and the parasitoid starts searching on a new leaf.
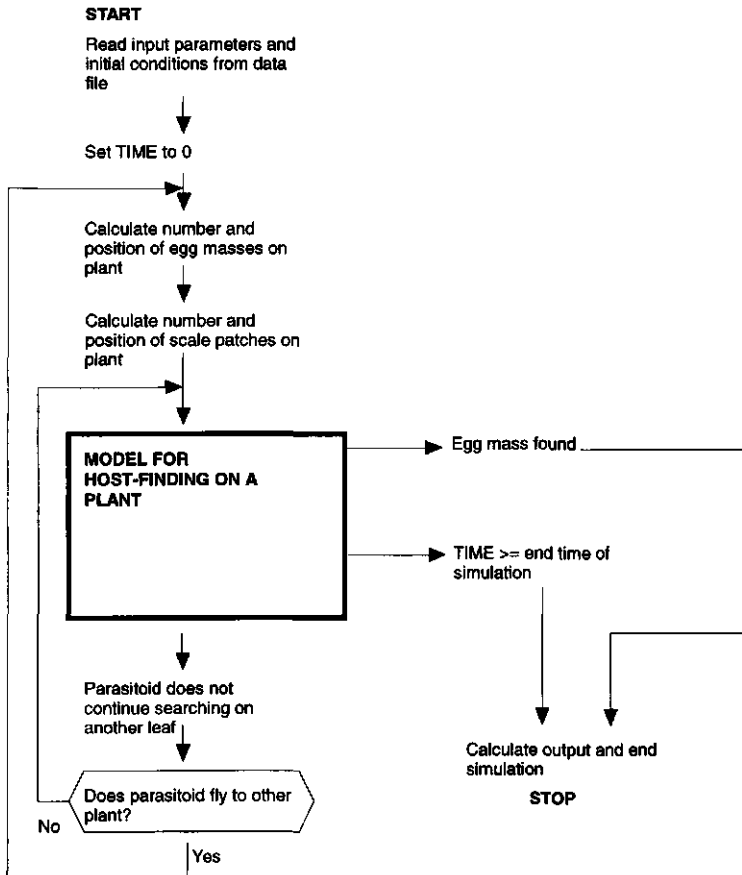

Figure 1.
Flowchart of the model simulating *Trichogramma brassicae* host searching in a maize crop.

In the model for searching in a crop, the location of the plants visited and the distance, direction and duration of between-plant movement are not taken into account. There are several reasons why this simplification is justified. In the first place, *O. nubilalis* egg masses are randomly distributed in the field (Hawlitzky *et al.*, 1994). This means that the chance that egg masses or scales are present on a plant does not depend on its position in the field (Chapter 2). In the second place, *T. brassicae* has a flight speed of about 2 m/s (Steenburgh, 1934), and most flights within the crop seem to be short (Chapter 4), so the duration of within field flights is negligible compared to the time spent walking and standing still on the plant. In the third place, the height at which the parasitoid leaves one plant by flight does not influence the height at which it lands on the next plant (Chapter 5). The simulation is finished when an egg mass is encountered or when the "end of the day" is reached (14-16 hours).

-Chance to fly away after a plant visit
If the parasitoid has finished searching, it can either stand still or fly away. At high temperatures, standing still for longer periods (>20 minutes) only occurs on the first leaf that is visited. At low temperatures (<20°C), most wasps stand still for most of the time. The wasps that visit more leaves by walking (i.e. the most active ones) appear to be the ones that are most likely to fly away to another plant (pers. observation). To quantify the chance that a parasitoid flies away to another plant, the residence time experiments in Chapter 6 were used. Wasps that ended their plant visit by standing still were given the value 0 and wasps that flew away the value 1. The number of leaves visited was used as x-value. Logit regression curves were then fitted to the data for each temperature, using iterative least square estimation. The chance to fly away after a plant visit increases with temperature and with the number of leaves visited on the plant.

$$CFLY(N) = e^{(A+B*N)}/1+e^{(A+B*N)}$$

$CFLY(N)$ = chance that a wasp flies away if plant visit is terminated after visiting the Nth leaf
A, B: temperature dependent variables
N: number of leaves visited during the current plant visit

$A = 20.647-1.236*TEMP$
$B = -29.735+1.664*TEMP$
TEMP: temperature (°C)

-Walking speed and walking activity
The influence of temperature on the parasitoids walking speed and walking activity were determined in Chapter 7. Walking speed increases linearly with temperature, while walking activity shows a logistic increase reaching a maximum at 25°C.

ACT = 0.95 / (1 + 69 x 0.69$^{(TEMP-6.64)}$)
WS = -1.08 + 1.02 x TEMP

ACT: Walking activity (time spent walking divided by total time)
WS: Walking speed (cm/minute)
TEMP: temperature (°C)

-Egg mass and kairomone distribution on the plant
Since *O. nubilalis* oviposits at random in the field (Hawlitzky *et al.*, 1994), the number of *O. nubilalis* visiting a plant in the model can be drawn from a Poisson distribution. On average, an *O. nubilalis* female visits 5.4 plants before laying an egg batch, with an mean of 1.7 landings on each plant visited (Chapter 2). With this data, the number of landings on the plant and their leaf position is calculated for each of the visiting moths. Finally, for each landing it is calculated whether scales are left and whether oviposition occurred, based on data from Chapter 2, to find the position of scale patches and egg masses on the plant.

SIMULATIONS

Encounter probability in the field

To quantify the encounter probability of *T. brassicae* with an egg mass in the field, searching was simulated at a host density of 0.1 egg mass per plant, maize plants that each had nine leaves with a total leaf area of 3814 cm² and a temperature curve based on mean temperatures measured in the field during the 1993 *Ostrinia* oviposition period. The concentration of scale patches is 0.14 per plant, 0.06 directly around egg masses and 0.08 not in the immediate vicinity of egg masses. These conditions were also used for the sensitivity analysis. 5000 iterations were run for each simulation.

Sensitivity analysis

The effect of single input parameters of the parasitoid, the host, the host plant and the environment on the encounter probability was simulated. The results were compared to the control using a G-test (Sokal and Rohlf, 1995). If multiple comparisons were made, the significance level was adjusted using the Bonferroni correction.

Parasitoid parameters
A sensitivity analysis of parasitoid parameters for the crop model was performed at the same conditions as for calculating the encounter probability. The effects of the following factors on the encounter probability were tested: width of the searching path of the parasitoid (WIDTH), walking speed (WS), walking activity (ACT), chance to land on the lower leaf side (LSIDE), residence time on first leaf (RTL1), residence time on second and subsequent leaves (RTL2), residence time on the lower leaf side (RTLOW), residence time on the upper leaf side (RTUP), chance to fly to another plant at the end of a plant visit (CFLY) and

chance to walk to another leaf (CWALK). Ten factors that describe the direction and duration of walking movements between leaves were also tested; these are described in detail in appendix 3. The chance to land on the lower leaf side (LSIDE), and the residence times on first (RTL1) and subsequent (RTL2) leaves were both increased and decreased by 25%. All other factors were increased by 25% in the sensitivity analysis.

Effect of different reactions of T. brassicae to host scales
In the model, the reactions of the parasitoid after encountering a scale patch are simulated in four ways: 1. by increasing the residence time on the current leaf side, 2. by increasing the residence time on the current leaf, 3. by increasing the chance to visit another leaf, 4. and by increasing the rate of encounter of an egg mass, if the scale patch is around the egg mass, by adding the scale patch diameter to the egg mass diameter. To study the effect of variation in these parameters on the encounter probability in the crop, they were increased and decreased by 25%. The simulation setup was the same as in the previous sensitivity analysis.

Oviposition behaviour of O. nubilalis
The number and position of *O. nubilalis* egg masses and scale patches used were based on data collected in the field and the laboratory (Chapter 2). The parameters determining the position and the number of egg masses and scale patches were increased or decreased to study what impact host parameters have on the encounter probability in the field.
- Egg mass distribution over the leaf levels (ODIS). Synchronisation in the distribution of landings by *T. brassicae* over the leaf levels and distribution of *O. nubilalis* oviposition over the leaf levels had a strong effect on the encounter probability in the model for searching on the plant, because it determined how much of the time the parasitoids were searching on leaves where egg masses could actually be found. To study what impact changes in the distribution of ovipositions over the leaf levels by *O. nubilalis* had on the encounter probability, the mean leaf level on which *O. nubilalis* oviposited was shifted one level up and one level down.
- Chance for *O. nubilalis* to land on lower leaf side (LSIDEON)
Egg masses of *O. nubilalis* are mainly found on the lower leaf side in the field (Chapter 2), caused by *O. nubilalis* landing more often on the lower leaf side than on the upper side according to our observations. To see the effect on the encounter probability if *O. nubilalis* lands more often on the lower leaf side, the chance to land on the lower leaf side was increased by 25%.
- Number of plant visits per oviposition (VISEGG). This parameter was based on insectary observations. If the number of plants visited per oviposition by *O. nubilalis* increases, there will be more plants in the field with scales but without egg masses. To see which effect this has on the encounter probability, the number of plants visited per oviposition was increased by 25%.

-Chance that scales are left on the plant after oviposition (CSCALEOV)
In our experiments, 63% of the egg masses had some moth scales within three centimeter of the edge of the egg mass (Chapter 2). To see how strong the encounter probability is influenced by these scales, the percentage of egg masses with scales was increased and decreased by 25%.
-Chance that scales are left on the plant at landings without oviposition (CSCALE)
As the model for searching on the plant has shown (Chapter 8), scale patches on the same leaf and the same leaf side as an egg mass had a strong effect on the parasitoid's encounter probability. Scale patches on uninfested leaves had little effect, and might even be detrimental in the field, because they could cause the parasitoids to spend more time on leaves and plants without egg masses. In the insectary observations, only one percent of the landings without oviposition resulted in scales on the leaf (Chapter 2). While *O. nubilalis* makes 8.25 other landings for each oviposition, the result is that 45% of the scales are close to an egg mass and 55% are not. To study the effect of scale patches that are not close to egg masses on the encounter probability, the chance to leave scales at a landing where no oviposition occurred was increased and decreased by 25%. An increase of CSCALE of 25% will mean that 60% of the scales are not close to egg masses, and a decrease of 25% will mean that 50% of the scales are not close to egg masses.
- Diameter of scale patches (PATCHDM)
Scale patches have different sizes and shapes (Chapter 2). In the model, it was assumed that all patches had the same size and were round. To see what effect an increased diameter of scale patches (both those around an egg mass and those in other places) had on the encounter probability, the diameter was increased by 25%.

Host egg mass density
Densities of *O. nubilalis* egg masses in Eastern Switzerland show a high variation (Bigler, unpubl.). The highest host density of 1982-1987 (0.4 egg masses per plant) was found in 1982. Host densities from 0.05 to 0.4 were used to simulate the effect of host density on the encounter probability.

Plant sizes
Maize plants have only three or four leaves at the beginning of the *O. nubilalis* oviposition period, but at the end of the oviposition period they can have up to 15 leaves. Leaf surface areas can increase more than tenfold. To study what effect this has on the encounter probability, five plant sizes from the 1993 *Ostrinia* oviposition period (May 23th, June 6th, July 4th, July 18th and August 2nd) were used as input for the simulation model (Garibay, unpubl.; Schubiger, unpubl.). The plant size used for the previous analyses (3814 cm^2) was used as a control.

Temperature
To show the effect of temperature in the field on the encounter probability, six leaf temperature curves were chosen to compare with the mean leaf temperature curve of seven field seasons. During the morning, leaf temperature is just above the air temperature. In the afternoon, it sinks below the air temperature (Singh and Jacobs, 1995). Air temperature data

recorded at the Swiss Federal Research Station were used to estimate the leaf temperature. Each day of the *O. nubilalis* oviposition period of 1985, 1986, 1987, 1988, 1990, 1992 and 1993 was ranked according to their mean leaf temperature during the day. The mean curves of the four quartiles were used as input data. In addition, leaf temperature from the days with the highest mean temperature (August 9th, 1992) and lowest mean temperature (July 9th, 1993) were used to study the effect of these extremes.

SIMULATION RESULTS

Encounter probability in the field

The simulated encounter probability of *T. brassicae* in the field was 0.057 ± 0.230 at a host density of 0.1 egg mass per plant, which means that about 94% of the females searched a whole day without encountering an egg mass. The mean number of encounters with scale patches was 0.54 ± 2.02.

Sensitivity analysis

Parasitoid parameters
The results a change of the first ten parameters are shown in Figure 2. None of the parameter changes resulted in significant changes of the encounter probability compared to the control (G-test). Increasing the width of the searching path (WIDTH), walking speed (WS), chance to land on lower leaf side (LSIDE+), and residence time on the lower leaf side (RTLOW) increase the encounter probability, while increasing residence time on the upper leaf side (RTUP) and chance to fly to another plant at the end of a plant visit (CFLY) decreased the encounter probability. Increasing the walking speed and the width of the searching path affect the chance that a parasitoid encounters an egg mass on the leaf side where it is searching, and increasing the chance to land on the lower leaf side and the residence time on the lower leaf side will make the parasitoid spent more time on the lower leaf side where the egg masses are present. Increasing the residence time on the upper leaf side will make the parasitoid spend more time on the leaf side without egg masses, and thus reduce the encounter probability.

Increasing the residence time on the first leaf visited (RTL1+) decreased the encounter probability, while decreasing the residence time on the first (RTL1-) and subsequent (RTL2-) leaves increased it. If the parasitoid stays shorter on each leaf, the chance to encounter an egg mass on that leaf is decreased, but it can visit more leaves and plants during the day. The net result is, according to the simulation, that the increase in the encounter probability as a result of more leaf and plant visits is stronger than the decrease caused by the lower encounter probability on each leaf. Increasing the walking activity (ACT) did not affect the encounter probability, probably because most egg masses are encountered in the warm part of the day when activity is high anyway.

Variation in the parameters that determine movement between leaves did not change the encounter probability significantly. Increasing the chance to move to other leaves while searching on the plant (CWALK) will cause the parasitoid to search more leaves per plant, which results in an increased plant residence time and a decreased number of plants visited

during the day. This decreases the encounter probability. This is consistent with the effect of increasing residence times on leaves.
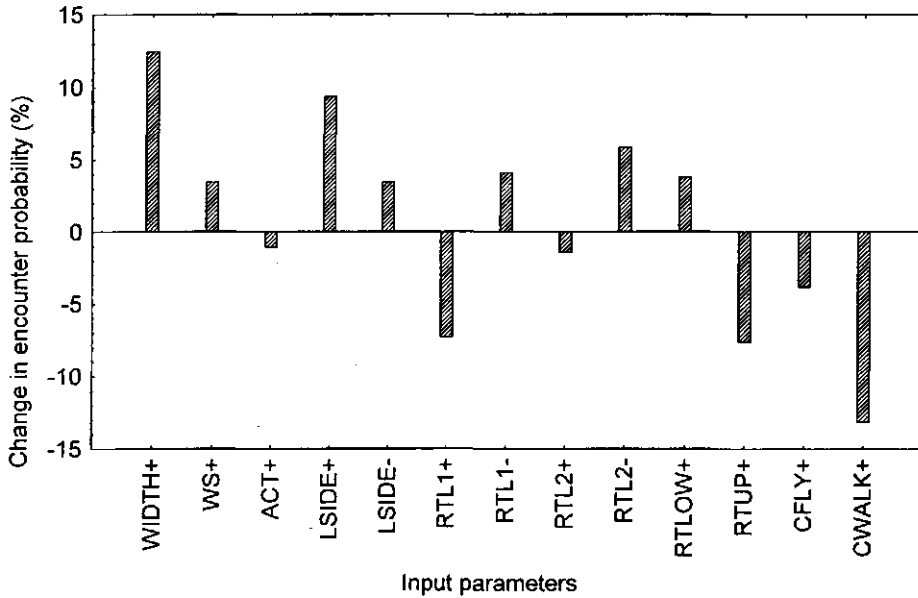
Figure 2.
Effect of *T. brassicae* parameters on the encounter probability. Parameters tested: width of the searching path of the parasitoid (WIDTH), walking speed (WS), walking activity (ACT), chance to land on the lower leaf side (LSIDE), residence time on first leaf (RTL1), residence time on second and subsequent leaves (RTL2), residence time on lower leaf side (RTLOW), residence time on upper leaf side (RTUP), chance to fly away at the end of a plant visit (CFLY) and chance to walk to another leaf (CWALK).
Chance to land on the lower leaf side (LSIDE), and residence time on first (RTL1) and subsequent (RTL2) leaves were both increased (+) and decreased (-) by 25%. All other factors were increased by 25% only.

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Effect of different reactions of T. brassicae to host scales
Changing the input parameters that describe the parasitoid's reaction to scale encounters by 25% did not cause significant changes in the encounter probability in the field (Figure 3). Increasing residence time on the leaf after encountering scales (IRTL+) did cause an increase in the encounter probability, while decreasing residence time on the leaf (IRTL-) decreased the encounter probability. Increasing the chance to visit another leaf (ICWALK), resulting in more leaves visited per plant. Decreasing the diameter of the scale patch (PATCHDM), making egg masses and scales patches smaller, also showed some effect on the encounter probability.
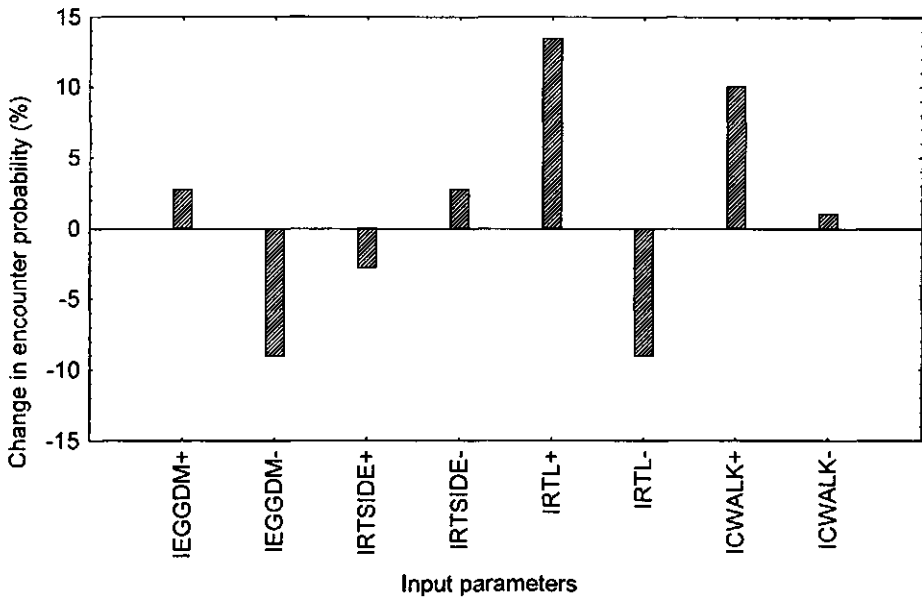
Figure 3
Effect of *T. brassicae* reactions to scales on the encounter probability. Scale reactions tested: increase in diameter of egg mass (IEGGDM), increase in residence time on leaf side (IRTSIDE), increase in residence time on leaf (IRTL) and increase in chance to visit another leaf (ICWALK). All four parameters were increased (+) and decreased (-) by 25%.

