Chemical Espionage by Parasitic Wasps

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in het bijzonder de Oecologie der Insekten

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Chemical Espionage by Parasitic Wasps

How *Trichogramma* Species Exploit Moth Sex Pheromone Systems

Proefschrift

ter verkrijging van de graad van
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Aan mijn ouders

Aan Donmanike

Stellingen

1. Het inschatten van de mogelijkheden voor toepassing van signaalstoffen in de biologische bestrijding van insektenplagen, en de wijze waarop toepassing dient te geschieden, vereist een goed begrip van de gedragsveranderingen van natuurlijke vijanden onder invloed van dergelijke stoffen. Hiervoor zijn direkte gedragsobservaties aan individuele dieren onontbeerlijk.

2. Een diffusie-olfactometer, zoals bijvoorbeeld het door Ferreira *et al.* (1979) ontwikkelde apparaat, is ongeschikt als instrument voor de bestudering van het oriëntatiegedrag van insekten onder invloed van vluchtige signaalstoffen.

   Ferreira, L.; Pintureau, B. & Voegelé, J. (1979). *Annales de Zoologie et Ecologie animale* **11**, 271-279.

   Dit proefschrift.

3. Indien de feromonale[1] en de kairomonale[2] werking van eispoelsels van *Pieris brassicae* op dezelfde substantie blijken te berusten is dit opnieuw een aanwijzing dat een functionele indeling van signaalstoffen de voorkeur verdient boven een gebaseerd op afkomst[3].

   [1] Klijnstra, J.W. (1982). *Proceedings 5th International Symposium Insect-Plant Relationships (Wageningen, 1982)*, pp. 145-151. Pudoc, Wageningen.

   [2] Noldus, L.P.J.J. & van Lenteren, J.C. (1985). *Journal of Chemical Ecology* **11**, 793-800.

   [3] Dicke, M. & Sabelis, M.W. (1988). *Functional Ecology* **2**, 131-139.

4. Om optimaal gereedschap voor de gebruiker te zijn dienen computerprogramma's zó ontworpen te worden dat de gebruiker slechts hoeft aan te duiden *wat het probleem is*, niet *hoe het opgelost dient te worden*. Hiervan zijn softwareontwikkelaars zich nog te weinig bewust.

   Noldus, L.P.J.J.; van de Loo, E.L.H.M. & Timmers, P.H.A. (1989). *Nature*, in press.

5. Het veelvuldig voorkomen van superparasitering en gastheervoeding door de sluipwesp *Encarsia formosa* doet vermoeden dat effectieve bestrijding van kaswittevlieg kan worden bereikt met geringere aantallen losgelaten sluipwespen dan tot nu toe gebruikelijk.

   Noldus, L.P.J.J. & van Lenteren, J.C. (1989). In *Critical Issues in Biological Control* (Ed. by M. Mackauer, L. Ehler & J. Roland). Intercept, Andover, in press.

6. Het door Vandersteen geschetste beeld van sluipwespen versterkt de wijd verbreide doch onjuiste opvatting dat biologische bestrijding met sluipwespen gemakkelijk tot wespenplagen kan leiden.

   Vandersteen, W. (1987). *Suske en Wiske. 211. De woeste wespen.* Standaard Uitgeverij, Antwerpen, 54 pp.

7. Gezien het korte tijdsbestek waarbinnen afwijzing van ongeschikte bladeren door de kaswittevlieg (*Trialeurodes vaporariorum*) plaatsvindt is het zeer onwaarschijnlijk dat selectie van voedingsplaatsen geschiedt op basis van de inhoud van zeefvaten.

   Noldus, L.P.J.J.; Xu, R.M. & van Lenteren, J.C. (1986). *Journal of Applied Entomology* **101**, 492-507.

   Janssen, J.A.M.; Tjallingii, W.F. & van Lenteren, J.C. (1989). *Entomologia experimentalis et applicata*, in press

8. Het verschil in groeisnelheid van schedelbeenderen tussen mannelijke en vrouwelijke bruinvissen (*Phocoena phocoena*) uit de noordelijke Stille Oceaan en de afwezigheid van een dergelijk verschil bij individuen uit de Noordzee ondersteunen de verdeling van *P. phocoena* in de subspecies *P. p. phocoena* en *P. p. vomerina.*

   Noldus, L.P.J.J. & de Klerk, R.J.J. (1984). *Zoologische Mededelingen Leiden* **58**, 213-239.

9. Het zou de objectiviteit bij de beoordeling van wetenschappelijke manuscripten voor publicatie ten goede komen indien niet alleen de beoordelaar maar ook de auteur anoniem zou blijven.

10. Het grote belang van geurprikkels bij de spionage door sluipwespen vraagt om een geschikte term. De Nederlandse taal dient derhalve - in aanvulling op 'afluisteren' en 'afkijken' - verrijkt te worden met het werkwoord 'afruiken'.

11. Analoog aan een roeiwedstrijd draait wetenschappelijk onderzoek om stellingen: primair is een goede opstelling en een precieze afstelling maar uiteidelijk gaat het om de juiste instelling.

12. Ter adstructie van de begrippen homologie en analogie verdient het werk van Gary Larson een plaats in het universitair curriculum Diermorfologie en Evolutiebiologie.

    Larson, G. (1989). *The Far Side Wall Calendar*. Andrews & McMeel, Kansas City, 365 pp.

13. De bewering dat vrijdag de dertiende geen geschikte dag zou zijn voor belangrijke evenementen is onjuist.

Stellingen behorende bij het proefschrift "Chemical espionage by parasitic wasps: How *Trichogramma* species exploit moth sex pheromone systems" door L.P.J.J. Noldus.

Wageningen, 13 oktober 1989

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Preface

Contrary to what the front page may suggest, what lies in front of you is not the result of the work of a single person but that of many people. At this place I would like to take the opportunity to thank all those who have contributed in one way or another to the completion of this dissertation.

First of all, my supervisor Joop van Lenteren. He was the one who aroused my interest for biological pest control and who guided my graduate research in Leiden. Due to his effort I have been able to work in China and the United States, and to continue and complete my doctoral research in Wageningen. From nearby or at a distance, directly or indirectly, he was always a support and source of inspiration. At the same time he gave me room to broaden my horizons and to conduct scientific research independently. Joop, thanks for the many ways in which you have been a 'promotor' in the literal sense of the word.

Furthermore, I want to thank my colleagues at the department of Entomology for their contribution to my scientific education. I recall with pleasure the inspiring evenings with the PREPAR-club and the many discussions with Louise Vet, Oscar Minkenberg, Ruurd de Jong, Peter Roessingh, Marcel Dicke and Gé Pak. I am very grateful for all their constructive comments on draft versions of various chapters. One particular aspect of the university environment that I have learned to appreciate is working in an international atmosphere. Through the many visiting scientists from abroad I got in touch with new scientific and cultural concepts. Thus I have benefited in several ways from the contact with Ring Cardé, Pingping Chen, Tom Mueller, Yugal Prasad, Bernie Roitberg, Jonathan Schmidt and Junji Takabayashi. I owe special thanks to Dan Papaj and David Karowe, for the many hours they spent on my manuscripts, their important scientific and linguistic advice, and their friendship. Thanks are also due to Maria Boccia, who was of great help during the preparation of one of the chapters; I was pleased to meet her in person after more than a year of correspondence.

During the year in Tifton my research was supervised by Joe Lewis. Due to his enthusiastic support and guidance, as well as that from Jim Tumlinson in Gainesville, it has been a very instructive period. I have enjoyed the friendship of Mike Keller and Ted Turlings and appreciated the contact with Yvonne Drost, Fred Eller, Franck Hérard and Olivier Zanen. In addition I thank Joe Lewis for being an excellent host and for teaching me the basics of snipe hunting.

In Wageningen my research depended on the assistance of numerous people. The men of the instrument shop – Gerrit van den Brink, Barend Tollenaar, Gerard Schuurman and Otto van Geffen – were always willing to help with the construction of experimental set-ups and to alter them over and over again. Thanks to Leo Koopman, Frans van Aggelen, Richard Pieters and Herman Dijkman, plants and insects were always in stock. Especially during the last months of preparation of the dissertation, the photographers Berry Geerligs, Jan Bakker, Wim van Hof and Hein Visser have done a great job with their skillful help. Frederik von Planta and Piet Kostense prepared many beautiful illustrations and designed the exquisite cover. Thanks are also due to Ans Klunder, Truus de Vries, Irene van Nes and Rob van Dijk for their administrative help.

During my research in Wageningen I have enjoyed guiding several graduate students, which was a new and very useful experience for me. The experiments of Roel Potting and Huibert Barendregt have been incorporated into this dissertation. Besides that, the work of Felix Wäckers, Inge de Groot, Koos Buser, Jan Dirven, Eddy Dijkstra, Ans Voesenek, Willem-Jan Boot and Theo Jetten has contributed significantly to the development of my ideas. I hope that they too have learned and benefited from our cooperation.

The past years have taught me how inportant it is to work in an environment where good contacts exist at working as well as personal level. Thanks to those contacts I have always felt genuinely at home at the department of Entomology. The PREPAR-evenings were not only useful but also very pleasant. Besides that, playing squash with Oscar or Dan and playing tennis with Marcel were always good methods to let off steam.

The evenings with the Leids Promovendi Dispuut "Drop-the-S" forced me to widen my horizon to other faculties and put things in perspective, and served as a pleasant preparation for the defence.

A special word of gratitude to my parents. Their continuing involvement, interest and encouragement during my studies and this research have always been an enormous stimulation for me. Well, Dad, here it is, twenty years after Part One: Part Two!

Last but not least I thank Donmanike, my help and stay, wherever I was, for giving a hearing to try-out talks, for rewriting texts into understandable English, and for continous moral and logistic support.

Lucas Noldus
July 1989

Samenvatting

Informatieoverdracht tussen insekten is hoofdzakelijk van chemische aard en verloopt door middel van *signaalstoffen*, ook wel *informatiestoffen* genoemd. Vrouwelijke nachtvlinders geven bijvoorbeeld specifieke vluchtige stoffen af om mannelijke soortgenoten te lokken. Deze stoffen heten *sexferomonen* (φερω = dragen, ὁρμαινω = aanzetten, prikkelen). Eiparasieten – insekten (sluipwespen) waarvan de larven zich ontwikkelen in eieren van andere insekten – maken bij het zoeken naar eieren gebruik van diverse chemische stimuli, waaronder geur- of smaaksporen van het gastheerinsekt. Zulke stoffen, waarvan de functie voordelig is voor de ontvanger maar niet voor de afzender, worden *kairomonen* genoemd (καιροσ = profijt, voordeel). Termen als *feromoon* en *kairomoon* zijn overigens niet verbonden aan stoffen als zodanig, maar aan functies van stoffen. Daardoor kan een en dezelfde stof in de ene context functioneren als een sexferomoon en in een andere context als een kairomoon. Dit proefschrift gaat over eiparasieten die sexferomonen van nachtvlinders als kairomonen gebruiken en zo het chemisch communicatiesysteem van hun gastheer uitbuiten. Men kan hier spreken van *chemische spionage*.

In het hier beschreven onderzoek is het verschijnsel chemische spionage bestudeerd aan de hand van twee systemen, elk bestaande uit een nachtvlinder en bijbehorende eiparasiet. Het ene systeem omvat de kooluil, *Mamestra brassicae*, een algemene mot die vaak een plaag vormt op kool en andere soorten groente, en de parasitaire wesp *Trichogramma evanescens*. Deze insekten komen algemeen voor in Europa en gematigd Azië. Tevens is een Amerikaans systeem bestudeerd: de katoenmot *Heliothis zea*, voorkomend op diverse gewassen en een grote plaag vormend op katoen en mais, en haar parasiet *Trichogramma pretiosum*. *Trichogramma* wespen zijn de meest toegepaste insekten ter wereld ten behoeve van de biologische bestrijding van insektenplagen. Plaagbestrijding met *Trichogramma* vindt momenteel plaats op verscheidene miljoenen hectaren veldgewassen.