Oviposition behaviour of O. nubilalis

The effects of *O. nubilalis* oviposition behaviour on the encounter probability are shown in Figure 4. The distribution of ovipositions over the leaf levels (ODIS) had a significant impact on the encounter probability. The more similar the distribution of landing by the parasitoid and oviposition by the host over the leaf levels is, the higher the encounter probability. The change for *O. nubilalis* to leave scales at oviposition (CSCALEOV) also had a significant effect on the encounter probability. Egg masses with scales in the direct vicinity are much easier to find. The ratio scales close to egg masses: scales not close to egg masses was 45:55. By increasing or decreasing the chance for *O. nubilalis* to leaves scales at landings without ovipositions (CSCALE), the ratio became 40:60 or 50:50. This had hardly an effect on the encounter probability. Scale patches that are not close to an egg mass did neither help or hinder the parasitoid searching for egg masses in the field.
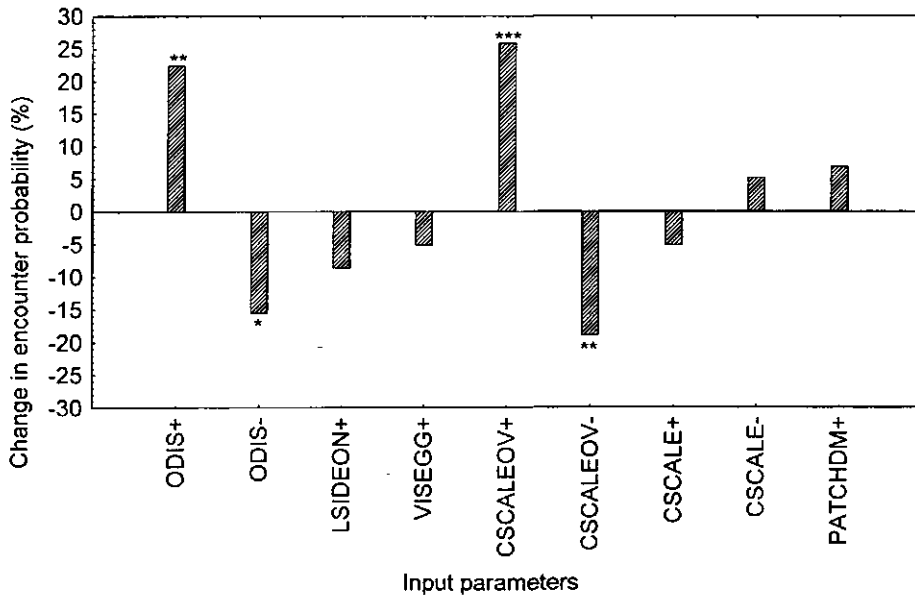
Figure 4

Effect of some aspects of *O. nubilalis* oviposition behaviour on encounter probability of *T. brassicae* with egg masses. Parameters tested: oviposition distribution (ODIS), chance for *O. nubilalis* to land on the lower leaf side (LSIDEON), number of plant visits per oviposition (VISEGG), chance that scales are left on the plant after oviposition (CSCALEOV), chance that scales are left on the plant at landings without oviposition (CSCALE) and diameter of scale patch (PATCHDM). Oviposition distribution was increased (+) or decreased (-) by one leaf level. The chance that scales are left on the plant after oviposition (CSCALEOV) and the chance that scales are left on the plant at landings without oviposition (CSCALE) were increased (+) and decreased (-) by 25%. All other parameters were increased by 25%. Asterisks indicate significant changes compared to the control (G-test, *: P<0.05, **: P<0.01, ***: P< 0.005)

Host egg mass density

The host density determines on how many of the leaves on which *T. brassicae* searches, eggs masses are present. There was a good linear correlation between host density and the encounter probability in the host density range simulated (Figure 5). All the encounter probabilities were significantly different from those at the next higher or lower density ($P<0.05$, G-test).
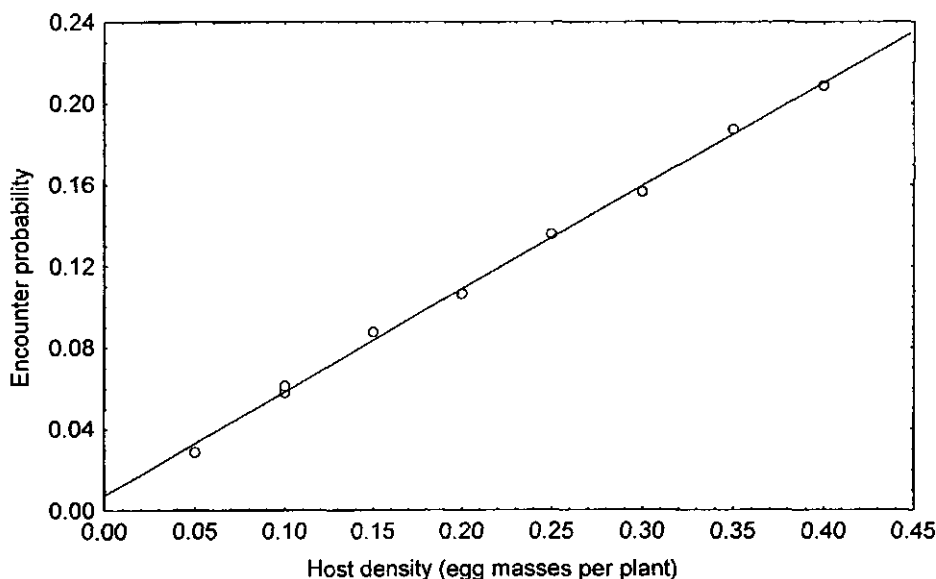
Figure 5

Effect of host density on encounter probability of *T. brassicae*. Significantly different encounter probabilities were obtained at all densities (G-test, $P<0.05$).

Plant size

On the smallest plants tested, the encounter probability was much higher than that found on the control plants, which was the plant size used in the sensitivity analysis (Figure 6). During May-July, the increase in leaf area (50-3000 cm^2, 3-15 leaves) leads to a strong decrease in the encounter probability. There was no significant difference (G-test with

Bonferroni correction) in the encounter probability on the plants of July 4th and July 18th, with a leaf area of 2840 and 4102 cm^2 leaf area, respectively. The encounter probabilities on the plants of the control and on the plants of July 18th and August 2nd, with leaf areas of 3814, 4102 and 4540 cm^2 were also not significantly different. As the fitted curve in Figure 6 shows, there is an exponential relationship between the leaf area and the encounter probability.
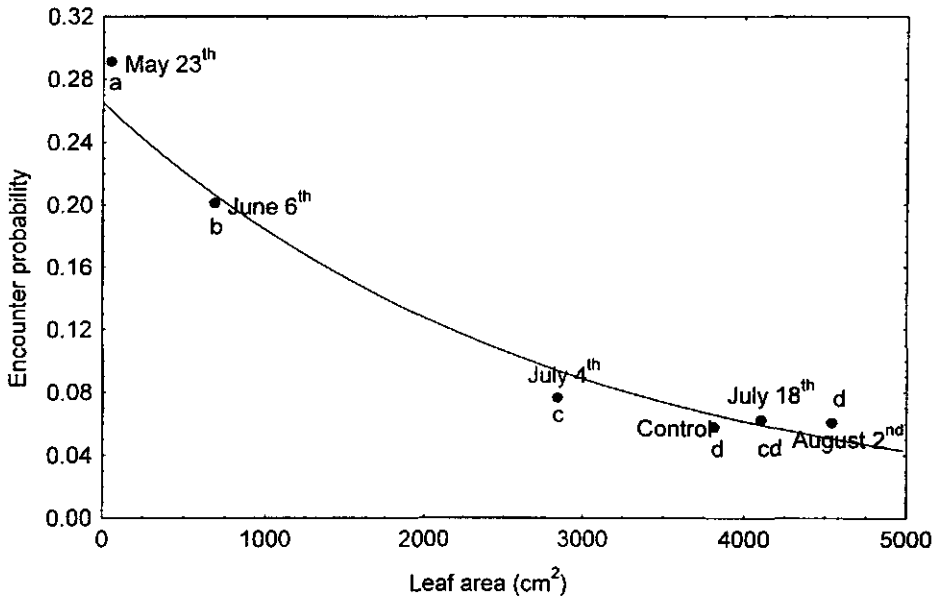
Figure 6
Effect of plant size on the encounter probability of *T. brassicae*. Maize plant sizes were measured during five days of the 1993 *O. nubilalis* oviposition period. The control is the plant size used in the sensitivity analysis. Different letters indicate significant differences (G-test with Bonferroni correction, P<0.05). Fitted curve: Encounter probability = 0.265 x e(Leaf area of plant x -3.7 x 10^{-7})

Temperature
Temperature had a strong effect on the encounter probability (Figure 7). It affects many of the input parameters of the model, like walking speed, walking activity, residence time on leaves, residence time on leaf sides, and the chance to fly away to other plants. The encounter probability was strongly correlated with both mean daytime temperature (Pearson product-moment correlation 0.9890/P<0.001) With an mean temperature curve from the

lowest quartile, the encounter probability is about 0.02, while an mean temperature curve from the highest quartile results in an encounter probability of 0.09. The encounter probability of the lowest and highest quartile were significantly different from the control ($P<0.05$, Bonferroni corrected G-test). The encounter probability of the fourth quartile was not significantly different from that of the third quartile ($P=0.1$). With the rapid temperature changes that are common in temperate climates, the encounter probability can increase or decrease fourfold in a couple of days. The daytime temperature sum is also strongly correlated with the encounter probability (Pearson product-moment correlation 0.9770/$P<0.001$).
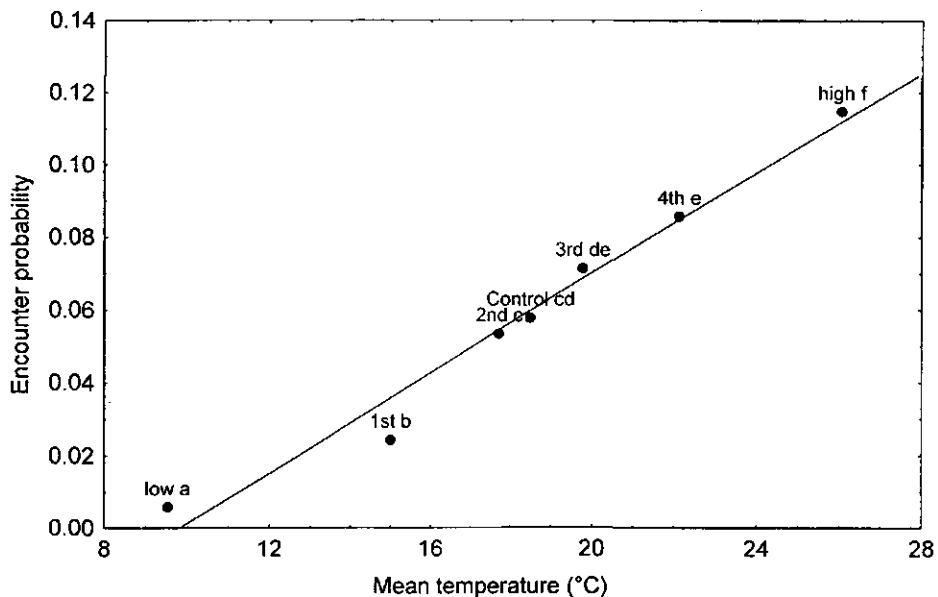
Figure 7
Effect of different leaf temperatures on the encounter probability of *T. brassicae*. Temperatures simulated: lowest temperature quartile (1st), second temperature quartile (2nd), third temperature quartile (3rd), highest temperature quartile (4th), mean temperature of 1993 (control), highest mean temperature and temperature sum (August 9th, 1992) (high), and lowest mean temperature and temperature sum (July 9th, 1993)(low). Different letters indicate significant differences (G-test with Bonferroni correction, $P<0.05$).

DISCUSSION

According to the model, the encounter probability of *T. brassicae* with *O. nubilalis* egg masses is only 0.06 under circumstances normally found in Swiss maize fields.

With a 16 hour day and 17 eggs per egg mass, this gives an host encounter rate of 0.06 eggs per hour. Van Roermund and van Lenteren (1997) found that *Encarsia formosa*, which has to find hosts on a leaf by random searching like *T. brassicae*, had a high host encounter rate on tomato leaflets with hosts (3.0 hosts per hour). In a greenhouse however, where the host, the whitefly *Trialeurodes vaporariorum*, was distributed in small clusters, only one percent of the parasitoids were searching on leaves with hosts (van Roermund *et al.*, 1997c). The resulting host encounter rate in the greenhouse was very low, 0.02 hosts per hour. In a natural habitat, the rate of encounter of parasitoids may also be low, if they have to spend most of their time locating areas with high host density. Host encounter rates of parasitoids in the field have rarely been studied, because direct observation of small, flying parasitoids is very difficult. Janssen (1989) studied host-finding behaviour of the Eucoilid and Braconid parasitoids *Leptopilina heterotoma* and *Asobara tabida* in the field. The host encounter rate with larvae of Drosophilids was 1.7 hosts per hour. This rate of encounter is much higher than that of *T. brassicae* or *E. formosa*, but the observations were made in an microhabitat with a high host density, so the time to locate this habitat was not included.

With an encounter probability of 0.06, only half of the parasitoid population would have encountered at least one egg mass after eleven days. In the laboratory *T. brassicae* females live 10-15 days (Bigler *et al.*, 1987). Longevity in the field is unknown, but expected to be shorter. Absence of oviposition increases longevity (Hohmann *et al.*, 1988), but lack of food (Bourarach and Hawlitzky, 1989) and environmental hazards will reduce it. *T. brassicae* is quite mobile and even if it lives for ten days or longer in the field, many parasitoids will have left the release field by then (Bigler *et al.*, 1990).

Because encountering egg masses is a rare event, a high host acceptance is important for successful biological control. Based on foraging theory (Godfray, 1994), the parasitoid should accept the first egg mass it encounters, since it is unlikely to find another egg mass. If the parasitoids are not egg-limited during their lifetime, a decrease in host acceptance will result in a decrease in parasitism. The main reason for the decline in efficacy of *T. brassicae* in Swiss maize fields was a strong decline of acceptance of *O. nubilalis* eggs because the released wasps had been reared for too many generations on a factitious host (Bigler *et al.*, 1982; van Bergeijk *et al.*, 1989). To extrapolate host acceptance ratios from lab experiments into the field is difficult, since the methods used to check host acceptance in the laboratory usually involve confining the parasitoid in a small tube with the eggs (Wäckers *et al.*, 1987; Frei and Bigler, 1994; van Lenteren *et al.*, 1994). The parasitoid will encounter the egg mass almost immediately, and many re-encounters will occur. In the field the parasitoid will encounter egg masses only after a long period of searching. For other parasitoids it was found that host-encounter interval and egg-load of the parasitoid determine host acceptance (Minkenberg *et al.*, 1992). If this is true for *T. brassicae*, then host acceptance in the field is likely to be high.

The encounter probability in a crop was not sensitive to parasitoid parameters such as width of the searching path, walking speed, walking activity, residence times, and chances to fly to other plants or to walk to other leaves. Bigler *et al.* (1988) tested *T. brassicae* strains with different walking speed in the field and found an effect on parasitism. However, the differences in walking speed between the strains tested were as high as 100%, and there may have been other quality differences (e.g. host acceptance) between the strains used. A quality index based on walking speed and host acceptance was made for *T. brassicae*. Parasitoids with a higher quality index gave higher parasitism in the field (Dutton *et al.*, 1996). Compared to the plant model (Chapter 8), the direction of the effect of the changes in the model for searching in a crop was the same for width of the searching path, walking speed, chance to land on the lower side, and residence times on the upper and lower leaf sides. An opposite effect was found for the residence time on leaves. Increasing the residence time on leaves will increase the chance to encounter an egg mass on a single plant, because the parasitoid has more time to search on that plant, but decrease the chance to encounter an egg mass in the field, because less plants can be visited, and most plants have no egg masses at all. Thus, making short visits to many plants leads to a higher encounter probability than searching less plants more thoroughly.

Of the host behaviour parameters, the effect of the distribution of *O. nubilalis* landings over the leaf levels, with its resulting effect on oviposition distributions, on encounter rate was the strongest. If the parasitoids mainly land on the leaf levels where most of the egg masses are present, the encounter probability will be highest. In the field, most *O. nubilalis* egg masses are found on the middle leaf layers (Chapter 2), but the leaf level with most of the egg masses can vary (Klinger, 1979). Hot and dry weather leads to more egg masses on the lower leaves (Chiang and Hodson, 1972; Despins and Roberts, 1986; Ohnesorge and Reh, 1987). The distribution of *T. brassicae* landings over the leaf levels is fairly constant (Chapter 5). If humidity and temperature cause changes in the oviposition level of *O. nubilalis* but not in the landing level of *T. brassicae*, an impact on the encounter probability would result.

Increasing the number of egg masses with host scales around them increases the encounter probability. An egg mass with scales has a significantly higher chance to be encountered than one without (Chapter 6; Chapter 8). This is confirmed by the crop model. Variation in the number of scale patches that were not close to an egg mass had no significant effect on the encounter probability. About 55% of the scale patches encountered on a plant are more than three centimeter away from an egg mass (Chapter 2). Apparently, the time lost by arrestment caused by these patches has no negative effect on the encounter probability.

Host densities are normally around 0.1 egg masses per plant in Switzerland. A strong positive linear correlation was found between host density and encounter probability when host density varied between 0.05 and 0.4 egg masses per plant. The chance for an egg mass to be encountered by at least one parasitoid during one day with a *T. brassicae* release of 60.000 females per hectare (0.6 females per plant) is 0.32 at a host density of 0.05 egg masses per plant and 0.27 at a host density of 0.4 egg masses per plant, according to the model. A 700% increase in host density will only cause a 15% decrease in the chance that an egg mass is encountered by at least one parasitoid. If a single encounter of a parasitoid

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with an egg mass is enough to parasitize the whole egg mass, parasitism is equivalent to the percentage of egg masses found. In this case, there is an insignificant inverse density dependence (lower percentage parasitism at higher host densities) in the field. The number eggs of *T. brassicae* is only enough to parasitize one or two egg masses. At higher host densities, some of the egg masses will be found by wasps that do not have enough eggs left to parasitize the whole egg mass. This would increase the inverse density dependence of parasitism by *T. brassicae*. Data of *T. brassicae* parasitism of *O. nubilalis* collected in Switzerland from 1980 to 1992 did not show any density dependence (Bigler, unpubl.). Inverse density dependence was also found in small scale experiments for *T. papilionis* on *Papilio xuthus* (Hirose *et al.*, 1976) and *T. pretiosum* on *Heliothis zea* (Morrison *et al.*, 1980). In a large field experiment, however, *T. pretiosum* showed direct density dependence on eggs of *H. zea* (Gross *et al.*, 1984).

Several authors studied the effect of plant size on *Trichogramma* parasitism (Need and Burbutis, 1979; Ables *et al.*, 1980; Burbutis and Koepke, 1981; Kanour and Burbutis, 1984; Maini *et al.*, 1991). In all cases, growth of the crop leads to lower parasitism rates. Kanour and Burbutis (1984) made a simple model to calculate the number of parasitoids to be released for a desired level of parasitism by *T. nubilale* against *O. nubilalis* in maize crops. They assumed that the level of parasitism decreased linearly with crop size, so their release strategy is based on a constant number of wasps per unit leaf area. However, our model shows an exponential rather than a linear relationship. When plants are large (from July onwards), a further increase in leaf area does not have an effect on the encounter probability.

Temperature had a strong effect on the encounter probability. The encounter probability on hot days can be four times as high as on cold days. This is not unexpected as many of the factors that influence searching of *T. brassicae* are temperature dependent, such as walking speed, walking activity, residence times and flight propensity. Bourchier and Smith (1996) found that temperatures and rainfall in the first three days after release can explain 75% of the variation in oviposition of *T. minutum* on *Choristoneura fumiferana* in a forest habitat. *T. brassicae* also shows a significant correlation ($r = 0.56$) between temperature sum above 18°C and parasitism (Dutton *et al.*, 1996).