In het begin van de jaren tachtig bleek uit veldexperimenten dat de parasiteringsgraad van eieren van de katoenmot (*Heliothis zea*) door van nature aanwezige *Trichogramma* wespen verhoogd kon worden door katoenvelden te behandelen met synthetisch sexferomoon van *Heliothis*. Deze vondst werd al snel gevolgd door speculaties over het gebruik van vlindersexferomonen ter verhoging van parasitering, door parasieten naar plaaggebieden te lokken. Zo zou men twee vliegen in één klap kunnen slaan: verwarring van vlinders door toepassing van sexferomonen zou hand in hand gaan met verhoogde parasitering door *Trichogramma*. Deze speculaties

waren echter slechts gebaseerd op *indirecte* gegevens; het gedrag dat ten grondslag lag aan de reactie van *Trichogramma* op vluchtige signaalstoffen was niet bekend. Het begrijpen van de basisprincipes die de interacties tussen parasiet en gastheer beheersen is van wezenlijk belang voor het vertalen van een bepaald verschijnsel naar een nieuwe plaagbestrijdingsmethode. Het hier beschreven onderzoek is dan ook uitgevoerd om een antwoord te krijgen op de volgende vragen:

1. Vertonen *Trichogramma* wespen een gedragsverandering bij het waarnemen van het sexferomoon van de gastheer?
2. Zo ja, *hoe* reageren deze wespen op het sexferomoon van de gastheer: welke gedragscomponenten zijn te onderscheiden, wat is het oriëntatiemechanisme?
3. Hoe specifiek zijn reacties van *Trichogramma* op geurstoffen?
4. Hoe functioneert het sexferomoon van de gastheer als een kairomoon in het veld: hoe kunnen deze wespen die slechts overdag actief zijn geuren waarnemen die 's nachts door motten worden afgegeven?

Alle experimenten behelsden direkte gedragsobservaties. Dit vereiste een geschikte methode voor het vastleggen, de tijdsregistratie en de analyse van opeenvolgende gebeurtenissen. Aangezien een dergelijk systeem niet voorhanden was aan het begin van het onderzoek, bestond de eerste stap uit het ontwikkelen hiervan. Het resultaat is een geïntegreerd softwarepakket voor computergestuurde registratie en analyse van gedragsgegevens. Het is zó ontworpen dat het geschikt is voor allerlei soorten gedragsonderzoek en verschillende typen computers.

De experimenten zijn voorts afhankelijk geweest van betrouwbare afgiftebronnen van het sexferomoon van de kooluil (*Mamestra*) en de katoenmot (*Heliothis*). Het sexferomoon van *Heliothis zea* is chemisch geïdentificeerd zodat er synthetisch materiaal gebruikt kon worden. De samenstelling van het sexferomoon van *Mamestra brassicae* is echter nog niet met zekerheid vastgesteld. Maagdelijke vrouwtjesmotten geven het sexferomoon af tijdens het 'roepen'; zij kunnen dus voor onderzoeksdoeleinden als afgiftebron van sexferomoon worden gebruikt, mits de omstandigheden waaronder dit gedrag optreedt worden geboden. Derhalve is het 'roepgedrag', de dagritmiek van het roepen, en de invloed van leeftijd en aantal uren licht per dag op het roepgedrag van maagdelijke *Mamestra* vrouwtjes onderzocht. Gebleken is dat bij een 16L:8D lichtcyclus (16 uur licht, 8 uur donker) roepgedrag consistent optreedt gedurende de tweede helft van de derde tot en met de vijfde donkerperiode na uitkomst uit de pop.

De eerste vraag – vertonen wespen een *gedrags*reactie op het gastheersexferomoon – werd onderzocht middels proeven in een vierarmige luchtstroomolfactometer (Figuur 5-1). Het antwoord was positief: *Trichogramma* wespen bleven langer in de zone waardoorheen de geur van roepende vrouwtjesmotten werd gevoerd dan in zones met schone lucht. Deze reactie trad niet op bij de geur van

mannetjesmotten of die van niet-roepende vrouwtjes.

Voor een meer gedetailleerde analyse van het oriëntatiegedrag van *Trichogramma* onder invloed van vluchtige kairomonen (vraag 2) zijn experimenten uitgevoerd in een windtunnel bij lage luchtsnelheid (Figuur 6-1). Een belangrijk resultaat van deze proeven is dat, in tegenstelling tot de verwachting, wespen niet werden *aangetrokken* door de geur van de gastheer. Wanneer ze werden blootgesteld aan licht van bovenaf en een horizontale luchtstroom met schone lucht dan wel lucht met gastheersexferomoon vertoonden de wespen een windopwaartse beweging *onafhankelijk van de geur*, terwijl de geur wel opwaartse vlucht onderdrukte en de loopsnelheid reduceerde. Het resultaat hiervan was een verlengde verblijfstijd op het substraat in geur-beladen lucht. In vliegproeven had het gastheerferomoon tot gevolg dat wespen kort na het wegvliegen weer landden. Deze resultaten zijn een aanwijzing dat deze wespen geurbronnen in het veld niet aktief localiseren. Opeenhoping van wespen in gebieden met plaagaantasting is wellicht eerder gebaseerd op een eenvoudig mechanisme van passief met de wind meedrijven, gevolgd door landing en zoekgedrag op het substraat, met daaropvolgend vertrek afhankelijk van locale windsnelheid en aanwezigheid van signaalstoffen. Een dergelijke strategie lijkt ook het meest geschikt voor deze wespen, die met hun circa 0.5 mm lichaamslengte nagenoeg niet in staat zijn om tegen de wind in te vliegen.

Met de reactie van *Trichogramma* op sexferomonen van motten – doorgaans mengsels van onvertakte koolwaterstoffen – is de vraag gerezen of wespen op elk willekeurig mengsel van dergelijke verbindingen zullen reageren indien dat naast schone lucht wordt aangeboden (vraag 3). Dit bleek niet zo te zijn: *Trichogramma pretiosum* reageerde niet op het sexferomoon van *Spodoptera frugiperda* (een Amerikaanse mot) noch op een willekeurig samengesteld mengsel van verzadigde acetaten. Deze resultaten komen overeen met het feit dat *Heliothis* een algemene gastheer is voor *Trichogramma pretiosum* in het veld, terwijl eieren van *Spodoptera* zeer zelden worden geparasiteerd. Dit laatste is vermoedelijk het gevolg van het feit dat deze mot haar eieren afzet in pakketten die ze bedekt met een dikke laag schubben, die de eieren onbereikbaar maken voor *Trichogramma*.

Zoals de naam al doet vermoeden geven nachtvlinders hun sexferomoon 's nachts af. *Trichogramma* wespen zijn echter alleen overdag actief. Een vraag die dus niet onaangeroerd kon blijven was: "hoe kunnen sexferomonen van motten functioneren als kairomonen voor wespen in het veld?" (vraag 4). In dit onderzoek is nagegaan of adsorptie van vluchtige stoffen aan de waslaag van bladeren de tijdskloof tussen afgifte door motten en waarneming door parasieten kan overbruggen. Wanneer een koolblad werd blootgesteld aan lucht die gevoerd was over een enkel roepend *Mamestra* vrouwtje (zie Figuur 8-1) bleek sexferomoon (waarvan slechts ca. 1×10^{-9} gram/uur wordt afgegeven) zodanig aan het blad te zijn geadsorbeerd dat het blad direkt na de behandeling gedragsreacties veroorzaakte bij zowel

mannelijke motten als bij wespen. *Mamestra* mannetjes werden niet aangetrokken door met geur behandelde bladeren in een windtunnel van een afstand van 1 meter, doch vertoonden duidelijke gedragsreacties op zeer korte afstand en na contact met behandelde bladeren. De verblijfstijd van *Trichogramma* was aanzienlijk langer op aldus behandelde bladeren dan op bladeren die waren behandeld met schone lucht of de geur van een niet-roepend vrouwtje. Op met feromoon behandelde bladeren brachten wespen ook relatief meer tijd door langs de rand en op de onderzijde in vergelijking met contrôlebladeren. Dat laatste is met name interessant, aangezien eieren van *Mamestra* voornamelijk op de onderzijde van bladeren worden gelegd. Alle gedragseffecten traden ook op na een periode van vier uur. Na 24 uur leidden met feromoon behandelde bladeren nog steeds tot verlengde verblijfstijden. Deze resultaten zijn het eerste voorbeeld van reacties van motten op geadsorbeerd sexferomoon afkomstig van één enkele vrouwtjesmot. Daarnaast tonen ze aan dat 's nachts afgegeven sexferomoon lang genoeg kan worden vastgehouden om overdag als stimulus voor zoekende parasieten te dienen.

De resultaten van dit onderzoek hebben mogelijke gevolgen voor het gebruik van *Trichogramma* in de biologische plaagbestrijding. Een van de hoekstenen van succesvolle biologische bestrijding is de selectie van een geschikte natuurlijke vijand. De reactie van *Trichogramma* op gastheersexferomoon zou als een van de criteria kunnen dienen bij de selectie van een soort of stam voor massale loslatingen. Er is echter nog verder onderzoek nodig om vast te stellen in hoeverre reacties van wespen op deze stoffen de kans op het vinden van eieren onder veldomstandigheden verhogen. Een ander belangrijk aspect van biologische bestrijding met *Trichogramma* is massakweek van wespen. Massakweek van natuurlijke vijanden onder onnatuurlijke omstandigheden heeft mogelijk negatieve gevolgen voor de reacties op die signaalstoffen die in het veld van belang zijn. Indien dit gevaar reëel blijkt te zijn dienen reacties op signaalstoffen een standaardonderdeel van kwaliteitscontrôle-procedures te worden. Tot slot kunnen signaalstoffen wellicht gebruikt worden om het gedrag van parasieten te manipuleren. Enerzijds zou dit gedaan kunnen worden in het veld, met nauwkeurige in achtneming van het oriëntatiegedrag van wespen in reactie op dergelijke stimuli. Anderzijds kan het gedrag mogelijkerwijs worden gemodificeerd door wespen voordat ze worden losgelaten in contact te brengen met signaalstoffen, bijvoorbeeld om aangeboren voorkeuren te versterken dan wel om te keren of om ongewenst vertrek uit het loslaatgebied te voorkomen. Het onderzoek naar de rol van dergelijke leereffecten in het zoekgedrag van *Trichogramma* staat echter nog in de kinderschoenen. Alhoewel er nog heel wat werk verzet moet worden, kunnen uiteindelijk de hier geschetste toepassingen van signaalstoffen hopelijk bijdragen tot een groter succes van biologische bestrijding van insektenplagen.

Summary

Interactions between insects are for a great deal mediated by *semiochemicals* (σημεῖον = sign, signal). For instance, female moths release specific volatile chemicals in order to attract males of the same species. These substances are called *sex pheromones* (φερω = to carry, ὁρμαινω = to excite). Egg parasitoids – i.e. insects of which the larvae develop in eggs of other insects – use various chemical cues in their search for hosts, including substances originating from the host insect. Such chemicals, of which the function is advantageous for the receiver but not for the emitter, are called *kairomones* (καιροσ = profit, advantage). The terms *pheromone* and *kairomone* are not attached to substances themselves but to functions of substances. One and the same substance may thus function as a sex pheromone in one context and as a kairomone in a different context. This dissertation deals with egg parasitoids that use moth sex pheromones as kairomones and thus exploit their host's chemical communication system. In other words, they commit *chemical espionage.*

In the research described here, the phenomenon of chemical espionage has been studied in two systems, each consisting of a moth and its egg parasitoid. The first includes the cabbage moth, *Mamestra brassicae,* a common pest on cabbage and other vegetables, and the parasitic wasp *Trichogramma evanescens.* These are common species in Europe and temperate Asia. The second is an American system, namely *Heliothis zea,* a moth with common names related to the crops on which it is a pest (e.g. cotton bollworm, corn earworm or tomato fruitworm), and its parasitoid *Trichogramma pretiosum. Trichogramma* species are the most widely used insects for biological control of insect pests in the world, with areas of application totalling several millions of hectares.

In the early eighties, field experiments showed that rates of parasitism of *Heliothis zea* eggs by naturally occurring *Trichogramma* wasps could be increased by treating cotton plots with synthetic sex pheromone of *Heliothis.* This finding was immediately followed by speculations that moth sex pheromones could be applied to increase levels of parasitism, by attracting parasitoids to target areas. A multitactic strategy to disrupt communication of the pest and to enhance parasitism by *Trichogramma* seemed possible. However, these inferences were merely based on *indirect* evidence; the behaviour underlying responses of *Trichogramma* to volatile semiochemicals was unknown. Understanding the basic principles that govern interactions between parasitoid and host is of basic importance if a given phenomenon is to be translated into a new pest control method. Therefore, the research described

here has been undertaken in order to answer the following questions:

1. Do *Trichogramma* wasps show a *behavioural* response to host sex pheromone?
2. If so, *how* do these wasps respond to host sex pheromone: which behavioural components constitute a response, what is the orientation mechanism?
3. How specific are responses of *Trichogramma* to odours?
4. How does host sex pheromone function as a kairomone in the field: how can these wasps that are only active during the day perceive odours released by moths during the night?