The model simulates the chance to encounter at least one egg mass, but an estimation of the mean number of different egg masses encountered per wasp during its lifetime can be made. It was observed that *T. brassicae* encountered the same egg mass several times within minutes, before the parasitoid started to oviposit (Suverkropp, unpubl.). The parasitoid is still close to the egg mass after leaving it, and has a very tortuous walking pattern after leaving an host egg (Gardner and van Lenteren, 1986). However, the chance to encounter a new egg mass is probably not increased by the first encounter, since the positions of egg masses are independent of each other. Thus, we can assume that the chance of the parasitoid to encounter a second egg mass is lower than for the first egg mass, especially as finding and examining or ovipositing the first egg mass takes time. Most of the egg-load of *T. brassicae* will be spent after parasitizing an egg mass, and parasitoids with a low egg-load are usually less active (Minkenberg *et al.*, 1992). If the daily encounter probability is 0.058, and 60.000 females are released per hectare maize with a host density of 0.1 egg masses per plant, the chance of an egg mass to be visited at least once during a day is 31%. After 5 days, the

chance that the egg mass is visited at least once is 84%. *T. brassicae* usually parasitizes most of the egg mass it encounters. If host acceptance is high, 84% of all egg masses are parasitized. This is very similar to the 80% rate of parasitism levels found in the field after *T. brassicae* releases (Bigler 1983; 1986).

To summarize, the following conclusions were drawn from the simulation of *T. brassicae* searching for egg masses in a maize crop: The influence of variation in each parasitoid parameter describing the searching process on the encounter probability is not strong, but temperature and plant size have a strong effect on the encounter probability. The encounter probability is density dependent. Within the range of host densities and the numbers of parasitoids released in Switzerland, the chance for egg masses to be found by *T. brassicae* is density independent, and parasitism is likely to be density independent also. Host encounters are relatively rare, but even this low encounter probability of 0.058 results in levels of parasitism comparable to those found in the field.

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Chapter 10

Summarising discussion

INTRODUCTION

Trichogramma brassicae, Ostrinia nubilalis **and maize**

The parasitoid wasp *Trichogramma brassicae* Bezdenko (Hymenoptera, Tricho-grammatidae) can be reared on the eggs of many lepidopteran species. This makes the species very suitable for inundative biological control of lepidopterans, since it can be cheaply mass-produced on eggs of stored product pests like *Sitotroga cerealella* or *Ephestia kuehniella. T. brassicae* is, among others, commercially produced for the biological control of the European corn borer in maize in Switzerland, Germany and France.

      The European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera, Pyralidae) is an important insect pest in maize in most countries of northern hemisphere. *O. nubilalis* can reproduce on many host plants, but its preferred host is maize, and the large scale cultivation of maize resulted in its rapid spread over large areas. Because the larvae spend most of their life inside the plant, chemical control is not easy, and there has always been interest in alternative control. In Switzerland, *O. nubilalis* is controlled with a combination of cultural techniques and inundative releases of *T. brassicae*.

Problem definition

Biological control of *O. nubilalis* with *T. brassicae* has been quite successful, but most of the techniques have been developed by trial and error methods. Little is known about the actual searching behaviour of the parasitoid in the field. Most experiments were either made at a field scale or in small laboratory setups. In field experiments, only levels of parasitism are measured, which gives limited information about the searching behaviour, as parasitism is the result of many connective processes. Laboratory experiments in petri dishes and olfactometers give precise quantitative data on some aspects of searching, but not about the behaviour on plants and in the field. Previous research has shown that the level of parasitism is influenced by many parasitoid, host, host plant and environmental factors, but the effect of these factors in a field environment is at best known in a qualitative way. This is an impediment for the improvement of *T. brassicae* biological control, and the development of quality control and parasitoid selection methods.

      The aim of this study was therefore to analyze the searching behaviour of *T. brassicae* in maize fields. In laboratory, greenhouse, insectary and field experiments, data were collected on dispersal, landing and host-finding of *T. brassicae*. These data were integrated in an explanatory simulation model. Using this model, the qualitative and quantitative effect of parasitoid, host, host plant and environmental factors on searching efficiency were estimated. Based on this, recommendations for biological control and quality control were made.

FIELD AND LABORATORY STUDIES WITH *O. NUBILALIS* AND *T. BRASSICAE*

O. nubilalis oviposition behaviour

There was sufficient information on the distribution of *O. nubilalis* egg masses over the crop and at the leaf level, but more detailed information on the distribution on the plant was lacking. There was also no information on the distribution of host-cues like scales of the moth on the plant. In **Chapter 2**, the oviposition behaviour of *O. nubilalis* was reviewed. Egg masses were collected in the field to determine the within-plant and within-leaf distribution. Landing and oviposition behaviour of *O. nubilalis* was also directly observed in cages and an insectary, and maize plants were inspected for visible host-cues (scales). Based on these experiments the following conclusions were drawn:

-*O. nubilalis* egg masses are mainly found on the lower leaf side of the middle leaves. Most egg masses are laid close to the mid-rib. A large proportion of egg masses are found on the middle section of the leaf relative to the plant stem, and oviposition near the tip of the leaf is rare.

-There is a close correlation between landing and oviposition sites. Few *O. nubilalis* move over the plant by walking, and those that do walk do so only for distances of three centimetre or less. This means that the distribution of egg masses also indicates the distribution of landing sites over the plant. Although landing and oviposition sites are similar, the number of landings is 9.3 times as high as the number of ovipositions. Abdominal probing suggests that *O. nubilalis* measures the quality of the host plant after landing.

-Because *O. nubilalis* does not walk over the plant, non-volatile host-cues will be spotwise distributed over the plant. Scales are found singly or in small dense patches (80%) or spread over parts of the leaf (20%). 63% of the egg masses have scales within three centimetres. These scales close to an egg mass account for 45% of the scales. The other scales (55% of the total amount) are not in the vicinity of an egg mass. Scales in the field will indicate that there probably is an egg mass on the plant or on the leaf, but this egg mass might not be close to the point where the scales are encountered.

Dispersal of *T. brassicae* in maize

In **Chapter 3** the aspects of *Trichogramma* behaviour that are most relevant to the host-finding process were reviewed, with an emphasis on *T. brassicae* behaviour in maize. *O. nubilalis* egg masses are randomly distributed over a maize field. *T. brassicae* is released at a limited number of points in a field, so the wasps initially have a clustered distribution in the field. The main mode of movement (flight, short jumps, or walking) between plants in the field was unknown. There was information available on the speed of dispersal, but much of the information was conflicting, and based on sampling of parasitized egg masses. Parasitism is of limited use in studying movement, because a parasitized egg mass proves that a wasp was present, but an unparasitized egg mass does not prove that wasps were not present. Glue-sprayed plants were used to study the initial flight distance of *T. brassicae*

and its dispersal speed in the field (**Chapter 4**). The following conclusions were drawn from the experiments:

-Most of the females make their first landing very close to the release point. Mean initial flight distance is only 60 cm.

-The number of wasps recaptured decreases sharply with distance on the first day of release within the sampling area (a circle with a diameter of 15 meter). On the second day, there is no correlation between distance and number of wasps recaptured.

-Landing is much lower on smaller plants.

-Flight is the most important mechanism for movement between plants. Landings on neighbouring plants are not concentrated at the positions where leaves touch.

-Most of the wasps have moved more than 7.5 meter away from the release point at the end of the first day, because a much smaller number is recaptured within the sampling area on the second and third day. For biological control, release points are 15 meter apart. This means that the parasitoids are evenly distributed over the field at the end of the first day of release.

-Males disperse slower than females. On the first and second day, they are still clustered around the release point.

-The correlation between parasitism and the number of wasps that actually visit a plant is very poor. This was shown by placing plants with egg masses next to glue-sprayed plants in the field.

-Parasitism is higher on the first day than on the second and third days, but does not decrease with distance from the release point within the sampling area.

Landing of *T. brassicae* on maize

Landing determines on which plant *T. brassicae* starts searching and where on the plant it starts searching. No observations on landing distributions of *T. brassicae* had been made before. It was also unclear if *Trichogramma* could detect host-cues while in flight, and select plants for landing by the presence of host-cues. Landing behaviour of *T. brassicae* was studied in the greenhouse and in the field, and direct observation and glue-sprayed plants were used for this in **Chapter 5**.

-The results of direct observation of landing and of trapping wasps on glue-sprayed plants were very similar. This means that the insect glue used was neither attractive nor repellent to the wasps.

-Landing occurs on all parts of the maize plant. Most wasps land on the middle leaves. The number of wasps landing per unit leaf area is higher on the upper leaf levels.

-The landing distributions were the same in the greenhouse, where wasps fly directly to the plants, and in the field, where the wasps landed on other plants before reaching the glue-sprayed plants. Landing and flying away repeatedly does not affect the distribution over the leaf levels.

-The effect of the height of the release point on landing positions is relatively small. Wasps that are released at ground level, land on average only eight centimetre lower on the first plant they encounter than wasps that are released at the plant middle, which is one meter above the ground.

-Wasps do not land more often on plants that are placed in the rows of a maize field compared to plants that are placed between rows.

-Host-cues have apparently no effect on landing. In one experiment, the host-cues were concentrated in a small area where an *O. nubilalis* female had been confined on the leaf. The resulting patches could contain scales, excrements, sex pheromones and eggs of *O. nubilalis*. In an other experiment, only volatile host-cues could reach the plants, that also contained fresh host-eggs and their odour, but no scales. In both experiments, the number of wasps landing on the plants with host-cues and the control plants was the same.

Host-finding of *T. brassicae* on maize plants

Once the parasitoid has landed, it starts searching for hosts by walking over the plant. There was no information about residence times and walking patterns on maize plants by *T. brassicae* available. Also, movement of *T. brassicae* between leaves had not been studied. Effects of host-cues had mostly been studied in olfactometers or petri-dish situations. Direct observations of *T. brassicae* moving over the plant were made and the effect of kairomones on host-finding and residence times was studied in **Chapter 6**.

In addition, the effect of temperature on walking speed and walking activity was studied, using direct observation and a computer image analysis system (**Chapter 7**).

The experiments led to the following conclusions:

-Temperature has a strong effect on residence times. Wasps spend an average of 44.9 and 20.8 minutes on the plant at 18°C and 25°C, respectively.

-Leaf level or leaf side where the wasp landed had no effect on residence time.

-Residence times are exponentially distributed.

-At 18°C, only 20% of the wasps flies away from the plant. The rest spend long periods standing still on the plant. At 25°C, 87% flies away and only 13% stands still for longer periods.

-The leaf part closest to the stem is the most visited and searched leaf area. At 18°C, wasps spent much time on the lower leaf side standing still.

-Wasps mainly walk from one leaf to another. If they fly, they rarely land on the same plant again.

-At 25°C, the mean number of leaves visited per plant is only 1.2, and 87% of the wasps visit one leaf only. At 18°C, 1.8 leaves per plant visited and 58% visit only one leaf.

-Although 18 to 24% of total searching time is spent in following the leaf edge and mid-rib, *Ostrinia nubilalis* host egg masses touching the mid-rib are not encountered more often than those away from the mid-rib. Host-finding is not influenced by the position of egg masses relative to the stem or by the leaf level.

-Naturally laid egg masses were found twice as often as artificially placed egg masses.

-Volatile host-cues that reach the plants through a layer of netting also affect host-finding and increase residence time on the plant.

-Walking speed increases linearly from 0.166 cm.s^{-1} at 12°C to 0.311 cm.s^{-1} at 20°C. At
    25°C, the wasps move even faster but speed could not be measured.
-Walking activity (time spent walking divided by total time) increases from 0.10 at 12°C
    to 0.90 at 25°C.
-At 25°C, walking activity is high during the whole day, at 20 and 16°C it decreases during
    the afternoon, and at 12°C the wasps are most active only at noon or later.
-At temperatures below 20°C, searching is more limited by low walking activity than by
    low walking speed.

The searching behaviour can be summarized as follows:
After hatching and mating, T. brassicae females spread rapidly through the field, making
short flights between plants. Most visits to plants are relatively short, where they inspect only
one or two leaves. At lower temperatures (<20°C), T. brassicae stays longer on the plant,
and visits more leaves, but the walking speed and especially the walking activity are much
lower than at temperatures above 20°C. If host-cues are encountered, the parasitoid also
stays longer on the plant and searches more intensively in the area where the host-cues are
encountered.

COMPUTER SIMULATION OF THE SEARCHING BEHAVIOUR OF *T. BRASSICAE*

Simulation models were developed of the searching behaviour of *T. brassicae*, using the
information collected in the previously described experiments. The models calculate the
encounter probability of *T. brassicae* on a maize leaf, a maize plant (**Chapter 8**) and in a
maize crop (**Chapter 9**), using Monte Carlo simulation. The encounter probability is the
chance that the parasitoid encounters at least one egg mass during a certain period of time.
The encounter probability was chosen instead of parasitism, because encounters on leaves and
plants can be directly observed, and because encounter probability is a result of searching
behaviour only, while parasitism also depends on host acceptance and host suitability. In
addition, the encounter probability is a very good indicator for the quality of *Trichogramma*
parasitoids.

Simulation of searching on a leaf

A simulation model for searching on leaves was developed. In this model, the leaf side on
which searching starts is selected and the total time that the parasitoid spends on the leaf is
calculated. During this time, the wasp can change from one leaf side to another or encounter
the egg mass, in which case the simulation ends. The effect of host-cues was not
incorporated. A validation experiment was made, where encounter probability on three leaf
sizes was measured. From the model and its validation the following conclusions were
drawn:
-Encounter probability on a leaf without host-cues ranged from 0.47 to 0.18 in the model and
    from 0.25 to 0.05 in the validation experiment. The effect of leaf size was well

simulated. The model overestimates the encounter probability on leaves. This is probably a result of the fact that in the experiments the wasps did often search only on a part of the leaf near the base, while in the model the whole leaf was searched.
-Leaf size has an impact both on leaf residence time and on the encounter probability. Wasps stay longer on larger leaves but have a smaller chance to encounter the egg mass.
-Following the leaf edge has a negligible effect on encounter probability.

Simulation of searching on a plant

The model for searching on a leaf was extended to searching on a plant. Searching on the leaf is similar as in the plant model, except for the effect of host scales. When scales are encountered, residence time on the leaf side and the leaf are increased, as well as the chance to visit another leaf. After searching on the leaf, the parasitoid may move upward or downward and visit another leaf. The simulation ends if an egg mass is encountered, if the parasitoid leaves the plant, if it stops walking or if the maximum searching time is reached. The model was validated with two experiments, for searching on plants without and with scales.

The following conclusions could be drawn:
-Encounter probability on a plant without host-cues ranged from 0.06 to 0.12 in the model and from 0.12 to 0.18 in the validation experiment. The effect of plant size is well simulated, but the model underestimates the encounter probability on plants.
-Encounter probability is decreases with increasing plant size.
-The sensitivity of the model to parasitoid and host factors depends on the temperature. At 25°C, only walking speed of the parasitoid has a significant impact on encounter probability. At 18°C, the diameter of egg masses, the parasitoids walking activity, leaf residence time and factors influencing between-leaf movement also have a significant effect on the encounter probability.
-The landing position of *T. brassicae* has a strong effect on encounter probability. The model shows that the distribution of landings over the plant leaf levels of *T. brassicae* should be similar to the egg mass distribution of *O. nubilalis* for optimal searching.
-Higher temperature leads to a higher flight propensity, which causes *T. brassicae* to spend less time on the plant and visit fewer leaves. The walking speed and walking activity increase with temperature. According to the model, the net result is that the overall encounter probability is higher at higher temperatures.
-The presence of host-cues doubled the encounter probability on plants with naturally distributed egg masses and scale patches. Four reactions of the parasitoid to host-cues (scales) are incorporated in the model: increased residence time on leaf side and leaf, increased chance to visit more leaves, and an increased diameter for egg masses with scales in the vicinity. Although the increased diameter for egg masses with scales in the vicinity has the strongest effect on encounter probability, only the combination of all four factors leads to the increase found in the validation.

-Scales that are close to an egg mass have the strongest effect on encounter probability, but also scales anywhere else on the lower side of the leaf where an egg mass is present, increase the encounter probability. At 18°C, scales on the lower side of the leaf above the one with the egg mass also significantly increase the encounter probability.

Simulation of searching in a crop

Searching in a maize field was simulated by extending the model for searching on one plant to searching on a series of plants during one day. Wasps move to new plants by flight. Temperature is not fixed, but dependent on the time of day. Actual dispersal is not simulated, since it is assumed that each plant has the same chance to have egg masses or scale patches. From the model, the following conclusions were drawn:

-Daily encounter probability in a field with plants with nine leaves and at average field temperatures is 0.06. This means that encountering an egg mass is a rare event for an individual wasp.
-Variation in single parasitoid parameters does not have a strong effect on the encounter probability.
-Increasing residence time on leaves will increase the chance to encounter the egg mass on a single plant, but decrease the chance to encounter egg masses in the field, because the wasp can visit fewer plants on a day. Apparently, *T. brassicae* has a sampling strategy which consists of visiting many plants, and searching only a small part of these plants.
-The landing distribution of *T. brassicae* and the distribution of *O. nubilalis* oviposition over the leaf levels have a significant effect on the encounter probability. This means that in the field the synchronisation between leaves on which parasitoids land and leaves on which egg masses are present is important.
-Scales close to egg masses are an important host-cue in the field. The encounter probability is sensitive to variation in the percentage of egg masses with scales in the immediate vicinity. Scale patches that are not close to the egg masses have neither a positive nor a detrimental effect on the encounter probability.
-There is a strong linear correlation between host density and encounter probability. The higher the host density, the higher the encounter probability. Although the encounter probability is higher, there are also more egg masses that have to be parasitized at higher host densities. The chance that an egg mass is encountered by at least one parasitoid during one day with a *Trichogramma* release rate of 60.000 females per hectare is 0.32 at a host density of 0.05 egg masses per plant and 0.27 at a host density of 0.4 egg masses per plant. Parasitism will thus be slightly lower at higher host densities. At higher densities than 0.4 egg masses per plant (which are however not found in Switzerland north of the alps) parasitism would decrease stronger, because of egg limitation.

-There was a clear effect of plant size on the encounter probability, which decreases with increasing plant size. The relation between plant size and encounter probability is, surprisingly, not linear but exponential. Increasing the leaf area of a large plant has less effect on the encounter probability than increasing the area of a small plant.
-Temperature has a strong effect on the encounter probability. On the coldest day of the *O. nubilalis* oviposition periods of seven years, the encounter probability was only 0.006, while on the hottest day, it was 0.115. Under normal field temperature conditions, the encounter probability ranges from 0.024 to 0.086.
-If the daily encounter probability is six percent and 60.000 females are released per hectare maize with a host density of 0.1 egg masses per plant, the chance of an egg mass to be encountered by *T. brassicae* at least once during a day is 31%. After 5 days, the chance that the egg mass is visited at least once is 84%. If host acceptance is good, this means that up to 84% of all egg masses are parasitized. This rate of parasitism is quite similar to parasitism rates found in the field with normal commercial releases (Chapter 9). This is an indication that the model gives an adequate description of the searching process of *T. brassicae*.

DISCUSSION

Biological control with *T.brassicae*

The biological control system for inundative releases of *T. brassicae* against *O. nubilalis* applied in Switzerland utilizes two releases of 60.000 females per hectare, separated ten days. The first release is made when *O. nubilalis* start ovipositing. The first fourteen days after release are decisive for the success or failure of the biological control. If *T. brassicae* fails to parasitize enough egg masses, the amount of damage caused by the *O. nubilalis* larvae emerging from the unparasitized eggs is too high.