All experiments involved direct observations of behaviour. This required a suitable method for recording, timing and analysing sequences of events. Since such a system was not available, the first step in this study has been to develop one. The result is an integrated software package for computer-aided event recording and data analysis in observational research. It has been designed in such a way that it can be used for many types of behavioural research and with different types of computers.

The experiments have further depended on reliable sources of the sex pheromones of *Mamestra* and *Heliothis*. The sex pheromone of *Heliothis zea* has been chemically identified and a synthetic blend is available, but the composition of the sex pheromone of *Mamestra brassicae* has not yet been established unambiguously. Virgin female moths release sex pheromone while 'calling'; they can thus be used as an experimental source of sex pheromone, as long as the conditions under which this behaviour occurs are provided. Therefore, the 'calling' posture, diel periodicity of calling, and the effect of age and photoperiod on calling behaviour of virgin females of *Mamestra* were studied. The results showed that under a 16L:8D cycle (16 h light, 8 h dark) calling occurs consistently during the second half of the third through the fifth night after emergence from the pupa.

The first question – is there a *behavioural* response of wasps to host sex pheromone? – was studied with experiments in a four-arm airflow olfactometer (Figure 5-1). The answer was positive: *Trichogramma* spent more time in the zones through which the odour of calling female moths was drawn than in zones with clean air. This response was not found with the odour of male moths or non-calling females.

A more detailed analysis of the orientation behaviour of *Trichogramma* in response to volatile kairomones (question 2) has been made in a wind tunnel at low air speed (Figure 6-1). An important result of these experiments is that, contary to expectations, wasps were not *attracted* by host odours. When exposed to overhead light and a horizontal stream of either clean or pheromone-loaded air, wasps exhibited upwind movement *independent of odour*, while odour suppressed upward flight and reduces walking velocity. The overall result was *arrestment*, or increased residence time, on the substrate in odour-loaded air. In flight tests, host sex pheromone caused

wasps to land shortly after take-off. These results suggest that wasps do not actively locate odour sources in the field. Aggregation in pest-infested areas may rather be based on a simple mechanism of passive downwind drift, followed by landing and locomotion, with subsequent take-off dependent on local wind velocity and presence of chemical cues. Such a strategy also seems feasible for these wasps which – due to a size of circa 0.5 mm – are hardly capable of upwind flight.

The response of *Trichogramma* to moth sex pheromones – which are typically mixtures of straight-chain hydrocarbons – raised the question whether wasps would respond to any blend of such compounds when contrasted with clean air (question 3). This turned out not to be the case: *Trichogramma pretiosum* did not respond to the sex pheromone of the fall armyworm moth (*Spodoptera frugiperda*) nor to an arbitrary blend of three saturated acetates. These results correspond with the fact that *Heliothis* is a common field host of *Trichogramma pretiosum*, whereas eggs of *Spodoptera* are very rarely attacked by this parasitoid. The latter is probably due to the fact that the eggs of this moth are laid in batches, covered by a thick layer of scales which cannot be penetrated by the wasps.

Sex pheromones of noctuid moths are released during the night, while *Trichogramma* is only active during the day. A question that could thus not be ignored was: "how can moth sex pheromones function as kairomones for parasitoids in the field?" (question 4). In this study the hypothesis was tested that adsorption of volatiles to the surface wax of plants can bridge the time gap between odour release by moths and perception by parasitoids. When a cabbage leaf was exposed to air passed over a single calling *Mamestra* moth (see Figure 8-1), sex pheromone (released at ca. 1×10^{-9} g/h) was adsorbed onto the leaf surface to such an extent that the leaf subsequently elicited behavioural responses in conspecific male moths as well as in wasps. *Mamestra* males were not attracted to odour-treated leaves in a wind tunnel from a distance of 1 m, but showed significant responses at very close distance and upon contact with treated leaves. On such treated leaves, *Trichogramma* wasps stayed significantly longer than on leaves treated with clean air or air passed over a non-calling female moth. In addition, on pheromone-treated leaves wasps spent relatively more time along the margin and on the leaf underside than on control leaves. The latter is of particular interest, since moth eggs are predominantly laid on the underside of leaves. All effects persisted for at least four hours. After 24 hours, pheromone-treated leaves still caused increased wasp residence times. These results are the first example of responses of moths to adsorbed airborne sex pheromone from a single female moth. In addition they indicate that sex pheromone released during the night can be retained long enough to function as a stimulus for searching parasitoids during daytime.

The results of this study have potential implications for the use of *Trichogramma* as biological control agents. One of the cornerstones of successful biological control is the selection of a suitable natural enemy. The response of *Trichogramma* to host sex pheromone might be used as one of the critria in the selection of a species or strain for mass releases. Obviously, this requires further study to establish to what extent responses of wasps to these chemicals increase their chance to find host eggs under field conditions. Another important aspect of biological control with *Trichogramma* is mass rearing of wasps. Mass rearing of natural enemies under artificial conditions may have a negative impact on responses to those semiochemicals which are important in the field. If this potential danger turns out to be real, responses to semiochemicals should become a standard component of quality-control procedures. Finally, semiochemicals may be used to manipulate the behaviour of parasitoids. This might either be done in the field, with careful consideration of the orientation behaviour of wasps in response to such cues. Alternatively, behaviour could possibly be modified by exposing wasps to semiochemicals prior to release, e.g. to strengthen or reverse innate preferences or to suppress unwanted dispersal out of target areas. However, research into the role of such learning in the foraging behaviour of *Trichogramma* is still in its infancy. Although still a lot of work remains to be done, it is hoped that the eventual application of semiochemicals as outlined here will contribute to a greater success of biological control of insect pests.

Publications

Publications resulting from this dissertation

The chapters of this dissertation have been, or will be, published as the following journal articles:

Chapter 2
Noldus, L.P.J.J. (1989). The Observer: an integrated software package for computer-aided event recording and data analysis in observational research. *Behavior Research Methods, Instruments & Computers*, submitted.

Noldus, L.P.J.J. & Boccia, M.L. (1989). Application of a computer system for observational studies of varying complexity. *Animal Behaviour*, submitted.

Chapter 3
Noldus, L.P.J.J. & Potting, R.P.J. (1989). Calling behaviour of *Mamestra brassicae* (Lepidoptera: Noctuidae): effect of age and photoperiod. *Entomologia experimentalis et applicata*, submitted.

Chapter 4
Noldus, L.P.J.J. & van Lenteren, J.C. (1985). Kairomones for the egg parasite *Trichogramma evanescens* Westwood. I. Effect of volatile substances released by two of its hosts, *Pieris brassicae* L. and *Mamestra brassicae* L. *Journal of Chemical Ecology* **11**, 781-791.

Chapter 5
Noldus, L.P.J.J. (1988). Response of the egg parasitoid *Trichogramma pretiosum* to the sex pheromone of its host *Heliothis zea*. *Entomologia experimentalis et applicata* **48**, 293-300.

Chapter 6
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Chapter 7
Noldus, L.P.J.J.; Lewis, W.J. & Tumlinson, J.H. (1989). Differential response of *Trichogramma pretiosum*, an egg parasitoid of *Heliothis zea*, to various olfactory cues. *Journal of Chemical Ecology*, submitted.

Chapter 8
Noldus, L.P.J.J.; Potting, R.P.J. & Barendregt, H.E. (1989). Moth sex pheromone adsorption to leaf surface: bridge in time for chemical spies. *Physiological Entomology*, submitted.

Chapter 9
Noldus, L.P.J.J. (1989). Semiochemicals, foraging behaviour and quality of entomophagous insects for biological control. *Journal of Applied Entomology*, in press.

Other publications

The author has also contributed to the following publications:

Noldus, L.P.J.J. & van Lenteren, J.C. (1983). Kairomonal effects on searching for eggs of *Pieris brassicae*, *Pieris rapae* and *Mamestra brassicae* of the parasite *Trichogramma evanescens* Westwood. *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **48**, 183-194.

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Xu, R.M.; Noldus, L.P.J.J. & van Lenteren, J.C. (1989). The parasite-host relationship between *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). XXII. Simulation models for the between-plant movement of adult greenhouse whiteflies. *Journal of Applied Entomology*, in press.

1 Chemical Espionage by Parasitic Wasps: Introduction and Overview

Chemicals as Transmitters of Information

Chemicals are by far the most important conveyors of information for insects. Although temperature, humidity, and visual, auditory and tactile cues are also part of the total sensory input to the central nervous system, chemoreception is the dominant perceptive modality. That chemical stimuli play a role in the behaviour of insects has been known for more than two thousand years: the first reports date back to Aristotle (384-322 B.C.) (Morge, 1973). However, the experimental study of behaviour-modifying chemicals did not commence until this century. A great leap forward in insect chemical ecology was the first identification of a sex pheromone, bombykol (Butenandt *et al.*, 1959). Advances in techniques for chemical isolation and identification and the development of a theory of olfactory transmission placed "... the study of chemical communication ... in the earliest stages of a rich and interesting history" (Bossert & Wilson, 1963). The past decades have verified Bossert & Wilson's prediction: the literature now abounds with examples how insects – across all major taxonomic groups and trophic levels – depend on chemical cues in their search for and choice of food, a sexual partner, or a habitat or host for their progeny, and during feeding, marking of resources or the avoidance of danger. Our increasing knowledge of the complex chemical interactions between organisms has called for a set of terms to facilitate communication between the investigators. Chemicals involved in the interactions between organisms are called *semiochemicals* (σημειον = sign, signal) (Nordlund, 1981). Since this term does not exclude poisonous or alimentary substances, Dicke & Sabelis (1988) proposed the term *infochemicals* to define those chemicals that "... in the natural context, convey information in an interaction between two individuals, evoking in the receiver a behavioural or physiological response that is adaptive to either one of the interactants or both". Since in this dissertation poisons or nutrients are not under discussion, the more familiar term semiochemicals is preferably used; in this context it is regarded as a synonym of infochemicals. Semiochemicals are divided into *pheromones*, which mediate interactions between organisms of the same species, and *allelochemicals*, which mediate interspecific interactions (Karlson & Lüscher, 1959; Dicke & Sabelis, 1988) (Figure 1-1).

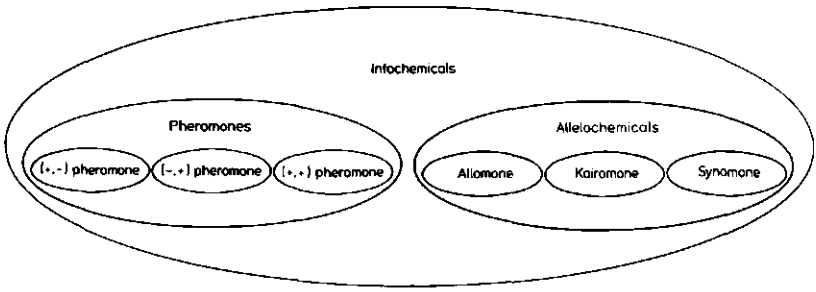

Figure 1-1. Structure of semiochemical (infochemical) terminology (from Dicke & Sabelis, 1988; reprinted with permission).

The word 'adaptive' in the definition of information-transmitting substances points at their evolutionary origin. As for any trait that is formed and maintained by natural selection, this brings up the matter of cost-benefit relationships. Depending on whether the benefit of the response to an allelochemical lies with the organism to whose biology the substance pertains, with the receiver, or with both, the substance is termed an *allomone, kairomone* or *synomone,* respectively (Dicke & Sabelis, 1988). The term kairomone has been criticized as evolutionary unsound because it refers to chemicals not adaptive for the organisms emitting them. For instance, Blum (1977) argued that there is only evidence of a secondary "kairomonal effect" of chemicals which primarily serve as pheromones or allomones. However, each chemically mediated phenomenon deserves independent consideration (Weldon, 1980), all the more since a substance that functions as a kairomone does not necessarily function as a pheromone or an allomone for the emitter. Recently, Dicke & Sabelis (1988) have proposed a similar subdivision of pheromones, based on a cost-benefit criterion, in (+,-) pheromones, (-,+) pheromones and (+,+) pheromones. In the following chapters the term pheromone refers to (+,+) pheromones as defined by Dicke & Sabelis (1988).

Of all semiochemicals, pheromones have traditionally received most attention, in particular sex pheromones. These are used in mate location and courtship behaviour by members of most insect orders (Cardé & Baker, 1984; Tamaki, 1985). The most spectacular examples of insect communication pertain to moth sex pheromones, such as the response of male silk moths (*Bombyx mori*) to a single molecule of female sex pheromone (Boeckh *et al.*, 1965) and the reported attraction of male moths to pheromone-releasing females from distances of several miles (e.g. Rau & Rau, 1929; Collins & Potts, 1932). Other examples of pheromones are the aggregation

pheromones of bark beetles (Birch, 1984), hemipteran alarm pheromones (Nault & Phelan, 1984; Blum, 1985), marking pheromones used by various herbivorous and parasitoid species to mark oviposition sites (van Lenteren, 1981; Prokopy *et al.*, 1984), trail pheromones of ants (Attygalle & Morgan, 1985) and caterpillars (Fitzgerald & Peterson, 1983; Roessingh, 1988) and pheromones of social insects such as bees (Duffield *et al.*, 1984; Free, 1987), ants (Bradshaw & Howse, 1984) and termites (Howse, 1984).

Allelochemicals often act between organisms in different trophic levels. Many kairomones and allomones originate from the members of the primary or secondary trophic level, while the responders belong to a higher level. For instance, plant odours and contact stimuli can serve as kairomones in host-plant location and acceptance by phytophages (Miller & Strickler, 1984; Visser, 1986). The phytophages themselves produce chemicals that are used by parasitoids during host finding and acceptance (Vinson, 1984*a*,*b*, 1985). Similarly, predators depend on prey kairomones during their search for food (Greany & Hagen, 1981; Sabelis & Dicke, 1985). Kairomones can be used to detect the presence of predators and thus to avoid attack. Alternatively, insects can scare off their predators by releasing repellent allomones, which have richly evolved in the Heteroptera (Aldrich, 1988). If such substances prevent the consumption of a toxic prey, they function as synomones rather than allomones. Comparably, plants can prevent herbivory by releasing chemicals that rebut potential feeders or by producing substances that reduce their palatability (Schoonhoven, 1981). The order of action can also be reversed, for instance by predators that release an allomone that attacts the prey (Huheey, 1984).

In the previous examples, either the receiver or the emitter benefitted from the chemical interaction. In addition, semiochemicals acting between the first and third trophic level can be advantageous for both. For instance, plants can release synomones to attract entomophagous insects and thus increase their efficiency in reducing herbivore damage (Vinson, 1981; Nordlund *et al.*, 1988; Williams *et al.*, 1988). Recent research has shown that the production and release of these attractants can be induced by the attack by herbivores, and thus represent a case of indirect inducible plant defence (Dicke & Sabelis, 1989).

Chemical Espionage

It should be stressed that terms used to describe semiochemical interactions between organisms are not attached to particular *substances* but to *functions* of those substances. In many cases there appears to be a *one substance – one function* coupling, but considerable overlap can exist. Evidence is now accumulating that this may be the rule rather than an exception. Functional chemical communication systems can be ex-

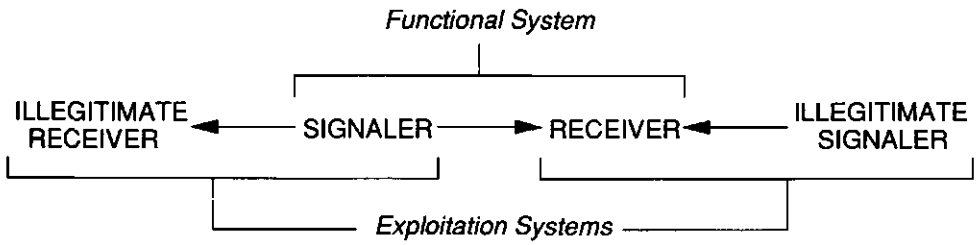

Figure 1-2. Diagram showing how signaling systems can be exploited by competitors and natural enemies in different ways. Exploitation systems depend upon the existence of functional communication systems (from Otte, 1974; reprinted with permission).

ploited on both sides, by illegitimate receivers of the signals, and by illegitimate signalers who deceive the receivers (Otte, 1974; Figure 1-2). Several examples of such exploitation have been described (Haynes & Birch, 1985). A well-documented case of an illegitimate signaler is that of female bolas spiders (*Mastophora* sp.) that attract their prey, male fall armyworm moths (*Spodoptera frugiperda*), by releasing a chemical mimic of the moth's sex pheromone (Eberhard, 1977). Illegitimate receivers of chemical signals – 'chemical spies' – have been unmasked among a variety of insect groups (Vinson, 1984*a*). Several examples are known of parasitoids and predators of bark beetles (Scolytidae) that are attracted by the aggregation pheromones of their hosts and prey, respectively (Wood *et al.*, 1982). Several species of myrmecophilic beetles (i.e. beetles that spend part of their life cycle in ant nests) are able to follow the chemical trail of their host army ants. This kairomonal effect is not detrimental for the emitter, since the beetles are symbionts of the ants (Haynes & Birch, 1985). Some parasitoids locate their hosts via their pheromones used to mark oviposition sites. For instance, *Opius lectus* responds to a substance deposited by *Rhagoletis pomonella* on infested fruit (Prokopy & Webster, 1978), and *Trichogramma* wasps react to a substance present on and around eggs of their butterfly host *Pieris brassicae* which contains an oviposition-deterring pheromone (Noldus & van Lenteren, 1985*b*; Pak & de Jong, 1987). A fourth group of communication signals that are exploited by chemical spies are sex pheromones. An example is the tachinid fly *Trichopoda pennipes* that is attracted by the sex pheromone of the southern green stink bug, *Nezara viridula* (Mitchell & Mau, 1971). Although the sex pheromone systems of Lepidoptera are the most extensively studied class of intraspecific semiochemicals, only a few cases of responses of parasitoids to these cues have been documented. Lewis *et al.* (1982) found that rates of parasitism by *Trichogramma* spp. could be increased by treating

cotton plots with the synthetic sex pheromone of *Heliothis zea*. *Telenomus remus* responds to components of the sex pheromone of its host *Spodoptera frugiperda* (Nordlund *et al.*, 1983).

Well known is the response of *Trichogramma* egg parasitoids to scales of their lepidopteran hosts, scattered around the area where they lay eggs (see *Chapter 9* for more details). The active substances in these scales are various alkanes. Although these kairomones appear merely by-products of the host's metabolism, they may also have an intraspecific function: in at least one moth species, alkanes in female body scales serve as a sex pheromone that elicits copulation attempts in a male upon contact (Grant *et al.*, 1987).

Trichogramma: Biocontrol Agent and Subject of Basic Research

The research described in this dissertation deals with the exploitation of sex pheromone communication systems of moths by *Trichogramma* egg parasitoids. The genus *Trichogramma* (Hymenoptera, Chalcidoidea, Trichogrammatidae) comprises more than 100 species (Voegelé, 1988) which predominantly attack eggs of Lepidoptera. *Trichograma* spp. have traditionally been considered as highly polyphagous (Mokrzecki & Bragina, 1916; Hase, 1925; Salt, 1935; Sweetman, 1958). However, evidence is accumulating that considerable inter- and intraspecific variation in host preference exists (Kot & Plewka, 1974; Stschepetilnikowa, 1974; van Dijken *et al.*, 1986; Pak, 1988; Hassan, 1989).

Trichogramma wasps are used more than any other entomophagous species for biological control of insect pests (Stinner, 1977; King *et al.*, 1985*b*). The Soviet Union and China lead in area of application, with reported use on together more than 20 million hectares (Ridgway & Morrison, 1985; Li, 1984; Gusev & Lebedev, 1988; van Lenteren, 1989). Control with *Trichogramma* is mostly attempted through mass releases, against at least 28 different herbivorous pest species on some 20 different crops (Hassan, 1988; Voegelé *et al.*, 1988). However, because of considerable variability in success of releases and little evidence of consistently successful application of *Trichogramma*, the usefulness of these parasitoids is currently strongly debated (van Lenteren, 1989). Judging by the numerous attempts of biological control with *Trichogramma* over the past 80 years, it is remarkable how little basic research has been conducted on these insects. The key process between release of an entomophagous insect in the field and successful parasitisation of hosts is the entomophage's searching behaviour. This is exactly where large gaps in our knowledge exist. The pioneer of basic research on *Trichogramma* is G. Salt, who made a detailed study of behavioural and physiological aspects of parasitism by

Trichogramma (Salt, 1940 and references therein). He was one of the first to realise the importance of the study of behaviour of parasitoids for their utilization as biological control agents (Salt, 1958). His work has been continued in recent years by, among others, H. Klomp, J.M. Schmidt and G.A. Pak (Klomp *et al.*, 1980; Schmidt & Smith, 1989; Pak, 1988; and references therein). Their studies have focused on the processes that occur after a wasp has contacted a host, i.e. host acceptance and host suitability (*sensu* Doutt, 1959). The process preceding host contact, i.e. searching behaviour, has received less detailed attention. Several authors have recorded responses of *Trichogramma* spp. to plant odours [see Nordlund *et al.* (1988) for review]. The notion that host cues play a role in searching behaviour of *Trichogramma* dates back to Laing (1937), who first showed that wasps responded to "traces" of host moths. The subject was left untouched for more than 30 years, until W.J. Lewis and coworkers initiated an in-depth study of the role of kairomones in the behavioural ecology of *Trichogramma* [see Lewis & Nordlund (1985) for review]. They showed that scales of moths contain a contact kairomone (Lewis *et al.*, 1972), and that a hexane extract of these scales, when properly applied, could increase rates of parasitism by *Trichogramma* (Lewis *et al.*, 1972, 1975*a*). Inhibition of flight and klinokinesis turned out to be the mechanism leading to intensified searching in contaminated areas and increased host finding (Beevers *et al.*, 1981; Morrison & Lewis, 1981; Gardner & van Lenteren, 1986; Shu & Jones, 1988).

Lewis *et al.* are one of the few groups that have investigated the effect of semiochemicals on parasitoid behaviour in the field, in the framework of potential use of these substances in biological control. Initially, a 'blanket treatment' of moth scale extract resulted in increased egg parasitism and seemed a very promising employment strategy for enhancing *Trichogramma*'s field performance (Lewis *et al.*, 1972, 1975*a,b*). However, with increasing plot size, this turned out to be true only at high host densities; at low or medium densities a uniform treatment led to arrestment of wasps in host-free areas and a decrease of effectiveness. The application of kairomone extract around host eggs gave the desired effect of enhanced local search without reduced movement at larger scale (Lewis *et al.*, 1979). This, however, was a very unpractical and labour-intensive method, and certainly not feasible for large-scale application. By simulating an increased host density with sterilized moth eggs at the start of the season, the problem of low density could be bypassed and a simpler kairomone application pattern could be used (Nordlund *et al.*, 1981; Gross *et al.*, 1981*a*, 1984). However, this was still not a practical solution. A correlation between high activity of moths and *Trichogramma* performance (Lewis *et al.*, 1979) suggested that volatile cues might be critical for consistent efficacy of released wasps independent of host density (Lewis *et al.*, 1985). This was supported by the finding that a synthetic sex pheromone blend of *Heliothis zea* increased rates of parasitism by *Trichogramma* spp. in cotton plots (Lewis *et al.*, 1982). Based on these findings, several authors have speculated

about the application of moth sex pheromones to enhance the efficacy of parasitoid releases: it might be possible to attract parasitoids to target areas (Wall, 1984; Vinson, 1986), and a multitactic strategy to disrupt mating of the pest and enhance parasitisation by *Trichogramma* appeared possible (Lewis & Nordlund, 1985). However, so far all evidence about the response of *Trichogramma* to host sex pheromones stemmed from *indirect* observations, and the underlying mechanism was unknown. First, the stimulus could act directly and cause an overt behavioural response, or act indirectly by causing a change in the responsiveness to other stimuli (Dickens & Payne, 1985; cf. *releaser* vs. *primer* pheromones [Wilson, 1963]). Second, assuming an overt behavioural response, the orientation mechanism was not known.