In Switzerland, *T. brassicae* is either released from cards that are hung on maize leaves above the middle of the plant, or from capsules that are distributed on the ground. As was shown in Chapter 5, release height has some effect on the leaf level of the first landing. After two or three landings this effect will have disappeared completely. Indeed, there seems to be no difference in the rate of parasitism by wasps released from cards and from capsules (Bigler *et al.*, 1989). The number of release points used at present is 50 cards per hectare, which means that the release points are about 15 meter apart from each other in the field. The results of Chapter 4 indicate that fewer release points could be used from a host-finding perspective, since the wasps disperse fast enough. Fewer release points could reduce the work in producing and distributing the release cards or capsules. Experiments in Southern Switzerland have shown that 16 release points per hectare gave good results. However, in this area *O. nubilalis* has two generations so a much larger number of *T. brassicae* (500.000 females and males) is released per hectare. The number of wasps released determines how often each egg mass is visited. An encounter probability of 0.06, assuming that the wasps can encounter and parasitize one egg mass per day and have an host acceptance of 100%, leads to a rate of parasitism of 82% after five days. If the number of wasps released is increased to 70.000, the daily chance of an egg mass to be encountered rises to 33% and parasitism after five days to 87%. A reduction to 50.000 results in a daily chance of encounter of 24% and a five-day rate of parasitism of 76%. It is clear that the number released is quite critical and that reductions of the standard number released are only feasible if a higher encounter probability than 0.06 is expected. These results are quite similar to those found by Hassan *et al.* (1990) in field experiments in Germany. Reduction of *O. nubilalis* was measured in the field with 19.000, 33.500, 75.000 and 300.000 females per release. *O. nubilalis* larval densities after *Trichogramma* releases were 51.0, 72.0, 88.8 and 93.8% of those in untreated fields. With the model for searching in a crop, parasitism after five days for these numbers released is 42.3, 62.1, 88.6 and 100.0%, respectively, assuming an encounter probability of 0.058.

It has been shown (Lewis *et al.*, 1975) that artificially spread kairomones in a field could increase parasitism by *Trichogramma evanescens*. The simulation model (Chapter 9) shows that the encounter probability is not very sensitive to scales that are not close to egg masses. Scales spread artificially as a kairomone source would usually not be close to egg masses. An uniform application of scales would be detrimental to parasitism, because the wasps will habituate to the scales if they do not find egg masses (Gardner and van Lenteren, 1986). Thus, in such a situation, they might no longer react to the scales close

to egg masses, which, as the model shows, have a significant impact on the encounter probability. Spotwise applied artificial kairomones do have an effect on parasitism because encountering them arrests the parasitoid, thus preventing it from migrating from the crop. If the density of these artificial kairomones becomes higher, encounter probability will decrease because the parasitoids are more and more retained on plants where no eggs are present. More information about the effects of kairomones on dispersal is necessary to find out at which density the decreased encounter probability is no longer offset by a higher number of wasps retained in the field.

The model shows the strong influence of temperature on the encounter probability (Chapter 9). Growers are advised not to release *T. brassicae* during periods of bad weather (low temperatures and rain). According to the model, the parasitoids have a very low encounter probability under such circumstances, so their release would not accomplish much. Fine tuning the number of wasps to release based on plant size and expected minimum temperatures would be possible. For example, to reach the same parasitism if plants are 2800 or 700 cm^2 instead of 3800 cm^2 (Chapter 9), 45.000 respectively 20.000 females instead of 60.000 are sufficient to reach 80% parasitism. The work involved to fine tune releases would probably cost more, however, than the money saved by releasing fewer *Trichogramma*. The same is probably true for specific, spotwise application of kairomones to raise parasitism. An increase in parasitism could be accomplished cheaper by releasing more parasitoids or by selecting parasitoid strains that show good dispersal characteristics within the release field but a low rate of migration from the release field.

Quality control and selection of *T. brassicae*

Quality of parasitoids reared for biological control is their potential to perform their intended function, i.e. to sufficiently parasitize the pest. The overall quality can be assessed directly only in the field. To maintain and measure the quality of mass-reared *T. brassicae*, quality control procedures have been developed (Bigler, 1994). A number of measurable traits that are thought to influence parasitoid quality are chosen, and measured in the laboratory. The results will show if the parasitoid material conforms to certain quality standards. The material used to start a mass rearing of *Trichogramma* for biological control should be the species and strain with the best quality for a specific pest and crop combination. Because *Trichogramma* species are often not monophagous, a large number of species and strains are usually available. Selection is therefore necessary, based on the same premises as quality control (Hassan, 1994).

Quality control guidelines of the IOBC for *T. brassicae* (van Lenteren *et al.*, 1994) mention host acceptance, fecundity, longevity, sex ratio and rate of emergence. Bigler (1994) and Cerutti and Bigler (1991; 1995) also include percentage wasps with deformed wings and walking speed. Based on the results of the crop simulation model, the significance of some of these traits can be reassessed.

The degree of host acceptance of *T. brassicae* is very important. In the model for searching in the crop parasitism is 80% after five days, although the encounter probability is only 0.058 (Chapter 9). This level of parasitism can be reached at such encounter

probabilities only if host acceptance is 90-100%. A decrease in host acceptance will lead to a proportional decrease in parasitism, if the parasitoid is not egg-limited. Although Bourchier and Smith (1996) found no effect of host acceptance on parasitism of *Choristoneura fumifera* by *T. minutum*, problems with low parasitism by *T. brassicae* in field releases were mainly due to a low host acceptance (Bergeijk *et al.*, 1989). Current host acceptance tests (Cerutti and Bigler, 1991; Frei and Bigler, 1994) do not mimic the situation where egg masses are encountered only after a long period of searching. Therefore, it is hard to say if the host acceptance as measured in the lab is the same as the host acceptance in the field. In the *T. brassicae* quality guidelines of the IOBC (van Lenteren *et al.*, 1994), no minimum value for the host acceptance is given. According to the results of the model for searching in the crop (Chapter 9), at least 90% of the wasps tested should accept the eggs.

The importance of fecundity depends on the number of egg masses that *T. brassicae* is expected to encounter in the field. This is dependent on the encounter probability and the longevity. In Chapter 9, an encounter probability of 0.058 was found for a typical field situation, with average temperatures of 1993, a host density of 0.1 and nine leaf plants. If the wasps search in the field for one week under these circumstances, 27 percent of the wasps will encounter one egg mass, 6 percent will encounter two egg masses and only 1 percent of the wasps will encounter more than two egg masses. *O. nubilalis* egg masses have a mean size of 17 eggs. Usually, two *T. brassicae* eggs are laid in each *O. nubilalis* egg. The fecundity of 40 eggs given as a minimum value in the IOBC guidelines (van Lenteren *et al.*, 1994) is therefore quite low. With an egg load of 40 eggs, parasitoids that encounter large egg masses or more than one egg mass will become egg-limited. If the encounter probability rises as a result of higher host densities, an egg load of 40 eggs is clearly too low. The level of parasitism is not dependent on the host density (Chapter 9), but this assumes that the wasps are not egg-limited and can parasitize all egg masses they encounter. If use of *T. brassicae* in an environment with high host densities is planned, a higher fecundity (>40 eggs) is necessary.

Sex ratio, rate of emergence from the rearing host (usually *Ephestia kühniella* Zeller or *Sitotroga cerealella* Olivier) and percentage of wasps with deformed wings determine how many females will be actually searching in a particular maize field after a certain number of parasitized eggs has been placed in that field. Since *T. brassicae* is dependent on flight to move through the field (Chapter 5), it is clear that parasitoids with deformed wings will hardly contribute to parasitism. It is shown in Chapter 9 that the level of parasitism is closely correlated with the number of wasps searching. 60.000 wasps per hectare is necessary to reach 80% parasitism after five days, if the encounter probability is 0.058. With 100.000 parasitized eggs per hectare, this means a normal sex ratio (>60% females) and emergence rate (>90%) are necessary. If this is not possible, the number of eggs per hectare should be increased. Low sex ratios and emergence rates and a high percentage of wasps with deformed wings indicate problems in production, storage or handling of the parasitized eggs (Cerutti and Bigler, 1995). Such material usually has a low overall quality and should not be used.

The encounter probability in the crop is not very sensitive to walking speed according to the simulation model. Experiments by Bigler *et al.* (1988) showed that parasitism in the field was influenced by walking speed. In this experiment, however, differences in walking speed were as high as 100%, and the strains that were compared were not tested for other

quality traits, e.g. host acceptance. It is questionable whether walking speed should be used as a general quality control trait. As will be discussed later, if it is used, it should be measured at lower temperatures.

Other quality traits than the ones discussed above have been proposed (Bigler, 1994). As the model shows, low temperatures cause a sharp reduction in the encounter probability. Daily parasitism during this time can be low, but still be sufficient for biological control, because *O. nubilalis* eggs also develop slower at lower temperatures. Cool days during the summer season in Switzerland are not exceptional, and might be a reason for insufficient levels of parasitism. A good walking activity at low temperatures (12-18°C) could prevent a disastrous decrease in parasitism during cold periods. The encounter probability in the model for searching on plants was sensitive to both walking speed and walking activity at low temperatures (Chapter 8). These traits could be measured in the lab at low temperatures to get an indication of the strain or species low temperature potential. An advantage of the activity test described in Chapter 7, which is based on direct observation of *T. brassicae* at different temperatures, is that no special equipment is needed.

Flight propensity is important, because it enables the wasps to sample many plants. Flight propensity of *T. brassicae* declines rapidly with temperature, and the wasps show little flight activity at 18°C (Chapter 6). Wasps with a higher flight propensity at low temperature would have a higher encounter probability. A quality test for measuring flight propensity has been developed by Dutton and Bigler (1995). This could be used to test the flight propensity at 18°C.

Recognition of host-cues has also been suggested as a quality trait. The model shows that reacting to scales close to egg masses increases the encounter probability. The response of *Trichogramma* is not specific to scales of a specific host species. Tricosane, the chemical compound to which the parasitoid reacts, is found in the scales of many lepidopterans (Jones *et al.*, 1973). It is not known whether there is much inter- and intra-species variation for *Trichogramma* in the responses to scales. Only if that is the case, measuring the responses would make sense for quality control.

A quality index was developed for *T. brassicae* (Cerutti and Bigler, 1991; 1995). This index is based on multiplication of seven traits, emergence rate, sex ratio, ratio of deformed females, longevity, walking speed, fecundity on the rearing host and fecundity in the target host. The last parameter is also a measure of host acceptance. Each trait was rated by comparison to the traits of a reference strain. This reference strain has (per definition) a value of one for each trait, resulting in a quality index of one. Other strains can be compared by their quality indices which should be about one or higher to indicate a good quality strain. A multiplicative index was chosen because it is clear that a very low value in any of the single traits would lead to very low parasitism. It was shown (Dutton *et al.*, 1996) that strains with a higher quality index always perform better in the field than strains with a low quality index. A multiplicative index also implies that very high values for one trait compensate for low values in other traits. Only traits that directly affect the actual number of female wasps searching in the field after release (emergence rate, sex ratio, ratio of deformed females) can directly compensate each other. A high fecundity can only compensate for low values in other traits if the parasitoid is egg-limited. This depends on the number of egg masses that the parasitoid is expected to encounter. At low host densities (<0.1 egg masses per plant), the

parasitoid is not egg-limited and in this case a higher fecundity will not result in a higher level of parasitism. The model also shows that the encounter probability is not very sensitive to the walking speed, so high walking speed will not compensate for low values in other traits either. It should be considered to omit walking speed from the quality index.

Host-location behaviour of *T. brassicae*

We may understand the host-location behaviour of *T. brassicae*, when we consider its host and host plant spectrum. Vet and Dicke (1992) listed three ways in which parasitoids can find hosts that are hard to detect: 1. by using synomones of the host plant induced by the host, 2. by using detectable cues like infochemicals from other host stages or 3. by linking easy to detect but unreliable cues with reliable but hard to detect cues through associative learning. They predict that a parasitoid that is a generalist in respect to both its hosts and its host plants will not have strong innate responses to host or host plant kairomones. If common chemical components occur in any of the volatile or contact infochemicals of the hosts or host plants, then a strong innate response to those components is expected. For an egg parasitoid, there are some special constraints in host-finding (Vet *et al.*, 1995): the parasitoid is small and has a short lifespan, and the host is especially hard to detect since it is immobile and usually does not interact with the host plant. Because the available hosts and the environment are not likely to change much during the short lifespan of the parasitoid, the parasitoid will not have to make many long range foraging decisions. The parasitoid might have to rely mainly on cues from the adult of the host to find the eggs (Noldus *et al.*, 1991).

The searching behaviour of *T. brassicae* seems, indeed, not to be influenced by specific plant or host-cues like Vet and Dicke (1992) predicted. *T. brassicae* in maize shows rapid dispersal (Chapter 4). Host-cues do not influence landing on plants (Chapter 5). Odour directed flight is not expected in very small parasitoids because the energy expenditure for upwind flight is too high (Chapman, 1982), and the parasitoid might be unable to reach the odour source at all. The presence of volatile host-cues does not seem to induce landing either. *T. brassicae* usually makes a short visit after landing on a plant (Chapter 6). Both volatile and non-volatile host-cues arrest the parasitoid on the plant (Chapter 6). Both lead to an increased encounter probability. Scales contain compounds that are common to most lepidopterans (Jones *et al.*, 1973). A strong innate reaction to scales is therefore expected, according to Vet and Dicke (1992). In the maize-*O. nubilalis-T. brassicae* system, scales are a reliable cue to egg masses. When *O. nubilalis* scales are encountered, there is a chance of 45% that there is an *O. nubilalis* egg mass within three centimetre (Chapter 2). The model for searching in the crop shows that scales close to egg masses have a significant influence on the encounter probability (Chapter 9). In Chapter 6, it was shown that *T. brassicae* also reacts with arrestment to volatile host-cues. In olfactometers, *T. brassicae* reacts to the sex pheromone of *O. nubilalis* females (Frenoy *et al.*, 1991). This might be the host-cue responsible for the arrestment responses (Chapter 6). Because of crop rotation, in Switzerland *O. nubilalis* mates outside the maize field, so sex pheromones are not present in the maize field (Derridj *et al.*, 1986; Cordillot, 1989). Volatile cues were therefore not incorporated in the model for searching in the crop. It is interesting that the generalist *T. brassicae* has an

innate response to a sex pheromone, which is usually host-specific. This contradicts the prediction that generalist parasitoids do not have strong innate responses to specific host kairomones (Vet and Dicke, 1992). *T. evanescens*, which is also a generalist, does react to the sex pheromones of at least five lepidopteran species (Noldus and van Lenteren, 1985; Zaki, 1985). The question arises whether sex pheromones possess common volatile compounds, beside the specific compounds.

A maize field with *O. nubilalis* egg masses is a very homogenous ecosystem. In this agro-ecosystem, some of the searching behaviour of *T. brassicae* will seem to be inefficient. *T. brassicae* spends 40-60% of its searching on the upper side of maize leaves (Chapter 6), where egg masses are rarely present. As described before, the parasitoid cannot use sex pheromones for host-finding, because they are not present in the maize field. Associative learning of *T. brassicae* will have little effect on the encounter probability inside the maize field. An increased response to maize odour does not increase the chance to encounter egg masses. It might lower dispersal for a while, if it makes the wasps stay longer on all plants in the field. The model for searching in a crop shows that a stronger reaction to scales will not increase the encounter probability either (Chapter 9).

In a natural ecosystem, the searching behaviour of *T. brassicae* might be more efficient than we observed in maize fields. The searching behaviour of *T. brassicae* has evolved in diverse ecosystems, with a large variation in hosts, host densities and host plants. If hosts of different species can be encountered, searching should not be restricted to one side of the leaf only. Although landing of *T. brassicae* is not influenced by volatile cues (Chapter 5), they are more detectable than scales and other contact cues (Chapter 6), and do influence encounter probability if they are present (chapter 6). Thus, sex pheromones may be an important cue indicating host presence for *T. brassicae* in natural habitats. Associative learning would also be profitable in a natural habitat. *T. brassicae* was shown to be able to associate host and plant odours with egg masses (Kaiser *et al.*, 1989). This leads to an arrestment response on the plant species on which eggs were encountered and to stronger arrestment when host-cues are encountered. In that way, the parasitoid will concentrate its searching on plants on which it has an increased chance to encounter eggs. This would result in a concentration of wasps in areas with a high host density. Increasing arrestment responses to odours of hosts that the parasitoid has already encountered would also lead to concentration on areas with high host density.

CLOSING REMARKS

Trichogramma species are used worldwide to control pests in many different crops. Reliable information on the success of *Trichogramma* performance, however, often lacks. In western Europe, the only large-scale application of *Trichogramma* is the use of *T. brassicae* against *O. nubilalis*. The research results and the simulation model presented in this thesis may contribute to the development of *Trichogramma* biological control systems against other pests and the improvement of the *T. brassicae/O. nubilalis* biological control system. Important contributions of the research described in this thesis are in the field of improved methods of selection and quality control. Improved reliability of *T. brassicae* releases and more efficient mass production will insure that biological control can stay competitive with other crop protection methods.

The future of biological control with *T. brassicae* is threatened by the introduction of genetically engineered maize that produces *Bacillus thuringiensis* toxins. These maize cultivars are fully resistant to *O. nubilalis* (Koziel *et al.*, 1993), which means that there would be no more need for *T. brassicae* releases. The continuous and all over presence of *B. thuringiensis* toxin in the environment that would be the result of a large scale use of the new maize cultivars is expected to lead to resistance against the toxins in *O. nubilalis*, if no resistance management is practised to prevent this. It would be an attractive and more sustainable solution to use maize with *B. thuringiensis* genes in combination with fields with other maize cultivars where *T. brassicae* is released. On the long term, if *O. nubilalis* becomes resistant against *B. thuringiensis* toxins, *Trichogramma* will once more be necessary to control it.

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Samenvatting

Zoekgedrag van de sluipwesp *Trichogramma brassicae* in maisvelden.

INLEIDING

Trichogramma brassicae, *Ostrinia nubilalis* en mais

Trichogramma brassicae Bezdenko (Hymenoptera, Trichogrammatidae) is een zeer kleine sluipwesp, slechts een halve millimeter lang. De soort heeft geen Nederlandse naam. *T. brassicae* werd voor het eerst gevonden in de voormalige Sovjetrepubliek Moldavië. De wesp plant zich voort door haar eieren in de eieren van vlinders en motten te leggen. De eieren vormen het voedsel voor de ontwikkeling van de larve van *T. brassicae*, die ook in het leeggegeten ei verpopt. *T. brassicae* is niet erg gastheerspecifiek, en kan daarom op de eieren van vele soorten vlinders en motten gekweekt worden. Hierdoor is de soort zeer geschikt voor inundatieve biologische bestrijding van vlinders en motten, omdat massaproduktie voor een lage prijs mogelijk is. *T. brassicae* wordt op commerciële schaal ingezet voor de biologische bestrijding van de maisstengelboorder in Zwitserland, Duitsland en Frankrijk.

De maisstengelboorder, *Ostrinia nubilalis* Hübner (Lepidoptera, Pyralidae) is een geelbruine (vrouwelijke dieren) of bruine (mannelijke dieren) mot met een spanwijdte van ongeveer drie centimeter. *O. nubilalis* komt voor in Europa, Noord Afrika, Noord Amerika en delen van Azië. Waarschijnlijk komt de soort oorspronkelijk uit Zuid Europa. Het is een belangrijk plaaginsect in mais. Het vrouwtje legt eimassa's op de bladeren van de maisplant. De larve eet eerst van het blad en gaat dan de stengel binnen, met als gevolg een lagere droge stof produktie en het breken van de stengel van de plant. *O. nubilalis* kan op veel plantensoorten leven, maar heeft een voorkeur voor mais. Het grootschalig verbouwen van mais wereldwijd zorgde ervoor dat de soort zich snel over een groot gebied kon verspreiden. Omdat de larven het grootste deel van hun tijd in de plant leven, is chemische bestrijding niet gemakkelijk, en is er altijd interesse geweest voor alternatieven. In Zwitserland wordt *O. nubilalis* bestreden met een combinatie van cultuurtechnische methoden en inundatieve vrijlatingen van *T. brassicae*. Dit functioneert als volgt: De mais wordt in de lente gezaaid. In mei verpopt *O. nubilalis*, die als rups in de maisstoppels overwintert. Uit de poppen komen de volwassen motten die paren. De vrouwtjes beginnen daarna hun eimassa's in de maisvelden te leggen. *O. nubilalis* eimassa's kunnen tussen het begin van juni en het eind van augustus gevonden worden, afhankelijk van het weer. Bij het eerste optreden van *O. nubilalis* eimassa's in het veld worden kaartjes of capsules in het veld verspreid, met eieren van de meelmot *Ephestia kühniella*, die door *T. brassicae* geparasiteerd zijn. Uit die eieren komen de volwassen parasitoïden. Na de paring beginnen de vrouwtjes vlindereieren te zoeken in het maisveld. De enige daar beschikbare eieren zijn de eimassa's van *O. nubilalis*, die door *T. brassicae* geparasiteerd worden. Tien dagen na de eerste vrijlating worden nog eens kaartjes of capsules met *T. brassicae* in het veld verspreid. Tien tot twintig dagen na de eerste parasitering, afhankelijk van het weer, beginnen de eerste *T. brassicae* uit de geparasiteerde eimassa's te komen. Gedurende de rest van de zomer, zolang er nog *O. nubilalis* eimassa's in het veld zijn, vormen de vrijgelaten *T. brassicae* en hun nakomelingen een populatie die de meeste van de beschikbare eimassa's parasiteert.

De larven van *O. nubilalis* die uit de niet geparasiteerde eieren komen boren zich uiteindelijk naar vlak boven de wortels van de maisplant, waar ze overwinteren. Daarom worden de stoppels in de herfst onder de grond geploegd, zodat de overwinterende rupsen niet meer kunnen verpoppen.

Onderzoeksdoelstelling

O. nubilalis kan succesvol met *T. brassicae* worden bestreden, maar de gebruikte technieken zijn langs empirische weg ontwikkeld. Er is weinig bekend over het eigenlijke zoekgedrag in het veld. De meeste onderzoeken naar het zoekgedrag zijn of direct in het veld of in kleine laboratoriumproeven uitgevoerd. In de veldproeven werd alleen het parasiteringsniveau gemeten, wat beperkte informatie over het zoekgedrag geeft, omdat parasitisme het resultaat is van veel verschillende processen. Laboratoriumproeven in petrischalen en olfactometers geven precieze kwantitatieve data over bepaalde aspecten van het zoeken, maar vertellen niets over het gedrag op planten en in het veld. Uit voorgaand onderzoek is gebleken dat de mate van parasitisme wordt beïnvloed door vele factoren van de parasitoïde, de gastheer, de waardplant van de gastheer en de omgeving, maar het effect van deze factoren in een veldsituatie is op zijn hoogst kwalitatief bekend. Dit is een belemmering voor de verbetering van biologische bestrijding met *T. brassicae* en de ontwikkeling van kwaliteitscontrole en selectiemethodes.

Doel van dit proefschrift is daarom om het zoekgedrag van *T. brassicae* in het maisveld te beschrijven. In laboratorium-, kas-, insectarium- en veldproeven werden data verzameld over dispersie, landing en beweging over de plant van *T. brassicae*. Deze data zijn geïntegreerd in een verklarend simulatiemodel. Met dit model werden de kwalitatieve en kwantitatieve effecten van de factoren van de parasitoïde, de gastheer, de waardplant en de omgeving op het zoekefficientie geschat. Gebaseerd op deze data worden aanbevelingen voor de biologische bestrijding en kwaliteitscontrole gegeven.

EXPERIMENTEEL WERK

Eileggedrag van *O. nubilalis*

Er was voldoende informatie beschikbaar over de verdeling van eimassa's van *O. nubilalis* over hele velden en over de bladniveau's, maar gedetailleerdere informatie over de verdeling over de plant ontbrak. Er was ook geen informatie beschikbaar over de verdeling van sporen van de gastheer, zoals schubben, over de plant. In hoofdstuk 2 wordt een korte literatuurstudie naar het eileggedrag van *O. nubilalis* beschreven. Eimassa's werden in het veld bemonsterd om de verdeling op de plant en op de bladeren te vinden. Landings- en ovipositiegedrag van *O. nubilalis* werd ook direct geobserveerd in kooien en in een insectarium. De maisplanten werden afgezocht op zichtbare sporen van de mot (schubben). Gebaseerd op deze experimenten werden de volgende conclusies getrokken:
-*O. nubilalis* eimassa's worden voornamelijk op de middelste bladeren gevonden, op de onderzijde van het blad. De meeste eimassa's worden dicht bij de middennerf gelegd. Een groot deel van de eimassa's wordt op het middelste stuk van het blad gevonden, en ovipositie op de punt van het blad is gering.
-Er is een nauwe samenhang tussen landing en eileg. Slechts weinig *O. nubilalis* vrouwtjes lopen over de plant, en dan slechts over afstanden van drie centimeter of minder. Dit betekent dat de verdeling van de eimassa's over de plant ook de verdeling van de landingen aangeeft. Hoewel de landingen en ovipositiepunten overeen komen, is het aantal landingen 9.3 maal zo hoog als het aantal oviposities. Vaak wordt na de landing het blad afgetast met de punt van het abdomen, wat erop wijst dat *O. nubilalis* de kwaliteit van de plant keurt na de landing.
- Omdat *O. nubilalis* nauwelijks over de plant loopt, zijn niet-vluchtige sporen van de gastheer geconcentreerd op kleine oppervlakten. Schubben worden enkel of in kleine dichte vlekken gevonden (80%) of verspreid over een deel van een blad (20%). 63% van de eimassa's hebben schubben binnen drie centimeter. Deze schubben, dicht bij eimassa's liggen, vormen echter slechts 45% van het totale aantal schubben. De andere schubben (55% van het totaal) liggen niet in de buurt van eimassa's. Schubben die de parasitoïde vindt in het veld geven aan dat er waarschijnlijk een eimassa op de plant of op het blad is, maar niet noodzakelijk in de buurt van de gevonden schubben.

Dispersie van *T. brassicae* in mais

In hoofdstuk 3 wordt een literatuurstudie beschreven naar het gedrag van *Trichogramma,* dat relevant is voor het zoeken naar gastheren, met een nadruk op het gedrag van *T. brassicae* in mais.

O. nubilalis* eimassa's zijn willekeurig verdeeld in een maisveld. *T. brassicae* wordt op een beperkt aantal punten vrijgelaten, zodat de wespen eerst een sterk gegroepeerde verdeling over het veld hebben. De manier waarop ze zich door het veld bewegen (lopend, met korte sprongen, of vliegend) was onbekend. Er was informatie over de dispersiesnelheid, maar deze was ten dele tegenstrijdig en voornamelijk gebaseerd op gemeten parasitering.

Parasitering is slechts beperkt bruikbaar om voortbeweging te bestuderen, omdat een geparasiteerd ei aangeeft dat een wesp aanwezig geweest is, maar een ongeparasiteerd ei niet bewijst dat er geen wespen geweest zijn. Om de reikwijdte van de eerste vlucht en de dispersiesnelheid van *T. brassicae* in het veld te bepalen, werden met lijm bespoten planten gebruikt (hoofdstuk 4). Dit leidde tot de volgende conclusies:

-De meeste vrouwtjes landen na hun eerste vlucht zeer dicht bij het vrijlatingspunt. De gemiddelde vluchtafstand is slechts 60 centimeter.

-Het aantal teruggevangen wespen neemt scherp af met de afstand van het vrijlatingspunt op de eerste vrijlatingsdag binnen het geobserveerde gebied (een cirkel met een diameter van 15 meter). Op de tweede dag is er geen correlatie meer tussen de afstand van het vrijlatingspunt en het aantal teruggevangen wespen.

-Op kleine planten landen veel minder wespen.

-Het belangrijkste voortbewegingsmechanisme van de wespen tussen planten is het vliegen. De landingen op aangrenzende planten zijn niet geconcentreerd op punten waar de bladeren elkaar raken.

-De meeste wespen hebben zich meer dan 7.5 meter van het vrijlatingspunt verwijderd aan het einde van de eerste dag. Op de tweede en derde dag worden namelijk veel minder wespen teruggevangen in het waarnemingsgebied. Voor biologische bestrijding liggen de vrijlatingspunten ongeveer 15 meter uit elkaar. Aan het einde van de eerste vrijlatingsdag zullen de wespen dus gelijkmatig over het veld verdeeld zijn.

-De mannetjes verspreiden zich langzamer dan de vrouwtjes. Op de eerste en de tweede dag zijn ze nog geclusterd rond het vrijlatingspunt.

-Er is geen verband tussen parasitering en het aantal wespen dat een plant bezoekt in de proef. Dit werd aangetoond door eimassa's te bevestigen aan planten naast de met lijm ingespoten planten.

-Op de eerste vrijlatingsdag is de parasitering hoger dan op de tweede en derde dag, maar er is geen correlatie tussen afstand en parasitering binnen het waarnemingsgebied.

Landing van *T. brassicae* op maisplanten

De landing bepaald op welke plant *T. brassicae* gaat zoeken en waar op de plant ze begint met zoeken. Er waren geen observaties van landingsverdelingen van *T. brassicae* bekend. Het was ook onbekend of *Trichogramma* soorten sporen van hun gastheren kunnen waarnemen terwijl ze vliegen. Verder was het onduidelijk of *Trichogramma* soorten planten om op te landen kunnen uitkiezen op grond van de aanwezigheid van sporen van de gastheer. In hoofdstuk 5 wordt het landingsgedrag van *T. brassicae* beschreven, in de kas en in het veld, zowel door directe observatie als met behulp van met lijm ingespoten planten. Dit onderzoek leverde de volgende conclusies:

-De resultaten van het direct observeren van landingen en het tellen van gevangen wespen op met lijm ingespoten planten waren vrijwel identiek. De lijm heeft dus noch een aantrekkend, noch een afstotend effect op de wespen.

-Er wordt overal op de maisplant geland. De meeste wespen landen op de middelste bladeren. Het aantal wespen dat per oppervlakte blad landt is hoger op de bovenste bladeren.
-De landingsverdelingen zijn hetzelfde in de kas, waar de wespen direct naar de plant konden vliegen, en in het veld, waar de wespen door of over rijen maisplanten moesten vliegen om de planten met lijm te bereiken. Een aantal tussenlandingen heeft blijkbaar geen effect op de verdeling van de landingen.
-Er is maar weinig effect van de vrijlatingshoogte op de landingsverdeling. Wespen die bij de grond worden vrijgelaten landen op de eerste plant die ze tegenkomen gemiddeld slechts acht centimeter lager dan wespen die een meter hoger worden vrijgelaten.
-Wespen landen evenveel op planten die in de rijen van het maisveld staan als op planten die ertussen staan.
-Sporen van de gastheer hebben geen effect op het landen. In een experiment werden de sporen kunstmatig op een klein deel van het blad geconcentreerd door een *O. nubilalis* vrouwtje een nacht lang op het bladdeel in te sluiten. Hierbij kunnen schubben, excrementen, sex-feromonen en eimassa's achtergelaten worden. In een ander experiment konden alleen vluchtige sporen van de gastheer de planten bereiken die kunstmatig van eimassa's waren voorzien. In beide gevallen was het aantal wespen dat op de behandelde en op de controleplanten landde hetzelfde.

Zoekgedrag van *T. brassicae* op maisplanten

Nadat de parasitoïde een plant bereikt heeft, begint ze gastheren te zoeken door over de plant te lopen. Er was geen informatie over verblijftijden en looppatronen van *T. brassicae* op maisplanten. Beweging tussen de bladeren van *T. brassicae* was nog niet bestudeerd. De effecten van sporen van de gastheer waren voornamelijk in olfactometers of kunstmatige arena's bestudeerd. Daarom werd het zoekgedrag van *T. brassicae* direct op de plant geobserveerd en werd het effect van sporen van de gastheer op zoekgedrag en verblijftijden bestudeerd (hoofdstuk 6).

In hoofdstuk zeven wordt het effect van temperatuur op de loopsnelheid en de loopaktiviteit beschreven, op basis van gegevens die door directe observatie en met behulp van een beeldanalysesysteem waren verkregen. Uit de experimenten werden de volgende conclusies getrokken:
-Temperatuur heeft een sterk effect op de verblijftijden. Wespen blijven bij 18 en 25°C gemiddeld 44,9 en 20,8 minuten op de plant.
-Het bladniveau of de zijde van het blad waar geland wordt heeft geen invloed op de verblijftijden.
-De verblijftijden zijn exponentieel verdeeld.
-Bij 18°C vliegt slechts 20% van de wespen weg van de plant. De rest eindigt met zoeken door een langere periode stil te staan. Bij 25°C vliegt 87% weg en slechts 13% gaat langer stil staan.
-Het deel van het blad dat het dichtst bij de stengel ligt wordt het meest bezocht. Bij 18°C brengen de wespen veel tijd stilstaand door op de onderkant van het blad.

-Wespen lopen voornamelijk naar andere bladeren van de plant. Als ze vliegen, landen ze zelden weer op dezelfde plant.
-Bij 25°C worden gemiddeld maar 1,2 bladeren bezocht, en 87% van de wespen bezoekt maar één blad. Bij 18°C worden gemiddeld 1,8 bladeren bezocht en bezoekt 58% van de wespen slechts één blad.
-Hoewel 18 tot 24% van de totale zoektijd wordt doorgebracht met het volgen van de bladrand en de middennerf, worden *O. nubilalis* eimassa's die tegen de middennerf liggen niet vaker gevonden dan eimassa's die niet tegen de middennerf liggen. Het bladniveau of de afstand van de stengel van de eimassa's hebben geen invloed op de kans, dat de eimassa gevonden wordt.
-Natuurlijk gelegde eimassa's worden meer dan twee keer zo vaak gevonden als kunstmatig op het blad bevestigde eimassa's.
-Vluchtige sporen van de gastheer, die de planten door een net bereiken, hebben een effect op de verblijftijden en het aantal gevonden eimassa's.
-De loopsnelheid neemt lineair toe van 0,166 cm.s^{-1} bij 12°C tot 0,311 cm.s^{-1} at 20°C. Bij 25°C lopen de wespen nog sneller, maar konden niet meer gemeten worden.
-De loopaktiviteit (de tijd dat de wesp loopt gedeeld door de totale tijd) neemt van 0,10 bij 12°C tot 0,90 bij 25°C toe.
-Bij 25°C is de loopaktiviteit de hele dag hoog. Bij 20°C en 16°C neemt de loopaktiviteit af in de namiddag, terwijl bij 12°C de wespen pas rond de middag actief worden.
-Bij temperaturen onder de 20°C wordt het zoeken meer beperkt door een lage loopaktiviteit dan door een lage loopsnelheid.

Het zoekgedrag van *T. brassicae* kan als volgt worden samengevat:
Na het uitkomen en de paring verspreiden de T. brassicae *vrouwtjes zich met (meestal korte) vluchten tussen planten over het veld. De meeste bezoeken aan planten zijn kort en er worden slechts één of twee bladeren bezocht. Bij temperaturen onder de 20°C blijft* T. brassicae *langer op de plant maar de loopsnelheid en vooral de loopaktiviteit zijn veel lager dan bij hogere temperaturen. Als sporen van de gastheer gevonden worden, blijft de parasitoïde langer op de plant en zoekt intensiever op de plek waar de sporen gevonden werden.*

SIMULATIES

Gebaseerd op de conclusies van de experimenten werden simulatiemodellen ontwikkeld. De modellen berekenen de kans voor *T. brassicae* om een eimassa te ontmoeten op een maisblad, een maisplant (hoofdstuk 8) en in een maisveld (hoofdstuk 9) met behulp van een Monte Carlo simulatie. De ontmoetingskans is de kans dat de wesp gedurende een vastgestelde periode (een bezoek aan een blad of aan een plant, of een dag in het veld) minstens één eimassa tegenkomt. Deze grootheid is gekozen in plaats van parasitering, omdat ontmoetingen met eimassa's op de plant direct geobserveerd kunnen worden en omdat ontmoetingskansen direct van het zoekgedrag afhangen, terwijl parasitering ook sterk door gastheeracceptatie en gastheergeschiktheid beïnvloed worden. De grootte van de ontmoetingskans is ook een goede indicator voor de kwaliteit van *Trichogramma* parasitoïden.

Simulatie van zoeken op een blad

Een simulatiemodel voor het zoeken op bladeren werd ontwikkeld. In dit model worden eerst de zijde van het blad waar de wesp begint te zoeken en de totale tijd op het blad bepaald. Gedurende de verblijftijd kan de wesp van de ene naar de andere kant van het blad gaan, of de eimassa tegenkomen. In het laatste geval stopt de simulatie. Er wordt geen rekening met sporen van de gastheer gehouden. In een validatie-experiment werd de ontmoetingskans op bladeren van drie verschillende groottes bepaald. Uit de simulatie en validatie werden de volgende conclusies getrokken:
-De ontmoetingskans op een blad zonder sporen van de gastheer loopt van 0,47 tot 0,18 in de simulatie en van 0,25 tot 0,05 in de validatie. Het effect van de bladgrootte werd goed gesimuleerd, maar het model overschat de ontmoetingskans op bladeren. De verklaring hiervoor is waarschijnlijk, dat het model niet ruimtelijk is, zodat de wespen over het hele blad zoeken, terwijl de wespen in de realiteit ernaar neigen slechts dat deel van het blad te doorzoeken waar ze landen.
-Bladgrootte heeft zowel op de verblijftijd als op de ontmoetingskans effect. Wespen blijven langer op grote bladeren maar hebben een kleinere kans eieren te ontmoeten.
-Het volgen van de bladrand heeft nauwelijks effect op de ontmoetingskans.

Simulatie van zoeken op een plant

Het model voor zoeken op een blad werd uitgebreid tot een model voor zoeken op de plant. In dit model wordt eerst het beginblad bepaald. Zoeken op het blad wordt gesimuleerd op dezelfde manier als in het model voor zoeken op een blad, maar bij het model voor zoeken op een plant wordt ook het effect van gastheersporen (schubben) gesimuleerd. Als deze ontmoet worden, neemt de verblijftijd toe op het blad en op de bladzijde waar de wesp is. De kans nog een blad te bezoeken neemt ook toe. Als de verblijftijd voor het eerste blad afgelopen is, wordt bepaald of de wesp een ander blad bezoekt en of ze naar boven of naar beneden loopt.

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De simulatie wordt beëindigd als een eimassa wordt ontmoet, als de parasitoïde wegvliegt, stopt met lopen, of als de maximum simulatietijd (twee uur) wordt overschreden. Het model werd met twee experimenten gevalideerd; één voor zoeken op planten zonder sporen van de gastheer, en één voor zoeken op planten met sporen van de gastheer.