Until recently, direct-observation studies on searching behaviour of *Trichogramma* were rare, and with respect to volatile semiochemicals such studies were absent at the onset of the present investigation. This must partly be due to the minute size of *Trichogramma* wasps (0.5 - 1 mm). Direct observations of searching by very small arthropods are not impossible, judging by detailed behavioural studies of predatory mites (Sabelis & Dicke, 1985). However, in contrast to predatory mites, *Trichogramma* wasps can fly, which complicates the design of bioassays. Notwithstanding these difficulties, the need for detailed studies on the foraging behaviour of and the use of various semiochemicals by *Trichogramma* has been stressed (Lewis & Nordlund, 1985).

Objective of the Research

The aim of this research was to unravel *how* and *why Trichogramma* exploit their host's communication system, i.e. use a volatile moth sex pheromone as a kairomone. It should be obvious from the preceding sections that the motivation for this topic comes from two sides: (1) chemical espionage by egg parasitoids as a phenomenon of fundamental ecological and evolutionary interest, and (2) the use of volatile semiochemicals by an important biological control agent. The order in which these two justifications are listed is not arbitrary: understanding the basic principles that govern host-parasitoid interactions is crucial before a given phenomenon can be translated into a new pest control method. The words 'how' and 'why' point at different approaches of the problem. 'How' refers to the *mechanism*, i.e. the *proximate causation*, while 'why' asks for the *function*, i.e. the *ultimate causation* (Mayr, 1982). As phrased by Hölldobler (1984), "... in order to answer the evolutionary *why* we first have to understand the physiological *how*". Therefore, I have started with analysing the mechanism underlying responses of *Trichogramma* to host volatiles. Subsequently, the function of these responses has been addressed.

Biologically sound bioassays are of prime importance in the study of semiochemically mediated behaviour (Kennedy, 1977; Baker & Cardé, 1984; Tumlinson & Teal, 1987). Especially with respect to volatile semiochemicals the availability of such techniques has been regarded as a limiting factor for progress in research (Lewis & Nordlund, 1985). Therefore, a considerable part of the present study has consisted of the development of proper research methodology and bioassays for the study of olfactory responses in *Trichogramma*. More specifically, I have attempted to answer the following questions:

— Does *Trichogramma* show an overt behavioural response to host sex pheromone?
— If so, how do wasps respond to host sex pheromone: which behavioural components constitute a response, what is the orientation mechanism?
— How specific are olfactory responses of *Trichogramma*?
— Is searching by *Trichogramma* in the field synchronized with release of host odours? If not, how does host sex pheromone function as a kairomone in the field: can diurnally foraging parasitoids perceive odours released by moths during the night?
— What is the adaptive value for parasitoids of responding to host sex pheromone: does it lead to increased host finding and higher reproduction?
— What is the relation between responses of parasitoids to semiochemicals and the quality of those parasitoids as biological control agents?

The Host-Parasitoid Systems

In this study, two *Trichogramma* species were used: *T. evanescens* and *T. pretiosum*. The former is a palearctic species, and has been studied in combination with one of its hosts, *Mamestra brassicae*. *Trichogramma pretiosum* only occurs in the New World; the same applies to *Heliothis zea*, one of its hosts. The experiments with *T. pretiosum* and *H. zea* were conducted at the Insect Biology & Population Management Research Laboratory in Tifton (Georgia, U.S.A.), in close cooperation with the Insect Attractants, Behavior & Basic Biology Research Laboratory in Gainesville (Florida, U.S.A.). Apart from the first experiments with *T. evanescens*, all other experiments were carried out in Wageningen.

Mamestra brassicae and *Trichogramma evanescens*

Mamestra brassicae (Lepidoptera; Noctuidae), the cabbage moth, is a common species throughout Europe (up to central Scandinavia) and temperate Asia to India and Japan (Figure 1-3). Adults are greyish-brown with a wingspan of 34-50 mm (Figure

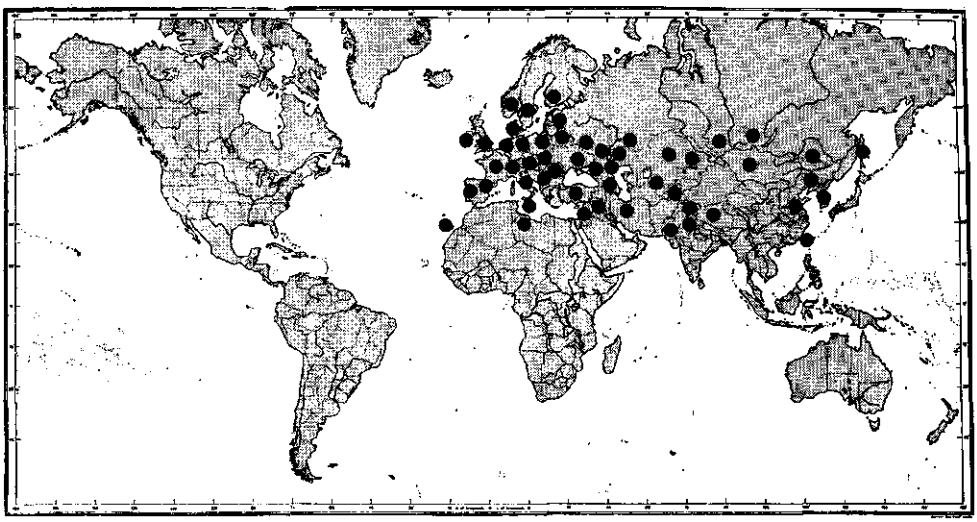

Figure 1-3. Geographic distribution af *Mamestra brassicae* (from CAB, 1984; reprinted with permission).

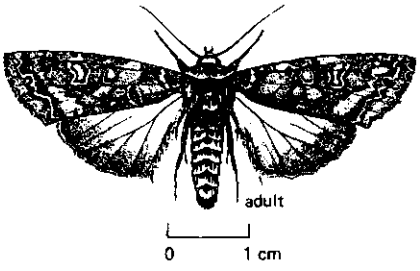

Figure 1-4. Adult moth of *Mamestra brassicae* (from Hill, 1987; reprinted with permission).

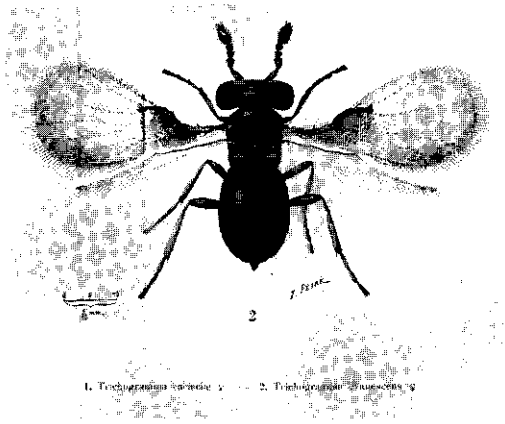

Figure 1-5. Adult female wasp of *Trichogramma evanescens* (from Marchal, 1936).

1-4). *Mamestra brassicae* has a distinct preference for Cruciferae, especially *Brassica* spp. However, the larvae are highly polyphagous, and the host range also includes a large number of weeds, flowers and trees. The number of generations per year is one or two, depending on the latitude. In the Netherlands the species is bivoltine; oviposition mostly occurs between June and September. Eggs are laid in clusters of 15 to over 100 on the underside of leaves. Under usual outdoor temperatures (15-20 °C) emergence takes place after ca. 8 days. The greenish young larvae feed from the leaf lamina and disperse over the plant; older instars darken to brown-black. On cabbage plants they tunnel into the heart of the plant, fouling it with frass. Brussels sprouts plants are particularly vulnerable late in the season when sprouts are present, which are preferably attacked by the larvae. Because of its feeding habits, *M. brassicae* is considered a serious pest of cabbage and other vegetable crops. This information was obtained from the following sources: South (1948), Stokoe (1948), Bonnemaison (1965), Theunissen & Freriks (1983), Carter (1984), Hill (1987) and Pak *et al.* (1989).

The *Trichogramma* species studied in combination with *M. brassicae* was *T. evanescens* Westwood (Westwood, 1833) (Figure 1-5). Although more than 60 host species have been listed for this species (Hase, 1925), distinct host preference exists, which varies among strains of *T. evanescens* (Pak, 1988). A strain is defined as 'the

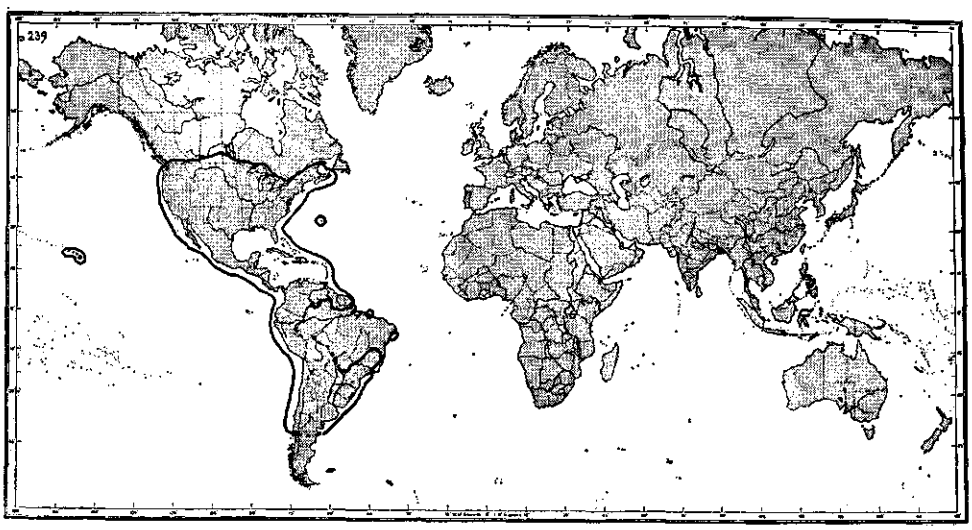

Figure 1-6. Geographic distribution of *Heliothis zea* (from CAB, 1967; reprinted with permission).

cultured offspring of a sample taken from a field population at a certain time and locality' (Diehl & Bush, 1984). This definition was also used by Pak (1988). In the first series of experiments (*Chapter 4*), we used a strain of *T. evanescens* collected in 1981 on *Mamestra brassicae* in the Netherlands (strain no. 11 in Pak & van Heiningen, 1985). According to a recent taxonomic revision, this strain is now referred to as *T. maidis*. However, in this context it is retained in *T. evanescens* for convenience. In all other experiments, I used a strain that had been collected on eggs of *Chilo* sp. in Egypt in 1981 (strain no. 57 in Pak & van Heiningen, 1985). This strain was chosen because it has a preference for *M. brassicae* (Pak, 1988) and has been most effective against *M. brassicae* in field-release experiments (Pak *et al.*, 1989).

Heliothis zea and *Trichogramma pretiosum*

Heliothis zea (Lepidoptera; Noctuidae) is a common moth of the New World, with a distribution covering the U.S.A. and most of Central- and South-America (Hill, 1987; Goodenough *et al.*, 1988; Figure 1-6). Adults are brown moths with a wingspan of 40-44 mm (Figure 1-7). The species is highly polyphagous, which is also reflected by the

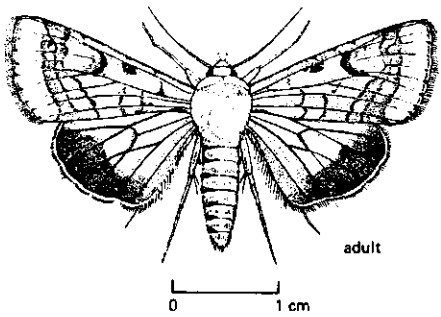

Figure 1-7. Adult moth of *Heliothis zea* (from Hill, 1987; reprinted with permission).

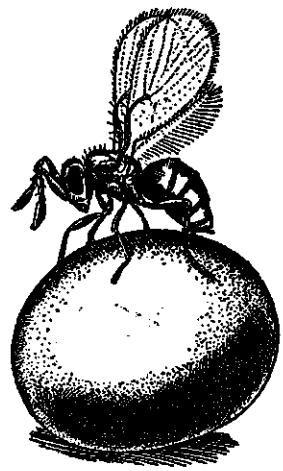

Figure 1-8. Adult female wasp of *Trichogramma pretiosum*, in the process of parasitising an egg of the brown-tail moth (from Howard & Fiske, 1911).

variety of common names of *H. zea*, e.g. cotton bollworm, corn earworm and tomato fruitworm. Besides the three plants mentioned, the host spectrum includes soybeans, tobacco, sorghum, cabbage, various other vegetables and a large number of weeds (Quaintance & Brues, 1905; Young & Price, 1975; Hillhouse & Pitre, 1976; Hill, 1987). *Heliothis zea* is multivoltine, the number of generations varying with the latitude. Mating and oviposition usually starts on the second complete night after emergence (Callahan, 1958; Phillips & Whitcomb, 1962). Eggs are laid singly on all parts of the plant; within-plant distribution of eggs is usually non-random but varies with the plant species (Wilson *et al.*, 1980; Farrar & Bradley, 1985; Terry *et al.*, 1987). Under usual outdoor temperatures, hatching of eggs takes only 2-4 days. The caterpillars go through 5-6 instars, reach a body length of 40-45 mm, and cause most damage by boring into the fruits of plants (e.g. cotton bolls, corn cobs, tomatoes) (Hill, 1987).