Uit de simulatie en de validatie werd het volgende geconcludeerd:

-De ontmoetingskans op planten zonder sporen van de gastheer ligt tussen 0.06 en 0.12 in het model en tussen 0.12 en 0.18 in het validatie-experiment. Het effect van de plantgrootte wordt goed gesimuleerd, maar het model onderschat de kansen eimassa's te ontmoeten.

-De ontmoetingskans neemt af bij toenemende plantgrootte.

-De gevoeligheid van het model voor eigenschappen van de parasitoïde en de gastheer hangt af van de temperatuur. Bij 25°C heeft alleen de loopsnelheid van *T. brassicae* een significant effect op de ontmoetingskans als deze met 25% verhoogd wordt. Bij 18°C hebben ook de diameter van de eimassa, de loopaktiviteit, de verblijftijden op het blad en enkele factoren, die beweging tussen de bladeren beïnvloeden, een significant effect op de ontmoetingskans.

-De landingsverdeling van *T. brassicae* heeft een sterk effect op de ontmoetingskans. Het model demonstreert dat de landingsverdeling van *T. brassicae* en de eilegverdeling van *O. nubilalis* moeten samenvallen voor een optimale ontmoetingskans.

-Hogere temperaturen leiden tot meer vlieggedrag, zodat *T. brassicae* korter op de planten blijft en minder bladeren per plant bezoekt. De loopsnelheid en activiteit nemen echter toe. Het model laat zien dat het netto resultaat een hogere ontmoetingskans bij hogere temperaturen is.

-De aanwezigheid van sporen van de gastheer verdubbelt de ontmoetingskans op planten met natuurlijk gelegde eimassa's en sporen van de gastheer. Vier reacties op sporen van de gastheer (schubben), namelijk een verhoogde verblijftijd op blad en bladzijde, een verhoogde kans om nog een blad te bezoeken, en een grotere diameter voor eimassa's met schubben erom heen, werden in het model verwerkt. Hoewel de grotere diameter het sterkste effect heeft, resulteert alleen de combinatie van de vier factoren de waargenomen verhoging in de ontmoetingskans.

-Schubben, die dicht bij een eimassa liggen hebben het sterkste effect op de ontmoetingskans, maar alle schubben, die op de onderkant van een blad met een eimassa liggen, verhogen de ontmoetingskans., Bij 18°C verhogen schubben op de onderkant van het blad, dat boven het blad met de eimassa's ligt, ook de ontmoetingskans.

Simulatie van zoeken in een maisveld

Het zoeken in een maisveld werd gesimuleerd door het model voor zoeken op een plant uit te breiden tot het zoeken op een aantal planten gedurende één dag. Wespen vliegen naar nieuwe planten, en voor elke nieuwe plant wordt het aantal en de positie van eventuele eimassa's en schubben berekend. De temperatuur is niet constant, maar hangt af van de tijd van de dag. Dispersie wordt niet gesimuleerd. Het wordt aangenomen, dat elke plant dezelfde kans heeft eimassa's of schubben te bevatten, zodat de positie van de wesp in het

veld de ontmoetingskans niet beïnvloedt. De wesp kan het veld ook niet verlaten. Uit het model kan het volgende geconcludeerd worden:

-De ontmoetingskans gedurende één dag in een veld met maisplanten met negen bladeren, een gastheerdichtheid van 0,1 eimassa per plant en gemiddelde temperaturen is 0,06. Dit betekent dat de wespen zelden eimassa's tegenkomen.

-De gemeten eigenschappen van de parasitoïde hebben geen sterk effect op de ontmoetingskans. Als deze eigenschappen met 25% veranderd worden, leidt dat niet tot significante veranderingen in de ontmoetingskans.

-Het verhogen van de verblijftijd op bladeren geeft een hogere ontmoetingskans op een enkele plant, maar een lagere ontmoetingskans in het veld. Blijkbaar is het voordelig voor de zoekstrategie van *T. brassicae* als veel planten bezocht kunnen worden, ook als die planten elk maar zeer oppervlakkig doorzocht kunnen worden.

-De landingsverdeling van *T. brassicae* en de eilegverdeling van *O. nubilalis* over de bladniveau's hebben een significant effect op de ontmoetingskans. Dit betekent dat ook in het veld de synchronisatie tussen de landingsverdeling van de parasitoïde en de eilegverdeling van de gastheer belangrijk is.

-Schubben die dicht bij eimassa's liggen vormen een belangrijke aanwijzing voor *T. brassicae* in het veld. Als het percentage eimassa's met schubben verhoogd wordt, gaat de ontmoetingskans ook omhoog. De schubben, die niet in de buurt van eimassa's liggen, hebben geen effect op de ontmoetingskans.

-Er is een sterke lineaire correlatie tussen de gastheerdichtheid en de ontmoetingskans. Hoe hoger de dichtheid van de gastheer, hoe groter de kans dat eieren gevonden worden. Bij hogere dichtheden zijn er echter ook meer eimassa's die gevonden moeten worden om hetzelfde parasiteringsniveau te bereiken. De kans dat een eimassa gedurende een dag door tenminste één parasitoïde wordt gevonden is 0.32 bij een dichtheid van 0.05 eimassa's per plant en 0.27 bij een dichtheid van 0.4 eimassa's per plant. Deze berekening is gebaseerd op 60.000 vrijgelaten *T. brassicae* vrouwtjes per hectare. Parasitering is dus iets lager bij hogere plaagdichtheden. Bij hogere dichtheden dan 0.4 eimassa's per plant (die echter in Zwitserland ten noorden van de Alpen niet voorkomen) zal de parasitering sterker afnemen, omdat de wespen zoveel eimassa's beginnen te ontmoeten, dat hun eigen voorraad eieren uitgeput raakt.

-Er is een duidelijk effect van de plantgrootte op de ontmoetingskans. De relatie tussen grootte en ontmoetingskans is niet lineair maar exponentieel. Het vergroten van de bladoppervlakte van een kleine plant heeft dus een sterker effect op de ontmoetingskans dan het verhogen van de bladoppervlakte van een grote plant.

-De temperatuur heeft een sterk effect op de ontmoetingskans. Op de koudste dag van zeven *O. nubilalis* veldseizoenen was de ontmoetingskans slechts 0,006, op de warmste dag 0,115. Bij normale veldtemperaturen fluctueert de ontmoetingskans tussen 0,024 en 0,086.

-Als de dagelijkse ontmoetingskans 0,06 is, en 60.000 *T. brassicae* vrouwtjes worden vrijgelaten in een veld met 0,1 eimassa's per plant, dan is de kans, dat een eimassa door tenminste één wesp bezocht wordt, per dag 0,31. Na vijf dagen is de kans dat de eimassa tenminste één keer bezocht is 0,84, als alle parasitoïden in het veld blijven. Als de gastheeracceptatie van de wespen goed is, kan een parasitering van 84% bereikt worden. Dit parasiteringsniveau valt goed binnen de gevonden praktijkwaarden in Zwitserland (hoofdstuk 9). Dit is een indicatie dat het model een adequate beschrijving van het zoekproces van *T. brassicae* in maisvelden geeft.

DISCUSSIE

Biologische bestrijding met *T. brassicae*

De inundatieve biologische bestrijding van *O. nubilalis* met *T. brassicae* in Zwitserland gaat uit van twee vrijlatingen van 60.000 vrouwtjes per hectare, tien dagen na elkaar. De eerste vrijlating wordt gemaakt wanneer de eerste *O. nubilalis* beginnen eieren te leggen. De eerste veertien dagen na de vrijlating zijn beslissend voor het succes of falen van de biologische bestrijding. Als *T. brassicae* onvoldoende eimassa's parasiteert, komen er uit de ongeparasiteerde eimassa's teveel *O. nubilalis* larven, die de planten beschadigen .
  In Zwitserland wordt *T. brassicae* vrijgelaten met kaartjes, die aan de middelste bladeren van de maisplant hangen of met capsules, die op de grond liggen. Zoals in hoofdstuk 5 beschreven heeft de hoogte, waarop de wespen worden vrijgelaten, waarschijnlijk alleen effect op de eerste landing op een plant. Na twee of drie landingen is het effect verdwenen. Er lijkt in het veld inderdaad geen verschil in het effect van kaartjes of capsules te zijn. Het aantal vrijlatingspunten is 50 per hectare, zodat de punten ongeveer 15 meter uit elkaar liggen. Door de snelle dispersie van *T. brassicae* moet het mogelijk zijn minder vrijlatingspunten te gebruiken, wat tot lagere werkkosten bij produktie en vrijlating kan leiden. Experimenten in Zuid-Zwitserland hebben aangetoond, dat met 16 vrijlatingspunten per hectare ook een goed resultaat bereikt wordt. Deze experimenten werden echter uitgevoerd in een gebied waar de maisstengelboorder twee generaties per jaar heeft, zodat een veel groter aantal (500.000) *T. brassicae* mannetjes en vrouwtjes per hectare wordt vrijgelaten. Het aantal vrijgelaten wespen bepaald hoe vaak elk ei gevonden wordt bij een bepaalde ontmoetingskans van de wespen. Als de ontmoetingskans 0,06 is, en de wespen per dag maximaal één eimassa parasiteren, dan is de kans dat een eimassa gevonden wordt elke dag 29%. Dit leidt tot een parasitering van 82% na vijf dagen (als de gastheeracceptatie 100% is en het aantal wespen in het veld constant blijft). Als het aantal vrijgelaten wespen wordt verhoogd tot 70.000, neemt de dagelijkse kans gevonden te worden voor een eimassa toe tot 33% en de parasitering na vijf dagen tot 87%. Een verlaagde vrijlating van 50.000 wespen leidt tot waarden van respectievelijk 24% en 76%. Het is duidelijk dat het aantal vrijgelaten wespen nogal kritisch is en dat verlagingen in het aantal vrijgelaten wespen alleen mogelijk zijn als een duidelijk hogere ontmoetingskans dan 0,06 verwacht wordt. Deze gesimuleerde waarden komen nauw overeen met resultaten van veldexperimenten in Duitsland. De vermindering van het aantal *O. nubilalis* larven in het veld werd gemeten bij

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vrijlatingen van 19.000, 33.500, 75.000 en 300.000 vrouwtjes. De afname in dichtheden van *O. nubilalis* waren 51,0, 72,0, 88,8 en 93,8% ten opzichte van die in onbehandelde velden. Het model voor zoeken in het veld voorspelt respectievelijk 42,3, 62,1, 88,6, en 100% parasitering, bij een ontmoetingskans van 0,058.

Kunstmatig verspreidde kairomonen kunnen parasitering door *Trichogramma evanescens* verhogen. Het simulatiemodel voor zoeken in het veld (hoofdstuk 9) laat zien dat de ontmoetingskans niet erg gevoelig is voor schubben die niet bij eimassa's liggen. Schubben die kunstmatig worden uitgebracht als een bron van kairomonen, zullen voor het grootste deel niet in de buurt van eimassa's liggen. Uniform over de planten aangebrachte kairomonen zullen tot lagere parasitering leiden, omdat gewenning aan de kairomonen optreedt. Ze kunnen dan niet meer de kairomonen bij de eimassa's gebruiken, die, zoals het model laat zien, een significante invloed op de ontmoetingskans hebben. Pleksgewijs aangebrachte kairomonen hebben een effect op parasitisme omdat ze de wespen in het vrijlatingsveld vasthouden. Als de dichtheid van deze kunstmatige kairomonen hoger wordt, zal de ontmoetingskans afnemen omdat de wespen steeds meer intensief zoeken op plaatsen waar geen eimassa's zijn. Meer informatie over het effect van ontmoetingen met kairomonen op het dispersiegedrag is nodig, om met het model te kunnen bepalen, bij welke kunstmatig verhoogde kairomoondichtheid de verlaagde ontmoetingskans niet langer gecompenseerd wordt door het hogere aantal wespen dat in het veld blijft.

Het model laat zien dat temperatuur een zeer sterk effect op de ontmoetingskans heeft (hoofdstuk 9). Telers wordt door de producenten geadviseerd *T. brassicae* niet tijdens periodes met slecht weer (lage temperaturen en regen) vrij te laten. Volgens het model hebben de parasitoïden bij zulk weer zeer weinig kans om eieren te vinden, zodat een vrijlating inderdaad weinig zin heeft.

Het is in principe mogelijk met hulp van het model het aantal vrijgelaten wespen per veld aan te passen op grond van plantgrootte en verwachtte minimumtemperaturen. Als bijvoorbeeld de plantgrootte 2800 of 700 cm^2 in plaats van 3800 cm^2 is (hoofdstuk 9), zijn 45.000 respectievelijk 20.000 vrouwtjes per hectare in plaats van 60.000 voldoende om een parasitering van 80% te bereiken. De hoeveelheid werk, om bij elke vrijlating het optimale aantal te bepalen kost waarschijnlijk meer dan de besparing die het vrijlaten van minder *Trichogramma* oplevert. Het zelfde geldt voor het uitbrengen van kunstmatige kairomonen ter verhoging van het parasitisme. Parasitisme kan goedkoper verhoogd worden door meer *T. brassicae* vrij te laten of door *T. brassicae* stammen te selecteren die een sterke dispersie in de vrijlatingsvelden vertonen maar een geringe dispersie tussen maisvelden.

Kwaliteitscontrole en selectie

De kwaliteit van parasitoïden die voor biologische bestrijding gekweekt worden is hun potentiaal om de functie te vervullen waar ze voor bedoeld zijn, namelijk om voldoende van de te bestrijden insecten in het veld te parasiteren. De algehele kwaliteit kan alleen in het veld direct bepaald worden. Om de kwaliteit van grootschalig geproduceerde *T. brassicae* te meten en in stand te houden zijn kwaliteitscontrole procedures ontwikkeld. Een aantal meetbare eigenschappen waarvan men verwacht dat ze de parasitering beïnvloeden, wordt gekozen en in het laboratorium gemeten. De resultaten geven aan of het gekweekte materiaal aan een gegeven kwaliteitsstandaard voldoet. Het beginmateriaal voor een *Trichogramma* kweek moet van de soort en stam met de hoogste kwaliteit voor een specifieke combinatie van gastheer en gewas zijn. Omdat de meeste *Trichogramma* soorten niet monophaag zijn, zijn er meestal vele soorten en stammen beschikbaar. Daarom is selectie noodzakelijk, gebaseerd op dezelfde

principes als kwaliteitscontrole.

In de richtlijnen voor kwaliteitscontrole van de Internationale Organisatie voor Biologische Bestrijding (IOBC) worden de eigenschappen gastheeracceptatie, fecunditeit, levensduur, sex-ratio en larvale mortaliteit genoemd. Anderen voegen hier nog het percentage wespen met gedeformeerde vleugels en de loopsnelheid aan toe. Op grond van de resultaten van het model voor zoeken in het veld kan het belang van deze eigenschappen voor de kwaliteit opnieuw ingeschat worden.

De gastheeracceptatie is zeer belangrijk. In het model voor zoeken in het veld is de parasitering na vijf dagen 80%, hoewel de dagelijkse ontmoetingskans slechts 0,058 is (hoofdstuk 9). Deze mate van parasitering kan bij zo'n lage ontmoetingskans alleen bereikt worden als de gastheeracceptatie 90-100% is. Een vermindering in gastheeracceptatie zal een evenredige vermindering in parasitering tot gevolg hebben, als de parasitoïden niet door hun eivoorraad beperkt worden. Hoewel er geen effect van gastheeracceptatie op de parasitering van *Choristoneura fumifera* door *Trichogramma minutum* werd gevonden, werden problemen met lage parasitering door *T. brassicae* in het veld in Zwitserland voornamelijk veroorzaakt door een slechte gastheeracceptatie. De op het moment gebruikte gastheeracceptatietests bootsen de situatie in het veld, waar de wesp eimassa's zelden tegenkomt en dan na een lange periode van zoeken, niet goed na. Het is daarom moeilijk te zeggen of de in het lab gemeten gastheeracceptatie goed overeenkomt met die in het veld. In de richtlijnen van het IOBC wordt geen minimum waarde voor de gastheeracceptatie gegeven. Op grond van de resultaten van het model voor zoeken in het veld is het bij de huidige vrijlatingspraktijk noodzakelijk dat tenminste 90% van de wespen de gastheereieren bij de eerste onmoeting accepteert.

Het belang van de fecunditeit hangt af van het aantal eimassa's dat *T. brassicae* naar verwachting zal ontmoeten in het veld. Dit hangt af van de ontmoetingskans en de levensduur. In hoofdstuk 9 werd een ontmoetingskans van 0,058 gevonden voor een typische veldsituatie, met gemiddelde temperaturen van 1993, een gastheerdichtheid van 0,1 en planten met negen bladeren. Als de wespen onder deze omstandigheden een week zoeken, zal 27 procent van de wespen één eimassa ontmoeten, zes procent twee eimassa's en slechts één procent van de wespen meer dan twee eimassa's. *O. nubilalis* eimassa's bevatten gemiddeld

17 eieren. *T. brassicae* legt normaal twee eieren in elk ei van *O. nubilalis*. De fecunditeit van 40 eieren die als minimumwaarde gegeven wordt in de IOBC richtlijnen is daarom aan de lage kant. Wespen met een eivoorraad van 40 eieren zullen gebrek aan eieren krijgen als ze grote eimassa's of meer dan één eimassa ontmoeten. Als de ontmoetingskans stijgt als een gevolg van hogere gastheerdichtheden, is een eivoorraad van 40 duidelijk te klein. Het parasiteringsniveau hangt niet af van de gastheerdichtheid (hoofdstuk 9), maar alleen als de wespen geen gebrek aan eieren hebben en alle eimassa's die ze ontmoeten kunnen parasiteren. Als *T. brassicae* in een milieu met hoge gastheerdichtheden wordt ingezet, is een hogere fecunditeit (>40 eieren) noodzakelijk.

Sex ratio, larvale mortatiteit in de kweekgastheer (gewoonlijk *Ephestia kühniella* Zeller of *Sitotroga cerealella* Olivier) en het percentage wespen met gedeformeerde vleugels bepalen hoeveel vrouwtjes daadwerkelijk in een maisveld zoeken, nadat er een bepaalde hoeveelheid geparasiteerde eieren is geplaatst. Omdat *T. brassicae* moet vliegen om zich efficiënt door het veld te bewegen (hoofdstuk 5), is het duidelijk dat wespen met gedeformeerde vleugels nauwelijks aan de parasitering zullen bijdragen. In hoofdstuk 9 wordt vermeld dat het parasiteringsniveau nauw samenhangt met het aantal vrijgelaten wespen. 60.000 wespen per hectare zijn noodzakelijk om 80% parasitering te bereiken na vijf dagen bij een ontmoetingskans van 0.058. Als er 100.000 geparasiteerde eieren per hectare in het veld worden geplaatst, is een normaal sex ratio (>60% vrouwtjes) en een lage larvale mortaliteit (<10%) noodzakelijk. Als deze waarden niet bereikt kunnen worden, moet het aantal eieren per hectare verhoogd worden. Lage sex ratio's en hoge larvale sterfte en percentages adulten met gedeformeerde vleugels duiden op problemen bij de produktie, opslag en verwerking van de geparasiteerde eieren. Zulk materiaal heeft over het algemeen een lage kwaliteit en kan beter niet gebruikt worden.

De ontmoetingskans in het veld wordt niet sterk door de loopsnelheid beïnvloed, volgens het model voor zoeken in het veld. In een experiment met verschillende *T. brassicae* stammen was parasitisme in het veld aan loopsnelheid gerelateerd. In dit experiment waren de verschillen in loopsnelheid echter groot (tot 100%). De stammen die vergeleken werden, waren niet getest op andere eigenschappen, bijvoorbeeld gastheeracceptatie. Het is de vraag of loopsnelheid gebruikt moet worden als een eigenschap voor kwaliteitscontrole. Als de loopsnelheid wel als eigenschap gebruikt wordt, kan ze het beste bij lagere temperaturen worden gemeten, om redenen die later besproken worden.