Since its original description by Riley (1879), the status of *Trichogramma pretiosum* (Figure 1-8) has been uncertain for a long time (e.g. Peterson, 1930; Flanders, 1968), until the description by Pinto *et al.* (1978). According to these authors, *T. pretiosum* resembles most closely the Old World species *T. evanescens*. Its geographic distribution includes the United States, Mexico, Central America and Columbia (Pak & Oatman, 1982). It is considered the most common member of its genus in North America, with a host range including members of at least 20 genera (Pinto et al., 1986). It is very frequently collected from *Heliothis zea* and the closely related species *H. virescens* (Lopez *et al.*, 1982; Segers *et al.*, 1984; Thorpe, 1984). *Trichogramma pretiosum* has been the subject of most of the semiochemical-related studies by W.J. Lewis and coworkers (Lewis & Nordlund, 1985). It has also been the species of choice in most inundative biological control programs in the U.S.A. (King *et al.*, 1985*a*).

Outline of the Research

A computer system for behavioural observations

All experiments described in this dissertation involved behavioural observations. The type of observations carried out in this study depend on a suitable method for recording and timing sequences of events. If possible, an event-recording system should be flexible with regard to experimental designs so that it can easily be adapted for various types of experiments. In addition, it should be compatible with currently available computer hardware. Since such a flexible system was not available at the start of this study, the first step has been to develop an integrated software package for event recording and data analysis in observational research. This system is described in chapter 2.

Calling behaviour of *Mamestra brassicae*

A study of the exploitation of moth sex pheromone systems by parasitoids depends on reliable odour sources, i.e. the sex pheromones of *Mamestra brassicae* and *Heliothis zea*. The sex pheromone of *H. zea* has been chemically identified and a synthetic blend is available (Klun *et al.*, 1980). However, the composition of the sex pheromone of *M. brassicae* has not yet been established unambiguously. Therefore, virgin female moths were used as source of sex pheromone in all experiments with the cabbage moth. Prior to this, the conditions under which 'calling' behaviour (and release of sex pheromone) occurs consistently had to be established. Thus, the calling posture, diel periodicity of calling and the effect of age and photoperiod on calling behaviour of virgin females of *M. brassicae* were studied (*Chapter 3*). The results showed that under a 16L:8D photoregime (16 h light, 8 h darkness), calling occurred consistently during the second half of the 3rd-5th night after emergence.

Behavioural mechanism of the response to host sex pheromone

The first question was: is there an overt *behavioural* response of wasps to host sex pheromone? Experiments carried out in a four-arm airflow olfactometer gave a positive answer: *T. evanescens* females showed a clear preference for the odour of calling *M. brassicae* moths over clean air, and did not respond to the odour of male moths or recently mated females (*Chapter 4*). Olfactometer experiments with *T. pretiosum* and *H. zea* gave similar results: the wasps spent significantly more time in the olfactometer field containing the sex pheromone released by calling virgin moths, than in control fields (*Chapter 5*). Besides these experiments with noctuid moths, a few experiments were done with a pierid butterfly: *Pieris brassicae* L. Virgin female butterflies were often observed in a posture that somewhat resembled the calling behaviour of a noctuid moth (but without extruded abdominal tip). *Trichogramma evanescens* showed a distinct preference for air passed over such a 'calling' butterfly compared to clean air. Again this response was not found with males or mated females as odour source (*Chapter 4*).

The small test chamber of a four-arm airflow olfactometer limits the expression of behavioural responses, which renders the apparatus less-than-optimal as a bioassay for the analysis of response mechanisms. Therefore, a more detailed study of the orientation behaviour of *Trichogramma* in response to host sex pheromone was made in a wind tunnel (*Chapter 6*). An important result of these experiments was that, contary to what had been suggested in the literature, wasps were not *attracted* by host sex pheromone. When exposed to overhead light and a horizontal air stream containing either odour-loaded air or clean air, wasps exibited (1) upwind anemotaxis *inde-*

pendent of odour, (2) odour-modulated positive phototaxis and (3) odour-induced inverse orthokinesis. Compared to clean air, pheromone-contaminated air increased residence times, walking times and path lengths on a platform, while it decreased walking velocity. In addition, host sex pheromone caused wasps to land shortly after take-off.

Responses to different olfactory cues

Sex pheromones of moths are usually blends of straight-chain aliphatic compounds (Tumlinson & Teal, 1987). This is true for the sex pheromone of *H. zea* (Klun *et al.*, 1980), and probably also for that of *M. brassicae*. Since *Trichogramma* spp. are usually found on several species of Lepidoptera in the field, we wondered if wasps would respond to any randomly chosen blend of straight-chain aliphatic compounds. Apart from that, the responses of *Trichogramma* to host sex pheromone recorded so far were obtained in laboratory set-ups, either a four-arm airflow olfactometer or a wind tunnel, in which host pheromone was tested against clean air. Due to the artificial environment, responses to stimuli recorded in such assays might represent a response to *something* vs. *nothing*, rather than a host-directed response (Jones, 1986).

To investigate these two hypotheses, comparative tests were carried out with *T. pretiosum*: the response to *H. zea* sex pheromone and clean air was compared with the response to two different odours: the sex pheromone of the noctuid moth *Spodoptera frugiperda* and a blend of three saturated acetates that has no known function as a semiochemical (*Chapter 7*). As in the previous experiments, in comparison to clean air, the synthetic sex pheromone of *H. zea* increased residence times, walking times and path lengths on a platform, while it decreased walking velocity; the odour also caused wasps to land shortly after take-off. These responses were not elicited by the sex pheromone of *S. frugiperda* nor by the blend of saturated acetates. These results corresponded with the fact that *H. zea* is a common field host of *T. pretiosum*, whereas eggs of *S. frugiperda* are very rarely attacked by this parasitoid.

Bridge in time between sex pheromone release by the host and its kairomonal function for the parasitoid

Sex pheromones of noctuid moths are released during the scotophase, while *Trichogramma* are only active during the day. A question that could not be ignored was: how can host sex pheromone function as a kairomone for parasitoids in the field? We have examined the possible role of plants in this respect, by testing the hypothesis that adsorption of volatiles to the epicuticular wax of plants can bridge

the time gap between odour release by moths and perception by parasitoids (*Chapter 8*). When cabbage leaves were exposed to air passed over a single calling *Mamestra brassicae* moth, sex pheromone was adsorbed onto the leaf surface to such an extent that it subsequently elicited behavioural respones in conspecific male moths, as well as in *T. evanescens* wasps. Male moths were not attracted to odour-treated leaves from a distance of 1 m, but showed significant responses close to and upon contact with treated leaves. On such treated leaves, *Trichogramma* wasps stayed significantly longer than on leaves treated with clean air or air passed over a non-calling female moth. This effect persisted for at least 24 hours, which indicates that sex pheromone released during the night is retained long enough to be available as a stimulus for parasitoids during daytime.

Why does *Trichogramma* use moth sex pheromone as a kairomone?

All the research described thus far dealt with the question *how* wasps use their host's sex pheromone as a kairomone. We should now turn to the question *why* this happens. It is tempting to interpret the results obtained in the present study in terms of parasitoid searching efficiency. To determine the adaptive value of the response of *Trichogramma* wasps to the sex pheromone of their hosts, we have to answer the question: do responses to moth sex pheromone lead to increased host finding and consequently a higher reproductive success? The field observations of Lewis *et al.* (1982) indicated that wasps responded to host pheromone but, since the number of wasps in the field was not known, the effect on the fitness of individual wasps could not be assessed. Therefore, we designed a series of field experiments in which the spatial distribution of parasitism by mass-released *T. evanescens* of *M. brassicae* eggs, artificially placed in concentric circles around a point source of moth sex pheromone, was related to a random search model. These experiments were unfortunately not successful, mainly due to adverse weather conditions during the summer of 1987 and severe viral infections of the *M. brassicae* rearing, which decimated the source of sex pheromone; the results have not been included in this dissertation. Obviously, additional studies in the field or semi-natural settings are necessary to determine more precisely the pay-off for wasps to respond to host sex pheromone.

Semiochemicals and Biological Control

Semiochemicals and quality of entomophagous insects

In the last chapter (*Chapter 9*) responses of parasitoids to host sex pheromones are placed in a broader context. Are responses to semiochemicals relevant to the quality of entomophagous insects for inundative biological pest control? To answer this question, four approaches arise: (1) behavioural manipulation with semiochemicals, (2) use of natural intraspecific variation in responses to semiochemicals, (3) artificial selection for intraspecific variation in responses to semiochemicals, and (4) application of simulation models of semiochemically mediated foraging behaviour. Apart from (1), these approaches have not yet been applied to *Trichogramma*. Future studies should provide more evidence of the importance of semiochemicals for *Trichogramma* as a biological control agent, i.e. to what extent responses of wasps to these chemicals increase their chance to find host eggs under field conditions.

Response of *Trichogramma* to host sex pheromones: practical implications

Although evidence of the importance of semiochemicals for biological control agents is limited, we can make some predictions about the potential implications of the results of the present study for the practical use of *Trichogramma*.

Essential for successful biological control with *Trichogramma* is the selection of a suitable natural enemy (Pak, 1988). Selection occurs by means of parameters that can be measured in the laboratory and which are related to field performance. Examples are low-temperature resistance (Pak & van Heiningen, 1985) and walking velocity (Bigler *et al.*, 1988). If variation in responses to host sex pheromones in the laboratory is related to field performance, then the response of *Trichogramma* to these cues might be used as one of the critria in the selection of a species or strain for mass rearing and release.

Another important aspect of inundative biological control is mass production of wasps. Mass rearing of natural enemies under artificial conditions may have a negative impact on responses to semiochemicals, but this has hardly been investigated so far (*Chapter 9*). If this turns out to be a real danger, responses to semiochemicals should become a standard component of quality-control procedures. In view of the possible negative effects of mass rearing, also on other traits than responses to semiochemicals, it is recommended that more attention is paid to the quality vs. the quantity of mass-reared insects, in other words to *product control* vs. *production control*.

Finally, semiochemicals may be used to manipulate the behaviour of parasitoids. This area of application has been the subject of much speculation (*Chap-*

ter 9). Behavioural manipulation might be done in the field, but only with careful consideration of the orientation behaviour of wasps in response to such cues. The present study has shown that wasps are probably not attracted to areas where moth sex pheromones are applied; retention of parasitoids in treated areas is more likely. However, from our simple laboratory tests we cannot yet predict how parasitoids will behave on natural plants, in a chemically variable environment. One of the applications that has been envisaged is the combined use of sex pheromones to disrupt pest mating and to increase parasitisation levels by *Trichogramma* (Lewis & Nordlund, 1985). In one study, numbers of beneficial insects were larger in cotton fields treated with the sex pheromone of *Pectinophora gossypiella* than in insecticide-treated fields (Campion *et al.*, 1989). A likely explanation is lower mortality of entomophagous insects in pheromone-treated fields. In addition the pest sex pheromone may have retained the beneficials. Since there were no untreated fields these two effects cannot be ascertained, but they deserve closer attention in future field studies.

Parasitoid behaviour may also be modified by exposing wasps to hosts or host-related cues prior to release. This might be applied to strengthen or to reverse innate preference patterns or to suppress unwanted dispersal out of target areas (*Chapter 9*). The role of prior experiences in the foraging behaviour of parasitoids is one of the most fascinating topics in current behavioural ecology research. While learning in herbivorous insects has been a focus of study for many years (Papaj & Prokopy, 1989), increasing numbers of entomologists are now trying to unravel what, how, when and why parasitoids learn and how their learning may be exploited by man for biological control purposes (Vet, 1988; Turlings *et al.*, 1989). With respect to *Trichogramma*, research into the role of learning in semiochemically mediated foraging behaviour is still in its infancy (Noldus *et al.*, 1988*b*; *Chapter 9*), and we still have a long way to go before we can play the sophisticated game of behavioural modification.

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2 The Observer: an Integrated Software Package for Computer-Aided Event Recording and Data Analysis in Behavioural Research[1]

ABSTRACT – A software package for event recording and data analysis in behavioural research is described. The program, named The Observer, is flexible with regard to experimental design, sampling method and event-recording hardware. It allows any IBM-compatible personal computer to serve as an event recorder. In addition, The Observer can *generate* event recording programs for three types of non-IBM compatible portable computers (TRS-80 Model 100, Tandy 102 and Epson PX-8), and transfer files between the PC and such computers.

Event recorder configurations can be precisely tailored to many different experimental designs. As many as 87 keys can be designated as events, and modifiers can be used to indicate the scope of an event, which can be useful in group observations. The program allows grouping of events in classes, distinction between mutually exclusive and non-exclusive events, and distinction between duration events and frequency events. Continuous behavioural records of one or more focal subjects can be made, timed to 0.1 s. During observations, miscellaneous notes can be made on an on-line electronic notepad. The program also includes on-line error correction. User comments as well as contingent variables can be stored together with the observational data.

Data files produced with the event recorder can be analyzed by the program on the PC. The user can select the type of analysis to be performed as well as the type of output file. The Observer calculates frequency of occurrence and durations for classes of events, individual events or combinations of events. For analysis of concurrence, the user can select the number of nesting levels as well as the order of nesting. Output can be generated in the form of sorted event sequence files, text report files and spreadsheet files. The latter can be used for further data analysis with other software packages.

Due to its flexibility, The Observer is suitable for various types of experimental research as well as for educational purposes. The program is fully menu-driven; it is resistant to most user-caused errors and on-line help screens are provided.

1 To be published as: (1) L.P.J.J. Noldus (1989). The Observer: an integrated software package for computer-aided event recording and data analysis in observational research. *Behavior Research Methods, Instruments & Computers*, submitted, and (2) L.P.J.J. Noldus & M.L. Boccia (1989). Application of a computer system for observational studies of varying complexity. *Animal Behaviour*, submitted.

Introduction

Computers in behavioural research

Many types of behavioural experiments involving direct observation of animals require that sequences of events and the time at which they occur are recorded. After the definition of behavioural parameters (Dunbar, 1976) and the choice of an appropriate sampling procedure (Altmann, 1974; Sackett, 1978; Slater, 1978), a suitable recording method must be selected. Although conventional methods such as recording on checksheets or verbal dictation on tape may still be useful or even indispensable in certain circumstances, computer event recorders are increasingly used (Sidowsky, 1977; Holm, 1978; Lehner, 1979; Martin & Bateson, 1986; Opp & Prokopy, 1986; Horner & Storey, 1989; Noldus *et al.*, 1989). They provide a time-saving aid to the researcher and reduce the occurrence of errors because data do not have to be transcribed. In addition, compared to other methods, they allow observations on a more accurate time base, more rapidly-occurring streams of events can be recorded, and observations can be of greater complexity (with regard to number of different events and/or subjects observed).

Event recorders

Dedicated event recorders

Over the past two decades, several types of event recorders have been developed for use in behavioural research. These usually consist of a specially designed keyboard with a number of pushbuttons and/or switches, the state of which is internally scanned at very short time intervals. Most early event recorders saved observation codes on magnetic tape for later storage and analysis on a mini or mainframe computer (Dawkins, 1971; White, 1971; Beauchamp & Scobie, 1973; Sackett *et al.*, 1973; Celhoffer *et al.*, 1977; Fitzpatrick, 1977; Stephenson & Roberts, 1977; Magyar & Fitzsimmons, 1979; van Lenteren *et al.*, 1980; Baker, 1981). More recently, event recorders have been developed with battery-powered electronic memory, which allows operation apart from a permanent data storage device and thus increases portability. Data can be printed directly (Buckley *et al.*, 1979) or be subsequently transferred to a larger computer (Torgerson, 1977; Smith & Begeman, 1980). Some event recorders have become commercially available, e.g. the Datamyte 900 (Torgerson, 1977), the SSR System 7 (Stephenson & Roberts, 1977), the OS-3 Event Recorder (Gagetalker Corp., Bellevue, WA, U.S.A.), and The Assistant (Human Technologies Inc., St. Petersburg, FL, U.S.A.; Paggeot *et al.*, 1988).

The systems described thusfar suffer from a number of limitations and disadvantages (Unwin & Martin, 1987; Whiten & Barton, 1988). All of them are *dedicated* event recorders, i.e. they can be used solely for event recording. The models that are commercially available are rather expensive, while other types have to be assembled from published electronic circuits, which requires technical expertise and considerable labour input if a reliable end product is to be obtained (cf. Kieras, 1981). The maintenance of such equipment also requires specialized personnel. In addition, most of them are rather inflexible because they have specialized keyboards and contain software in machine code which is inaccessible for non-experts.

Using a microcomputer as event recorder

With the advent of microcomputers a new age has dawned for computer-aided event recording. Since commercially available microcomputers – when used as event recorder – allow data entry directly into the computer (i.e. keyboard keys are redefined as 'events'), custom-designed keyboards are no longer necessary for most purposes. Furthermore, one can rely on dealer networks for maintenance. Most important is the great flexibility offered by microcomputers. They can be programmed in high-level languages such as BASIC, Pascal and C. In addition, most computers are equipped with a printer port, allowing a hard copy of collected data directly after an observation session, and a more or less large screen, allowing direct visual feedback during operation, which is indispensible for trapping and correcting user errors (Flowers & Leger, 1982; Martin & Bateson, 1986). Interaction via the screen – as far as possible during an observation session – also helps to take away the concern about the 'invisibility' of computer-collected data compared to written records (Lehner, 1979; Whiten & Barton, 1988). Most of these options are not available with dedicated event recorders. Finally, microcomputers are already available that are far less expensive than dedicated event recorders (and prices are going down continuously), and they can of course be used for many other purposes than event recording.

The type of hardware selected depends on various aspects of the observational study. For *data collection*, experimental design (sampling method, number of event categories, number of events to be recorded per unit time) and location of observations are decisive factors. Of course, the budget of the researcher plays a role as well. For example, complex continuous observations with many different events, occurring in rapid succession require a fast computer, while a sampling method including irregular, unformatted notes needs the flexibility of a checksheet. For some observational studies in the laboratory a desktop PC can be used, when space is not a constraint. However, experiments in a crowded experimental chamber require the compactness of a portable computer. Observations outdoors always require a portable

device, which can be a laptop computer, e.g. for sedentary observations (Unwin & Martin, 1987), or a hand-held pocketcomputer (Whiten & Barton, 1988). For experiments with multiple observers Pamment & Stephens (1981) designed an apparatus consisting of a number of specialized keyboards connected to a central encoding unit; such experiments can now be handled with inexpensive portable computers used as stand-alone event recorders. The environmental settings in which the equipment is to be used limit the choice of hardware with respect to robustness and resistance to temperature extremes, humidity and dust. Experiments under low-light conditions require a computer with either a backlit or an externally illuminated display.

As far as *permanent data storage* and *analysis* is concerned, currently available personal computers are powerful enough (with respect to memory, disk capacity and processing speed) to perform most of the tasks that required a minicomputer only less than a decade ago. That means that in some circumstances, the same type of device can be used for event recording and data analysis (e.g., an Apple II (Flowers, 1982) or an inexpensive IBM PC 'clone' for data collection and a powerful IBM AT-type computer for data analysis). However, in many circumstances there will be no direct hardware-compatibility between the apparatus on which data are collected and the one on which they are to be analyzed, and some kind of data transfer medium will have to be implemented. For example, currently available IBM-compatible laptop computers are still rather expensive and unable to operate on batteries for more than a few hours, which makes them unsuitable for field work.

In summary, today's researcher can choose from a range of different hardware types for behavioural observations. In fact, any computer that is equipped with a

Table 2-1. Systems for which event recording and data analysis software has been published.

Event recording	Data analysis	Reference(s)
Apple II	Apple II	Bernstein & Livingston (1982), Flowers (1982), Flowers & Leger (1982), Krauss *et al.* (1988)
Apple Macintosh	Apple Macintosh	Deni (1987)
TRS-80 Model 100, Tandy 102	TRS-80 Model 4	Deni *et al.* (1983)
TRS-80 Model 100, Tandy 102	Apple Macintosh	Deni (1987)
Epson PX-8	BBC Model B	Unwin & Martin (1987)
HP41, HP71	–	Whiten & Barton (1988)

real-time clock (which is standard on almost all recent types) can be used as an event recorder. This is also reflected by the variety of commercial microcomputers for which event recording software has been described, ranging from the desktop Apple Macintosh to HP41 and HP71 pocketcomputers (Table 2-1). At the time this chapter was written, the author was not aware of any published event-recording programs for the IBM PC.

Event recording software

To function as an event recorder, a computer must be programmed, and here several problems may arise for the typical behavioural scientist. First of all, writing an efficient event recording program requires programming experience, which may be a major obstacle for many potential users of computer event recorders. An event recording program should be adapted to the machine on which it has to be run, as well as to the experimental design for which it will be used. An event-recording program should preferably do more than simply respond to a keypress with storing a code representing the key and the time at which it was pressed. It is the flexibility of microcomputers and high-level programming languages that facilitates programs with error trapping and correction (e.g. after the user has pressed a non-defined key), distinction between mutually exclusive and non-exclusive events, storage of contingent (independent) variables (e.g. stimulus, treatment, environmental conditions), etc. However, as soon as such features are built into a program, one has to choose between two options: one can make them a fixed part of the program, making the program dedicated for a particular research application, or one can include a facility which allows the user to configure the program according to his/her personal desires. The former approach implies that any change in experimental design will require a change in the event-recording program, which means that the tedious and error-prone process of rewriting and debugging the program must be repeated. On the other hand, programs of more general nature may easily become unsuitable to be run on compact portable computers with limited memory and processing speed. The larger the amount of program code, inherent to the program's flexibility, the less memory space is left for data storage during operation. Also, the more evaluation (by the program) needed to process each individual keypress, the slower the program will become. That means that software above a certain level of sophistication would have to be written in machine code, which is an unattractive alternative for most non-expert programmers (Unwin & Martin, 1987).

Finally, a change from one type of computer to another (e.g. if one wants to change from a lab to a field setting, or if the model one has chosen ceases to be produced or supported) demands a new event recording program. This means either

writing a new program or adapting a program written by someone else. In the latter case there is a fair chance that data files will be in a different format, which makes them incompatible with data files produced so far.

As far as data analysis is concerned, most published programs are part of an 'event recording / data analysis system' designed for a particular set of computers (Table 2-1). However, several of these models are no longer made and most researchers now use other computers. There seem to be no general-purpose data analysis programs available.

An integrated approach

These problems, outlined above, were the impetus for developing an integrated software package for event recording and data analysis, according to the following criteria:

1. It should free the researcher completely from programming.
2. It should be easy to use and intuitively understandable, even for people with little or no computer experience. Wherever possible, descriptive (mnemonic) labels rather than cryptic codes should be used. It should be robust against user errors.
3. It should be flexible with regard to hardware, i.e. it should support a variety of commercially available microcomputers to be used as event recorders. This should include powerful desktop systems as well as simple portable models. The system should also be easily adapted to new types of computers.
4. It should be flexible with regard to experimental designs, i.e. it should support designs ranging from studies with few behavioural categories to complicated group observations. It should be possible to make complete behavioural records, while having the flexibility of a checksheet. The program should support various sampling methods; after all, the event recording method should be adapted to the research design, and not vice versa.
5. It should allow complex and sophisticated event recording on small portable computers, yet with a minimum amount of program code and maximum execution speed.
6. It should analyse observational data and provide the user with statistics of a moderate level of sophistication.
7. For further analysis, output files should be compatible with commercially available software packages.

The result of this effort is a software package, called The Observer. The package was designed for IBM-type personal computers and includes:

a. configuration of a computer as an event recorder, precisely tailored to the user's experimental design;
b. event recording on the PC;
c. data analysis;
d. an *event-recording program generator* for non-IBM compatible portable computers: currently the TRS-80 Model 100, Tandy 102 and the Epson PX-8;
e. file transfer between the PC and non-IBM compatible computers.

Current trends in software development indicate that program generators are increasingly used. As far as the author is aware, The Observer represents the first application of this technique in observational research.