Andere eigenschappen dan de tot nu toe gebruikte zijn voorgesteld voor kwaliteitscontrole. Het model laat zien, dat bij lage temperaturen ook de ontmoetingskans zeer klein wordt. De dagelijkse parasitering gedurende periodes met koud weer kan laag zijn en toch voldoende, omdat ook *O. nubilalis* eieren zich langzamer ontwikkelen. Maar als de parasitering te laag wordt kan dit tot het falen van de biologische bestrijding leiden. Omdat koele dagen tijdens de Zwitserse zomer geen uitzondering zijn, kan het effect van lage temperaturen een verklaring zijn dat de parasitering soms lager is dan gewenst. Een sterke neiging tot vliegen en een hoge loopaktiviteit bij lagere temperaturen (12-18°C) kunnen een te sterke teruggang van de parasitering helpen voorkomen. Het model voor zoeken op de plant was bij lage temperatuur zowel gevoelig voor loopsnelheid als voor loopaktiviteit (hoofdstuk 8). Deze beide eigenschappen kunnen bij lage temperatuur gemeten worden, om

een indruk te krijgen van de kwaliteiten van een stam of soort bij lage temperaturen in het veld. Een voordeel van de activiteitstest zoals beschreven in hoofdstuk 7, waarbij *T. brassicae* bij verschillende temperaturen geobserveerd wordt, is dat er geen speciale apparatuur voor nodig is.

De neiging tot vliegen van de wespen is belangrijk omdat vliegen de wespen in staat stelt vele planten te onderzoeken. De neiging tot vliegen neemt bij *T. brassicae* sterk af bij dalende temperatuur, en bij 18°C is er weinig vliegactiviteit meer (hoofdstuk 6). Wespen, die een sterkere neiging tot vliegen hebben bij lagere temperaturen, zullen een hogere ontmoetingskans hebben. Er zijn tests ontwikkeld, om de neiging tot vliegen te meten, die gebruikt zouden kunnen worden om de neiging tot vliegen bij 18°C te meten.

Het herkennen van en reageren op sporen van de gastheer is ook genoemd als een kwaliteitseigenschap. Het model laat zien, dat het herkennen van en het reageren op schubben die dicht bij eimassa's liggen, inderdaad de ontmoetingskans verhoogd. De respons van *Trichogramma* is niet specifiek op schubben van één soort gastheer. Tricosan, de chemische stof waar de wesp op reageert, wordt in de schubben van vele vlinders en motten gevonden. Het is onbekend in hoeverre er veel intra- en interspecifieke variatie in de respons op schubben bestaat. Alleen als dat het geval is, zal het de moeite zijn de respons te meten als kwaliteitseigenschap.

Er is een kwaliteitsindex voor *T. brassicae* ontwikkeld. Deze index is gebaseerd op de vermenigvuldiging van de scores voor zeven eigenschappen, namelijk larvale mortaliteit, sex- ratio, percentage vrouwtjes met gedeformeerde vleugels, levensduur, loopsnelheid, fecunditeit op de kweekgastheer en fecunditeit op *O. nubilalis*. De laatste eigenschap wordt ook als maat voor de gastheeracceptatie gebruikt. De score voor elke eigenschap is een ratio ten opzichte van een standaardstam. Deze heeft (per definitie) voor elke eigenschap de score één, zodat de kwaliteitsindex ook één is. Andere stammen kunnen worden vergeleken door hun kwaliteitsindices die ongeveer één of hoger moeten zijn om zeker te zijn van een goede kwaliteit. Een multiplicatieve index is gekozen omdat het duidelijk is, dat een zeer lage score in één van de eigenschappen tot zeer laag parasitisme zal leiden. Het is gebleken, dat stammen met een hogere kwaliteitsindex in het veld ook een hogere parasitering geven. Een multiplicatieve index impliceert ook, dat zeer hoge waarden voor één eigenschap lage waarden in andere eigenschappen compenseren. Alleen eigenschappen, die direct het aantal vrouwtjes dat in het veld zoekt beïnvloeden (larvale mortaliteit, sex ratio, percentage vrouwtjes met gedeformeerde vleugels) kunnen elkaar direct compenseren. Een hoge fecunditeit kan alleen lage waarden bij andere eigenschappen compenseren, als de wesp gebrek aan eieren heeft. Dit hangt af van het aantal eimassa's dat de wesp naar verwachting ontmoet. Bij lage gastheerdichtheden (<0.1 eimassa's per plant) krijgt de wesp meestal geen gebrek aan eieren en zal een hogere fecunditeit dus niet tot een hoger parasiteringniveau leiden. Het model laat ook zien dat de ontmoetingskans slechts weinig door de loopsnelheid beïnvloed wordt. Een hoge loopsnelheid zal dus niet lage waarden voor andere eigenschappen kunnen compenseren. Het kan overwogen worden de loopsnelheid geheel weg te laten uit de kwaliteitsindex.

Zoekgedrag van *T. brassicae*

Het gastheerzoekgedrag van *T. brassicae* moet in de context van het gastheer- en waardplantspectrum van deze soort gezien worden. Er zijn drie manieren waarop parasitoïden gastheren kunnen vinden die zelf geen gemakkelijk te ontdekken signalen afgeven: 1. door synomonen te gebruiken die de waardplant afgeeft als reactie op het plaaginsect (de gastheer), 2. door eenvoudig te detecteren signalen zoals informatiestoffen van andere levensstadia van de gastheer te gebruiken, of 3. door gemakkelijk te detecteren maar onbetrouwbare signalen te koppelen aan moeilijk te detecteren maar betrouwbare signalen door middel van associatief leergedrag. Parasitoïden die zowel gastheergeneralisten als waardplantgeneralisten zijn, zullen geen sterke aangeboren responsen voor kairomonen van gastheren of waardplanten hebben. Als er gemeenschappelijke chemische stoffen voorkomen in de vluchtige of niet-vluchtige infochemicaliën van de gastheer of waardplant, dan wordt voor deze stoffen wel een sterke aangeboren respons verwacht. Een eiparasitoïde heeft nog enkele speciale beperkingen bij het zoeken van de gastheer. De parasitoïde is klein en heeft een korte levensduur. De gastheer is extra moeilijk te vinden, omdat eieren onbewegelijk zijn en de plant meestal niet beïnvloeden. Het kan zijn dat eiparasitoïden voornamelijk op signalen van het adulte stadium van de gastheer aangewezen zijn.

Het zoekgedrag van *T. brassicae* lijkt inderdaad niet door specifieke signalen van de waardplant of de gastheer beïnvloed te worden. *T. brassicae* in mais vertoont een snelle verspreiding (hoofdstuk 4). Signalen van de gastheer beïnvloeden het landen op planten niet (hoofdstuk 5). Een gerichte vlucht naar een geurbron is bij kleine parasitoïden niet te verwachten, omdat de energiekosten van windopwaarts vliegen te hoog zijn, en de parasitoïde wellicht helemaal niet in staat is de geurbron te bereiken. De aanwezigheid van vluchtige signalen van de gastheer induceren ook geen landingsgedrag. *T. brassicae* brengt normaal een kort bezoek aan de plant na het landen (hoofdstuk 6). Als hierbij vluchtige of niet-vluchtige signalen van de gastheer gevonden worden, wordt de verblijftijd hoger (hoofdstuk 6). Dit leidt tot een verhoogde ontmoetingskans met eimassa's. Schubben van vlinders en motten bevatten een aantal stoffen die bij de meeste soorten aangetroffen worden. Het is dan ook te verwachten dat *T. brassicae* een sterke aangeboren reactie op schubben heeft. In het mais-*O. nubilalis-T. brassicae* systeem zijn schubben een betrouwbare aanwijzing voor de aanwezigheid van eimassa's. Als *O. nubilalis* schubben ontmoet worden, is er een kans van 45%, dat er binnen drie centimeter een eimassa ligt (hoofdstuk 2). Het model voor zoeken in het veld laat zien dat schubben, die dicht bij eimassa's liggen, een significante invloed hebben op de ontmoetingskans (hoofdstuk 9). *T. brassicae* reageert met langere verblijftijden op vluchtige signalen van de gastheer (hoofdstuk 6). In olfactometers blijkt *T. brassicae* te reageren op het sex-feromoon van *O. nubilalis* vrouwtjes. Dit kan het signaal van de gastheer zijn geweest dat verantwoordelijk was voor de langere verblijftijden die in hoofdstuk 6 werden gevonden. Als gevolg van vruchtwisseling paart *O. nubilalis* in Zwitserland niet in de maisvelden. Daarom werd de respons voor vluchtige signalen niet in het model verwerkt. Het is interessant, dat de generalist *T. brassicae* een aangeboren respons heeft voor sex-feromonen, die normaal soort-specifiek zijn. Dit is in strijd met de voorspelling dat generalistische parasitoïden geen sterke aangeboren responsen voor soortspecifieke signalen hebben. *T. evanescens*, ook een generalist, vertoont een respons op de sex-feromonen van

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minstens vijf soorten vlinders en motten. Het is de vraag of sex-feromonen naast de soortspecifieke stoffen ook stoffen bevat die bij elke soort gelijk zijn.

Een maisveld met *O. nubilalis* eimassa's is een zeer homogeen ecosysteem. In dit agro-ecosysteem zullen sommige delen van het zoekgedrag van *T. brassicae* inefficiënt lijken. *T. brassicae* brengt 40-60% van de zoektijd door op de bovenkant van de bladeren (hoofdstuk 6) waar nauwelijks eimassa's voorkomen. Zoals hiervoor beschreven, kan de parasitoïde sex-feromonen niet voor het zoeken gebruiken, omdat ze niet in het maisveld voorkomen. Associatief leren van *T. brassicae* zal weinig effect hebben op de ontmoetingskansen in het maisveld. Een versterkte respons op de geur van mais zal de kans eimassa's te ontmoeten niet verhogen. Het kan voor een tijdelijke verlaging van de dispersie zorgen, als de wespen op alle maisplanten in het veld langer blijven. Het model voor zoeken in het veld laat zien, dat een sterkere reactie op schubben ook de ontmoetingskans niet verhoogt (hoofdstuk 9).

In een natuurlijk ecosysteem kan het zoekgedrag van *T. brassicae* efficiënter zijn. Het zoekgedrag van *T. brassicae* is geëvolueerd in heterogene ecosystemen, met een grote variatie aan gastheren, gastheerdichtheden en waardplanten. Als gastheren van verschillende soorten ontmoet kunnen worden, is het nuttig als het zoeken zich niet tot één zijde van het blad beperkt. Hoewel het landen van *T. brassicae* niet door vluchtige signalen beïnvloed wordt (hoofdstuk 5), zijn deze signalen eenvoudiger te detecteren dan schubben en andere contactsignalen (hoofdstuk 6). Daarom zijn sex-feromonen misschien in natuurlijke habitats een belangrijk signaal voor *T. brassicae* voor de aanwezigheid van gastheren, nadat de wesp op een plant geland is. Associatief leren kan ook nuttig zijn in een natuurlijke habitat. *T. brassicae* is in staat geuren van de gastheer en de waardplant te associëren met eimassa's. Dit leidt tot een langer verblijf op de soort plant waarop de eieren gevonden zijn. Het resultaat is dat de wespen zich zullen concentreren in gebieden met een hoge gastheerdichtheid. Een sterkere respons op signalen van gastheren die al eerder gevonden zijn, leidt ook tot een concentratie in gebieden met een hoge gastheerdichtheid.

TOT SLOT

Trichogramma wordt wereldwijd gebruikt voor biologische bestrijding in vele gewassen. Betrouwbare informatie over het succes van de vrijlatingen ontbreekt echter vaak. In West-Europa is de enige grootschalige toepassing van *Trichogramma* de vrijlating van *T. brassicae* tegen *O. nubilalis* in mais. Ik hoop dat dit proefschrift kan bijdragen tot de verbetering van de biologische bestrijding van *O. nubilalis* met *T. brassicae* en tot ontwikkeling van biologische bestrijdingsmethoden met *Trichogramma* tegen andere schadelijke insecten. Ik denk dat de resultaten van dit onderzoek met name voor het ontwikkelen van betere selectie en kwaliteitscontrolemethoden van belang zijn. Een hogere betrouwbaarheid van *T. brassicae* vrijlatingen en efficiëntere massaproductie zijn noodzakelijk, opdat biologische bestrijding ook in de toekomst kan blijven concurreren met andere bestrijdingsmethoden.

De toekomst van de biologische bestrijding van *O. nubilalis* met *T. brassicae* wordt bedreigd door de invoering van genetisch gemanipuleerde mais die *Bacillus thuringiensis* toxine produceert. Deze mais is momenteel geheel resistent tegen *O. nubilalis*, zodat het gebruik van *T. brassicae* overbodig wordt. De massieve aanwezigheid van *B. thuringiensis* toxine in het milieu die grootschalig gebruik van deze maiscultivar zal veroorzaken zal vroeger of later tot een resistentie van *O. nubilalis* leiden, tenzij er vanaf het begin bij de toepassing maatregelen worden genomen om dit te voorkomen. Het zou een aantrekkelijke en duurzamere oplossing zijn om het gebruik van mais met *B. thuringiensis* genen te combineren met velden met andere *B.t*-loze cultivars, waar *T. brassicae* wordt ingezet. Als er op de lange duur een wijdverbreide resistentie tegen *B. thuringiensis* toxine ontstaat, zal men weer op *T. brassicae* moeten teruggrijpen.

APPENDIX 1

Input data for simulation of *T. brassicae* host-finding on a maize leaf

Time

Timer statements in minutes
The length of the simulation is determined by the starting time (STTIM) and the finishing time (FINTIM). The time step of the model (DELT) is usually chosen as 1/10th of the smallest time coefficient of the model.
STTIM = 0
FINTIM = 120
DELT = 0.020

Temperature data

Leaf temperature in °C
Leaf temperature (TEMP) during the experiment.
TEMP = 20

Plant data

Leaf area in cm^2
Size of one side of a maize leaf (AREA).
Large leaf: AREA = 334.6
Medium leaf: AREA = 123.9
Small leaf: AREA = 24.3

Ostrinia nubilalis data

Number of egg masses
Number of egg masses per leaf (EGGMASS)
EGGMASS = 1

Diameter of O. nubilalis egg mass in cm
The diameter of *O. nubilalis* egg masses (EGGDM) was calculated by averaging the length and width of 30 *O. nubilalis* egg masses.
EGGDM = 0.36

Trichogramma brassicae data

Probability for T. brassicae to land on the lower leaf side
The probability for *T. brassicae* to land on the lower leaf side (LSIDE) was measured in direct observations (chapter 6).
LSIDE = 0.5

Width of T. brassicae searching path in cm
The width of the searching path of *T. brassicae* depends on the tactile, visual and olfactory range of the parasitoid. Although visual ranges of 2 mm were found for more distinctive eggs, direct observation showed that *T. brassicae* rarely changes direction close to an egg. It was thus assumed that eggs are only found within the tactile range. The width of the searching path of *T. brassicae* (WIDTH) was found by multiplying the mean head width of 20 *T. brassicae* females by two.
WIDTH = 0.0360

Walking speed of T. brassicae in cm/min
Walking speed of *T. brassicae* (WS) at 20°C. Data from chapter 7.
WS = 19.32 cm/min

Walking activity of T. brassicae
walking activity (ACT) of *T. brassicae* at 20°C. Data from chapter 7.
ACT = 0.64

Coefficient of variation of walking activity
The coefficient of variation of the walking activity (CVACT) was calculated from direct observations of *T. brassicae* (chapter 6) after an arcsin-transformation.
CVACT = 0.1

Residence time of T. brassicae on a leaf in minutes
Mean residence time on leaves without scales (RTL) at 20°C. Measured in validation experiment.
Small leaves: RTL = 10.13
Medium leaves: RTL = 10.69
Large leaves: RTL = 26.57

Residence time of T. brassicae on lower leaf side in minutes
Mean residence time on lower leaf side (RTLOW) at 20°C. Data from chapter 6.
RTLOW = 7.26

Residence time of T. brassicae on upper leaf side in minutes
Mean residence time on upper leaf side (RTUP) at 20°C. Data from chapter 6.
RTUP = 8.48

APPENDIX 2

Input data for simulation of *T. brassicae* host-finding on a maize plant

Time

Timer statements in minutes
See appendix 1.

Temperature data

Leaf temperature in °C
Leaf temperature (TEMP) during the experiment.
TEMP = 18 or TEMP = 25

Plant data

Leaf area in cm^2
Size of one side of a maize leaf (AREA) at each leaf level.

Leaf level	AREA
1	50.0
2	113.6
3	177.2
4	204.0
5	219.4
6	208.5
7	179.1
8	135.9
9	74.9
10	18.6
11	4.6

Number of leaves on a plant
Number of leaves on a plant (MXLEAF)
MXLEAF = 11

Ostrinia nubilalis data

Diameter of O. nubilalis egg mass in cm
See appendix 1.

Trichogramma brassicae data

Distribution of T. brassicae landings over the leaf levels
The landing distribution of *T. brassicae* over the leaf levels (LL) was found in chapter 5.
Level 1 is the leaf level closest to the ground.

Leaf level	proportion of wasps landing
1	0.007
2	0.030
3	0.083
4	0.120
5	0.149
6	0.171
7	0.165
8	0.138
9	0.112
10	0.024
11	0.002

Probability for T. brassicae to land on the lower leaf side
See appendix 1

Width of T. brassicae searching path in cm
See appendix 1

Walking speed of T. brassicae in cm/minute
Walking speed of *T. brassicae* (WS) at 20°C. Data from chapter 7.
18°C: WS = 17.28
25°C: WS = 19.89

Walking activity of T. brassicae
Walking activity (ACT) of *T. brassicae* at 20°C. Data from chapter 7.
18°C: ACT = 0.47
25°C: ACT = 0.88

Coefficient of variation of walking activity
See appendix 1

Residence time of T. brassicae on a leaf in minutes
The relationship between mean residence time on the first leaf after landing (RTL1), leaf temperature (TEMP) and leaf area (AREA) is calculated according to the equation
RTL1 = (1/60)* ALRT * EXP(BLRT)*EXP(CLRT*AREA)/(DLRT+ELRT*TEMP) that was fitted to data from chapter 6 and from the validation experiment for the simulation of searching on leaves.
ALRT = 1.406
BLRT = 6.219
CLRT = 0.002405
DLRT = 0.198
ELRT = 0.033

The mean residence time on the second and subsequent leaves (RTL2) can be calculated from the residence time on the first leaf: RTL2 = RTL1*(ALRT2+BLRT2* TEMP). This is based on data from chapter 6.
ALRT2 = 1.067
BLRT2 = -0.024

Residence time of T. brassicae on a leaf side in minutes
The residence time of *T. brassicae* on the upper (RTUP) and lower (RTLOW) leaf side is influenced by the leaf size (AREA) and by temperature (TEMP). It can be calculated from the mean residence time on a leaf (RTL1), but there is a stronger influence of temperature. Because higher walking speed and activity are higher at higher temperatures, the parasitoid meets the leaf edge more often.
RTUP = (1000*RTL1)/(TEMP^2*BUSI)
RTLOW = (1000*RTL1)/(TEMP^2*BLSI)
BUSI and BLSI are temperature dependent indices. Data from chapter 6 were fitted to calculate the relationship.
At lower temperatures, *T. brassicae* spends more time on the lower leaf side.

temperature	BUSI	BLSI
18	5.04	6.65
25	7.55	6.90

Chance to walk to another leaf
The chance to walk to another leaf, as opposed to terminating the plant visit, of *T. brassicae* (CWALK) is temperature dependent. It also increases with the number of leaves visited. It is calculated using the equation
$$CWALK(N) = (1/(A+B*N))/(1/(A+B*(N-1)))$$
$$CWALK(1) = 1/(A+B)$$
with N = number of leaves visited on the plant.
The equation was fitted to data from chapter 6.

temperature	A	B
18°C	0.61	1.86
25°C	-0.06	5.25

The resulting chances to walk to another leaf are given in the table.

Leaves visited(N)	18°C	25°C
1st	0.40	0.19
2nd	0.57	0.50
3rd	0.70	0.67
4th	0.77	0.75
5th	0.81	0.80
6th	0.84	0.83
7th	0.86	0.86

Walking movements of *T. brassicae* between leaves
All parameters are based on data from chapter 6.

Direction of T. brassicae movement on the stem
The direction in which *T. brassicae* walks on the stem is determined by the following probabilities: the chance to walk upwards when leaving a leaf from the upper side (CWUU) and the change to walk upwards when leaving a leaf from the lower side (CWLU).
CWUU = 0.889
CWLU = 0.567

Leaf side after walking between leaves
The leaf side on which *T. brassicae* starts searching after walking over the stem from another leaf is determined by the following probabilities: change to start searching on the upper side when arriving from above (CAUU) and change to start searching on the upper side when arriving from below (CALU).
CAUU = 0.632
CALU = 0.215

Returning to the same leaf

T. brassicae may return to the same leaf after walking on the stem. The chance that this happens is determined by the following probabilities: the chance to return to the same leaf after leaving from the upper side (CRU) and the chance to return to the same leaf after leaving from the lower side (CRL). Whether a returning parasitoid continues searching on the same leaf side is determined by the following probabilities: the chance to return to the upper leaf side after leaving from the upper side (CRUSU) and the chance to return to the upper leaf side after leaving from the lower side (CRLSU).
CRU = 0.333
CRL = 0.516
CRUSU = 0.778
CRLSU = 0.281

Residence time of T. brassicae on the stem in minutes
The mean residence time of *T. brassicae* on the stem when moving to a different leaf (RTS) is longer than the mean residence time on the stem when returning to the same leaf (RTSR). *T. brassicae* spends only 5% of its total searching time on the stem.
RTS = 1.08
RTSR = 0.86

Reaction of *T. brassicae* to host scale encounters

There are four effects on *T. brassicae* of an encounter with host scales: an increase in the residence time on the leaf where the scales are encountered (IRTL), an increase of the residence time on the leaf side where the scales are encountered (IRTSIDE), an increase in the chance to visit another leaf on the plant (ICWALK) and, if the scales are close to an egg mass, a direct increase in the probability to encounter that egg mass. The last effect is simulated by adding the diameter of the scale patch (KAIDM) to the diameter of the egg mass (EGGDM). All parameters are based on data from chapter 6.

IRTL = 2.0
IRTSIDE = 1.5
ICWALK = 0.20

Data used for validation of plant model

Leaf area in cm²
T. brassicae landing distribution over the leaf levels
O. nubilalis oviposition distribution over the leaf levels
For each leaf level, the area of one leaf side (AREA), the chance for *T. brassicae* to land on that level (LL) and the chance that an *O. nubilalis* egg mass is present (ODIS) are given. All data from the validation experiment for the model for searching on plants.

Large plants

Leaf level	AREA	LL	ODIS
1	50.0	0.007	0.000
2	113.6	0.030	0.000
3	177.2	0.083	0.000
4	204.1	0.120	0.000
5	219.4	0.149	0.222
6	208.5	0.171	0.444
7	179.1	0.165	0.222
8	135.9	0.138	0.111
9	74.9	0.112	0.000
10	18.6	0.024	0.000
11	4.6	0.002	0.000

Small plants

Leaf level	AREA	LL	ODIS
1	25.0	0.006	0.000
2	55.6	0.037	0.000
3	236.6	0.132	0.000
4	137.9	0.184	0.444
5	141.2	0.322	0.444
6	86.6	0.264	0.111
7	4.2	0.056	0.000

Chance for T. brassicae to land on lower leaf side
The chance for *T. brassicae* to land on the lower leaf side (LSIDE) was directly taken from the validation experiment for the model for searching on plants.
Large plants: LSIDE = 0.4
Small plants: LSIDE = 0.5

Data used for validation of effect of scales

Leaf area in cm²
T. brassicae landing distribution over the leaf levels
For each leaf level, the area of one leaf side (AREA) and the chance for *T. brassicae* to land on that level (LL) are given. Landing level of *T. brassicae* is based on results of Chapter 5. Leaf area was measured in the validation experiment for the effect of scales.

Leaf level	AREA	LL
1	217.8	0.007
2	316.5	0.012
3	374.1	0.046
4	525.8	0.103
5	602.6	0.143
6	582.3	0.173
7	535.2	0.181
8	474.2	0.159
9	422.9	0.128
10	218.9	0.042
11	176.2	0.004
12	53.5	0.002

Distribution of egg masses and scales of O. nubilalis
The number of eggs at each leaf level in the validation experiment for the effect of scales
are given. The number and position of the scales are based on the data from Chapter 2.

Leaf level	Egg masses with scales	without scales	Other scale patches upper side	lower side
1	1	0	0	0
2	0	0	0	1
3	1	0	0	0
4	2	0	0	3
5	1	2	1	4
6	1	2	0	1
7	2	1	1	0
8	1	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0

APPENDIX 3

Input data for simulation of *T. brassicae* host-finding in a maize crop

Time

Timer statements in minutes
T. brassicae is only active during daylight. The length of the simulation is determined by the starting time (STTIM) and the finishing time (FINTIM). The time step of the model (DELT) is usually chosen as 1/10th of the smallest time coefficient of the model.
STTIM = 360.
FINTIM = 1260.
DELT = 0.020

Temperature data

Leaf temperature in °C
Leaf temperatures (TEMP) during daylight are based on mean air temperatures of 1993 oviposition period.

Time (hr)	TEMP
6.00	13.5
7.00	15.2
8.00	17.0
9.00	18.7
10.00	20.2
11.00	21.6
12.00	23.1
13.00	24.0
14.00	24.3
15.00	24.2
16.00	23.6
17.00	22.2
18.00	19.7
19.00	15.9
20.00	12.2
21.00	9.5
22.00	9.5

Plant data

Leaf area in cm^2
Size of one side of a maize leaf (AREA) at each leaf level, based on data by Dutton collected in 1993 (unpubl.). Level 1 is the leaf level closest to the ground.

Leaf level	AREA
1	113.9
2	230.5
3	313.8
4	468.9
5	518.7
6	552.7
7	521.6
8	572.8
9	521.6

Number of leaves on a plant
Number of leaves on a plant (MXLEAF)
MXLEAF = 9

O. nubilalis data

Density of O. nubilalis egg masses in egg masses/plant
The density of *O. nubilalis* egg masses per plant (DENSIT) was based on data by Dutton (unpubl.).
DENSIT = 0.1

Diameter of O. nubilalis egg mass in cm
See appendix 1

Number of O. nubilalis plant visits per oviposition
The number of plant visits by *O. nubilalis* (VISEGG) can be calculated from the number of egg masses. This is based on direct observation of *O. nubilalis* (chapter 2). Each plant visit consists of one or more leaf visits.
VISEGG = 5.37

Number of O. nubilalis leaf visits per plant visit
This table gives the probability that a certain number of leaf visits (landings) is made
during a plant visit, based on observations from chapter 2.

number of leaf visits	probability
1	0.741
2	0.119
3	0.044
4	0.030
5	0.015
6	0.012
7	0.010
8	0.009
9	0.007
10	0.006
11	0.004
12	0.003

Distribution of O. nubilalis egg masses over the leaf levels
The distribution of the egg masses over the leaf levels (ODIS) is similar to the distribution
of *O. nubilalis* landings over the leaf levels, observed in the insectarium (chapter 2). Level
1 is the leaf level closest to the ground.

leaf level	proportion of egg masses
1	0.030
2	0.089
3	0.158
4	0.273
5	0.181
6	0.133
7	0.083
8	0.035
9	0.018

Vertical movement of O. nubilalis between leaf visits
Between two landings, *O. nubilalis* may fly to a higher or a lower leaf level (chapter 2).

Change in leaf level	probability
-3	0.032
-2	0.065
-1	0.194
0	0.516
1	0.129
2	0.032
3	0.032

Change for O. nubilalis to leave scales at oviposition and other leaf visits
When *O. nubilalis* lands on the plant, there is a chance that scales will be left behind. This chance is higher at landings where *O. nubilalis* oviposits (CSCALEOV) then at landings where it does not oviposit (CSCALE) (chapter 2).
CSCALEOV = 0.62
CSCALE = 0.098

Chance for O. nubilalis to land on the lower leaf side
The chance that *O. nubilalis* land on the lower leaf side (LSIDEON) was measured in the insectary (chapter 2).
LSIDEON = 0.84

Diameter of an O. nubilalis scale patch in cm
By averaging 83 scale patches found (chapter 2), the mean diameter of a scale patch (PATCHDM) was found. The four large areas covered with scales were not included.
PATCHDM = 0.6

T. brassicae **landing**

Distribution of T. brassicae landings over the leaf levels
The landing distribution of *T. brassicae* over the leaf levels (LL) was found in chapter 5.
Level 1 is the leaf level closest to the ground.

Leaf level	proportion of wasps landing
1	0.007
2	0.030
3	0.083
4	0.120
5	0.149
6	0.171
7	0.165
8	0.138
9	0.137

Probability for T. brassicae to land on the lower leaf side
The probability for *T. brassicae* to land on the lower leaf side (LSIDE) was measured in
direct observations (chapter 6).
LSIDE = 0.5

T. brassicae **searching on leaves**

Width of T. brassicae searching path in cm
See appendix 1

Walking speed of T. brassicae in cm/min
The walking speed (WS) is a linear function of the temperature (chapter 7).

temperature	walking speed
2	0.96
12	11.16
16	15.24
20	19.32
25	24.42
30	29.52
35	34.62
40	39.72

Walking activity of T. brassicae
The walking activity (ACT) is a logistic function of the temperature (chapter 7). At very high temperatures, wasps are no longer active.

temperature	walking activity
0	0.000
5	0.000
7	0.015
9	0.032
11	0.065
12	0.091
14	0.173
16	0.306
18	0.471
20	0.640
22	0.772
24	0.856
25	0.883
27	0.917
29	0.934
31	0.942
33	0.946
35	0.948
40	0.000

Coefficient of variation of walking activity
See appendix 1

Residence time of T. brassicae on a leaf in minutes
See appendix 2

Residence time of T. brassicae on a leaf side in minutes

The residence time of *T. brassicae* on the upper (RTUP) and lower (RTLOW) leaf side is influenced by the leaf size (AREA) and by temperature (TEMP). It can be calculated from the mean residence time on a leaf (RTL1), but there is a stronger influence of temperature. Because higher walking speed and activity are higher at higher temperatures, the parasitoid meets the leaf edge more often.

RTUP = (1000*RTL1)/(TEMP^2*BUSI)

RTLOW = (1000*RTL1)/(TEMP^2*BLSI)

BUSI and BLSI are temperature dependent indices. Data from chapter 6 were fitted to calculate the relationship.

temperature	BUSI	BLSI
0	2.17	6.36
10	2.17	6.36
25	7.55	6.90
40	8.50	7.44

Termination of *T. brassicae* searching on leaves

Chance to walk to another leaf

The chance to walk to another leaf, as opposed to terminating the plant visit, of *T. brassicae* (CWALK) is temperature-dependent. It is calculated using the equation

CWALK(N) = (1/(A+B*N))/(1/(A+B*(N-1)))

CWALK(1) = 1/(A+B)

N = number of leaves visited. The equation was fitted to data from chapter 6.

temperature	A	B
0	0.80	0.80
13	0.80	0.80
14	0.78	0.90
15	0.77	1.10
16	0.74	1.30
17	0.69	1.57
18	0.61	1.86
19	0.51	2.34
20	0.42	2.83
25	-0.06	5.25
30	-0.54	7.68
33	-0.82	9.13
39	-0.92	9.62

Chance to fly away at the end of a plant visit
The chance for *T. brassicae* to fly to another plant at the end of a plant visit, instead of standing still for a longer period of time (CFLY) is temperature dependent (chapter 6). It can be calculated using the following equation
$$CFLY(N) = e^{(A+B*N)}/1+e^{(A+B*N)}$$

N = number of leaves visited during the current plant visit
A = 20.647-1.236*TEMP
B = -29.735+1.664*TEMP

Maximum number of leaf visits on one plant
Because the probability to continue searching on the same plant increases with the number of leaf visits on that plant, an artificial boundary (MXVIS) was set to prevent wasps from spending all their searching time on one plant only.
MXVIS = 60

Walking movements of *T. brassicae* between leaves
See appendix 2

Reaction of *T. brassicae* to host scale encounters
See appendix 2

Data for effect of host density, plant size and leaf temperature

Density of O. nubilalis egg masses in egg masses/plant
The maximum density of *O. nubilalis* egg masses per plant (DENSIT) found in Switzerland north of the Alps was 0.4 (Bigler, unpubl.).
DENSIT = 0.4
DENSIT = 0.35
DENSIT = 0.3
DENSIT = 0.25
DENSIT = 0.2
DENSIT = 0.15
DENSIT = 0.1
DENSIT = 0.05

Temperature data: Leaf temperature in °C
Mean leaf temperatures (TEMP) during daylight from the 25% coldest (1st quartile) to the
25% hottest (4th quartile) days of the *O. nubilalis* oviposition periods 1985-1993.

Time (hr)	1st	2nd	3rd	4th
6.00	12.6	12.9	14.8	15.9
7.00	13.7	14.9	16.0	17.0
8.00	14.5	16.5	18.0	19.5
9.00	15.5	17.9	19.7	22.0
10.00	16.3	19.2	21.4	24.1
11.00	17.2	20.4	22.9	25.9
12.00	17.9	21.9	24.5	27.9
13.00	18.5	22.8	25.7	29.4
14.00	18.5	23.0	25.9	29.8
15.00	18.2	23.0	25.9	29.6
16.00	17.4	22.4	25.1	28.7
17.00	16.7	20.8	23.5	26.7
18.00	14.7	18.3	20.3	23.2
19.00	11.7	14.3	16.0	18.1
20.00	8.6	10.6	11.6	13.0
21.00	7.2	8.5	9.0	8.6
22.00	7.2	8.5	9.0	8.6

Leaf temperatures on the coldest (July 11th, 1993) and hottest (July 25th, 1985) days of the *O. nubilalis* oviposition periods 1985-1993.

Time (hr)	July 11th, 1993	July 25th, 1985
6.00		16.0
7.00	10.1	17.8
8.00	10.5	20.8
9.00	10.8	23.2
10.00	10.5	26.5
11.00	11.8	28.5
12.00	11.8	30.6
13.00	14.3	33.0
14.00	10.4	33.1
15.00	10.1	33.8
16.00	9.7	33.5
17.00	11.0	30.9
18.00	9.9	27.6
19.00	8.1	21.5
20.00	4.9	15.3
21.00	4.3	15.3
22.00	4.3	

Plant size data: leaf area in cm^2

Size of one side of a maize leaf (AREA) at each leaf level, based on data from 1993 by Schubiger (unpubl.) and Garibay (unpubl.). Level 1 is the leaf level closest to the ground.

Leaf	May 23th	June 6th	July 4th	July 18th	August 2nd
1	14.1	14.2	20.8	102.8	157.0
2	21.6	27.3	44.9	172.0	262.8
3	10.8	56.4	83.4	255.0	351.4
4	2.9	95.5	146.4	347.9	441.8
5		120.7	226.7	432.4	527.1
6		120.4	317.9	512.3	581.1
7		117.5	384.1	550.0	586.9
8		85.5	436.2	556.2	544.5
9		34.2	409.0	404.7	468.5
10		10.4	319.0	392.8	335.5
11		1.6	213.5	206.4	183.6
12			125.0	105.3	99.6
13				65.3	37.2
14				38.5	
15				9.9	
Total	49.4	683.7	2840.6	4075.1	4539.8

Curriculum vitae

Bastiaan Paul Suverkropp werd op 5 februari 1964 in Beek (Limburg) geboren. Reeds op jonge leeftijd was hij zeer in insecten geïnteresseerd. Aan de Scholengemeenschap St. Michiel behaalde hij in 1982 zijn Gymnasium-B diploma. Datzelfde jaar begon hij aan de studie Plantenziektenkunde aan de Landbouwuniversiteit Wageningen. Als eerste afstudeervak deed hij een onderzoek naar het effect dat de celsluiting van honingbijen op het binnendringen van broedcellen door de *Varroa*-mijt, bij Drs. J. Beetsma van de vakgroep Entomologie. In 1988 deed hij een stage-onderzoek van zes maanden naar superparasitisme bij de wittevlieg-parasiet *Encarsia formosa* aan het Department of Biology van Beijing Shifan Daxue (Beijing Normal University) in de Volksrepubliek China, onder begeleiding van Prof. Xu Rumei. Zijn tweede afstudeervak deed hij samen met M.T.T. Poolman-Simons bij de sectie Dierecologie van de vakgroep Entomologie, onder begeleiding van Dr. L.E.M. Vet, naar het leervermogen bij de generalistische sluipwesp *Leptopilina heterotoma* en de nauw verwante specialistische soorten *L. boulardi* en *L. fimbriata*. Hij studeerde in 1989 af. Na zijn studie deed hij een half jaar onderzoek aan oriëntatiegedrag van de malariamug *Anopheles gambiae* bij de sectie Vectorbiologie van de vakgroep Entomologie van de Landbouwuniversiteit Wageningen. In 1991 begon hij aan het promotieonderzoek aan de Eidgenössische Forschungsanstalt für landwirtschaftliche Planzenbau te Zürich, waarvan dit proefschift het resultaat is. In de herfst van 1995 deed hij voor de OESO een onderzoek naar de neven-effecten van de vrijlating van *T. brassicae* op vlindersoorten die in aangrenzende semi-natuurlijke habitats leven. Hij is getrouwd met Jannie Atzema en heeft een dochter, Zina.

Resume

Bastiaan Paul Suverkropp was born on February 5th, 1964 in Beek (Limburg), in the Netherlands. He graduated from the Scholengemeenschap St. Michiel in 1982. In the same year, he started studying Crop Protection at Wageningen Agricultural University. His first M.Sc. research project was a study of brood cell capping by honeybees and its effect on the Varroa-mite, under supervision of Dr. J. Beetsma of the Department of Entomology. In 1988, he spent six month in China doing research on superparasitism in the whitefly parasite *Encarsia formosa*, under supervision of Prof. Xu Rumei, at the Department of Biology of Beijing Normal University. He did his second M.Sc. research project, together with M.T.T. Poolman-Simons at the section Animal Ecology of the Department of Entomology, under supervision of Dr. L.E.M. Vet. Its subject was learning behaviour of the generalist parasitoid wasp *Leptopilina heterotoma* and the closely related specialist species *L. boulardi* and *L. fimbriata*. He graduated in 1989. After his studies, he worked for six month on the orientation behaviour of the malaria mosquito *Anopheles gambiae* at the section Vector-biology of the Department of Entomology, Wageningen Agricultural University. In 1991, he started the Ph.D. research project at the Swiss Federal Research Institute for Agronomy in Zurich, Switzerland, that resulted in this thesis. In the autumn of 1995 he did a research project for the OECD on side-effects of *T. brassicae* releases on butterflies living in semi-natural habitats. He is married to Jannie Atzema and has a daughter, Zina.