The Observer has been written in Microsoft QuickBASIC 4.5. It includes many assembly-language routines from the QuickPak Professional library, version 1.25 (Crescent Software, Stamford, CT, U.S.A.). The program modules are stand-alone executable files. Details of the system are outlined below.

Hardware used with The Observer

The Observer uses any IBM-type personal computer, including the IBM PC, XT, AT, and PS/2 series and compatible 'clones' (hereafter collectively referred to as IBM PC) as host computer and as event recorder. This includes laptop PC's such as Toshiba or Zenith portables. The IBM PC has been chosen because it has become the standard PC in office and laboratory automation, and with the new PS/2 series hardware compatibility is guaranteed for at least another decade, preventing the program from becoming obsolete.

Besides the IBM PC, three types of small portable computers are supported as event recorders: the TRS-80 Model 100 and its successor the Tandy 102 (Tandy Corp., Fort Worth, Texas, U.S.A.), and the Epson PX-8 (Epson Corp., Japan). For details about the features of the TRS-80 Model 100 see Berman (1983), Deni *et al.* (1983), Cameron (1984) and Gilreath (1985). For the merits of the Epson PX-8 as an event recording device, the reader is referred to Martin & Bateson (1986) and Unwin & Martin (1987). All three computers are inexpensive and offer a compact combination of a programmable computer, a solid-state random-access memory (RAM) that keeps data stored even when the machine is turned off, an RS-232 port for data transfer, and an eight-line display which allows menu-driven operations. They use batteries as well as AC-voltage as power source. Because of these features, they can be used in settings where most personal computers cannot be used, e.g. in the field. The com-

Table 2-2. Comparative data of four different types of portable computers that can be used as event recorder.

	TRS-80 Model 100	Tandy 102	Epson PX-8	Toshiba T1000[1]
CPU	80C85	80C85	Z-80	80C88
Random-access memory (kb)	32	32	64 (max. 192)	512
Data storage	external disk or tape drive	external disk or tape drive	internal tape drive, external disk drive	internal disk drive
Weight (kg)	1.6	1.3	2.3	2.9
Character size (mm)[2]	5.5	5.5	3.5	4.5
No. of screen columns	40	40	80	80
No. of screen rows	8	8	8	25
Screen type	built-in	built-in	flip-back	flip-back
Maximum time on batteries (h)	20	20	15	4

1 The T1000 is the simplest model of the series. Other models are far more expensive, and heavier.
2 Height of capital letters.

puters come with a BASIC interpreter as well as other ROM software, including an editor. For an immediate hard copy of collected data in the field, the Epson can be connected to a battery-operated printer (Unwin & Martin, 1987).

There are some differences between the three types, relevant to use as event recorders. Table 2-2 lists a number of characteristics, together with data for the Toshiba T1000 as an example of a portable PC. The TRS-80 Model 100 and Tandy 102 are based on a 80C85 processor which is considerably faster than the Epson's Z-80. Therefore, the Epson should not be used for observations where speed and accuracy of time recording are crucial. The characters on the screen of the Epson are smaller, and thus harder to read from a distance. Further, the Tandy's are lighter than the other models and can be more easily carried during operation, e.g. in the field where they have proven to be very convenient (Berry *et al.*, 1987). They can be protected against rain or dust by putting them in a transparent plastic bag, so that one can type through the bag. This cannot conveniently be done with computers with a flip-back screen. The Epson has more memory than the Tandy (although a considerable part is occupied by the CP/M operating system). Although this may be a consideration for very long observations, the Tandy's limited memory is usually not a problem. For intermediate data storage in the field one can use a portable disk drive. With a portable

PC, the batteries rather than the data storage capacity will most probably limit the length of observation sessions in remote places.

Description of the System

General features

The Observer is 'menu driven': it centers around a main menu, from which the various program modules and sub-menus can be accessed (Figure 2-1). The program has been designed according to, what can be called, standards for user-friendly software (Simonson, 1986). The system includes program screens with a standardized lay-out, and prompts the user for appropriate inputs, using standard editing keys. The program contains an on-line manual through which the user can browse, and various context-sensitive 'pop-up' help screens. Further, the program is resistant to most user-caused errors, which are trapped and return a relevant error message. Any entry or decision made by the user can be cancelled by pressing the 'Escape'-key. A schematic representation of the system is given in Figure 2-2. The components of the system are described in more detail below. The use of the various features of system configuration and data analysis is illustrated with a hypothetical case study of the social behaviour of monkeys (Appendix A).

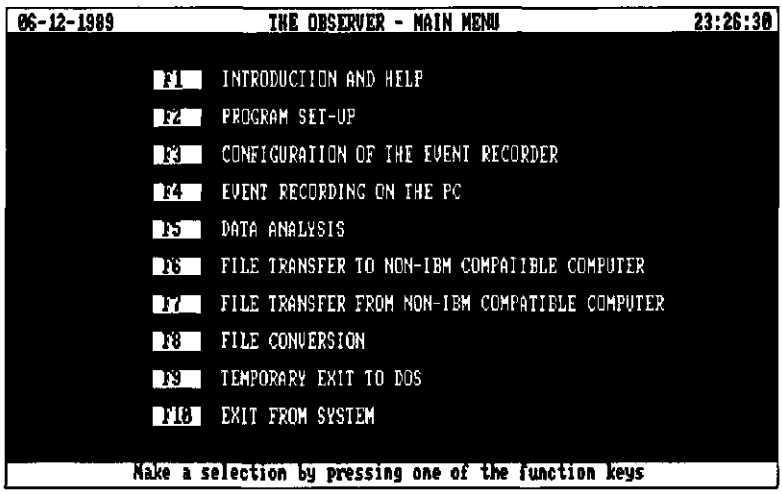

Figure 2-1. Main menu of The Observer.

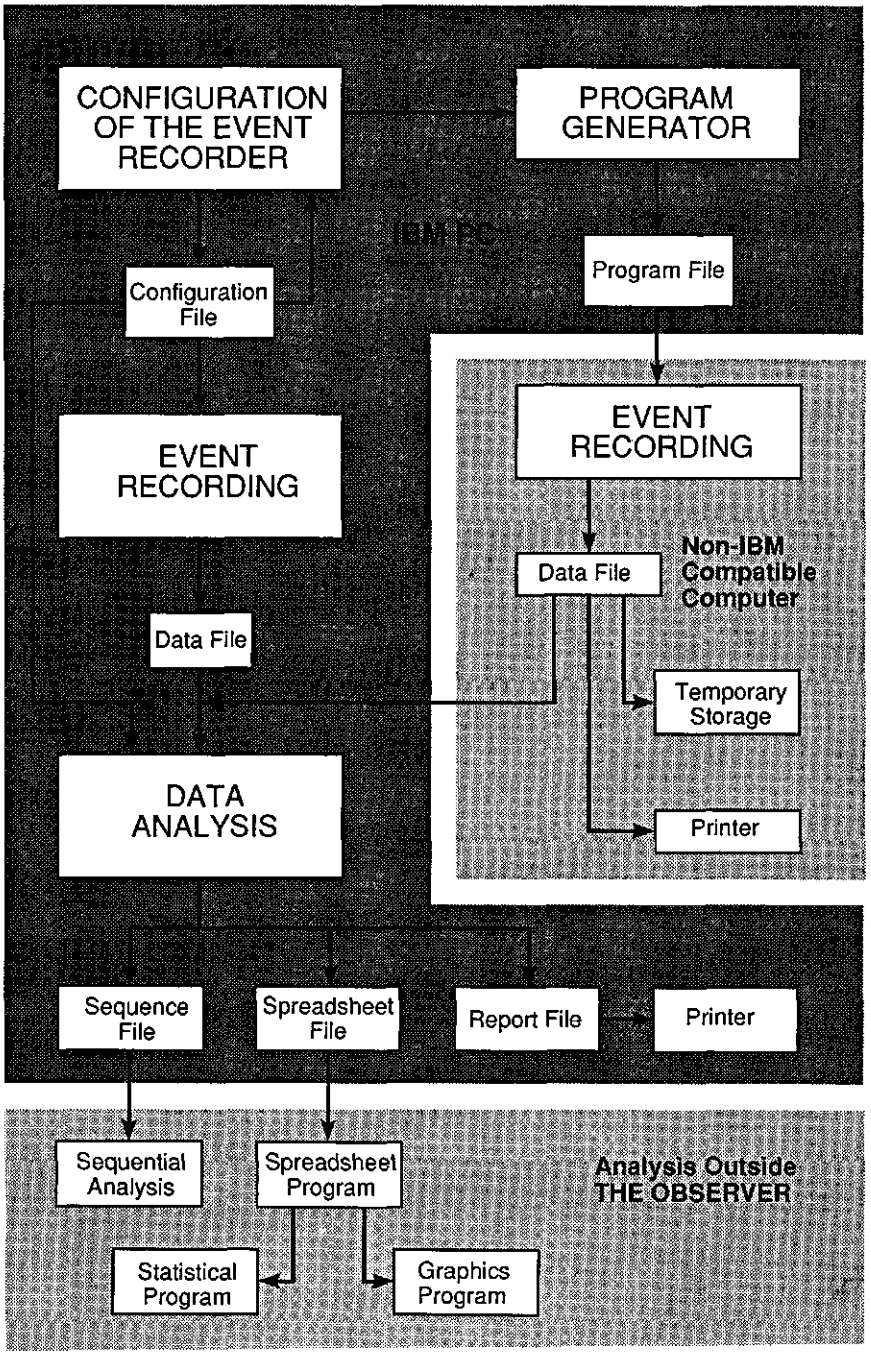

Figure 2-2. Schematic representation of the components of The Observer.

Configuration of the event recorder

This part of the program contains a number of screens in which the user enters information relevant to the configuration of the event recorder. This allows him/her to tailor the event recorder exactly to the experimental design. All information is eventually saved on disk in a *configuration file* which is used by the program at the start of an event recording session on the PC as well as during data analysis (Appendix B). The configuration file can also be retrieved from disk for further editing. A complete event recording configuration has the following elements:

1. *Type of event recorder.* This refers to the type of computer to be used as event recorder. The user can select between IBM PC, TRS-80 Model 100, Tandy 102 and Epson PX-8.
2. *Maximum time for observations.* If one selects 'yes', one can enter a maximum time (in min) after which an observation will be terminated automatically. Apart from this, observations can always be terminated by the user. In the example, the maximum time has been set to 10 min.
3. *Data storage method.* One can let The Observer store data in two ways: by optimizing speed, i.e. as fast as possible, or by optimizing space, i.e. as compact as possible. The former implies a little less efficient memory or disk usage, while the latter takes a little more time. This is only relevant if one of the non-IBM compatible computers is used as event recorder.
4. *Auditory feedback during event recording.* If set to 'yes', The Observer produces a short tone with a unique pitch at the depression of each event key during observations. This can be useful if one has to operate the event recorder 'blindly'.
5. *Modifiers.* These are keys that are used to indicate the limits of an event. For instance, in observations of social interaction, one may not only want to record the behaviour of a focal subject, but also the recipient of certain behaviours. By attaching two modifiers to an event, observations of more than one focal subject are possible, with initiator and recipient of any behaviour recorded. One chooses whether or not to include modifiers in the configuration, and if 'yes', one can enter the total number of keys to be defined as modifiers. This can be any number, as long as the total number of events and modifiers does not exceed 87 (i.e. the total number of alphanumeric keys on the keyboard, minus a few that have a special function in the program). In the example, *beat, touch, play* and *groom* are events, while *John, Rob, Pete* and *Charlie* are modifiers that are used to indicate who is beaten, touched, played with or groomed.
6. *Labels for events and modifiers.* The user has the option to define descriptive labels for display on the event recorder's screen. If this option is selected, the user can also define the length of labels (2-4 characters). If labels are used, the label corresponding with a key rather than the key is displayed during event recording. In

the example, the *e* key is defined as *near pool*, and *po* is defined as a label for that key.

7. *Number of event classes.* Events can be grouped in up to eight *classes*. This has two possible applications:
   a. During data analysis, statistics can be calculated per class of events. With our example, the program could calculate the time spent on *social behaviours* (class with 4 behaviours) vs. *other behaviours* (2 behaviours).
   b. Events within a class can be made *mutually exclusive*. This is discussed below.
8. For each *class*, one has to specify:
   a. The *name* of the class. Examples: *location, locomotion behaviours, social behaviours.*
   b. The *number of events* in the class. This can be any number, as long as the total number of events does not exceed 87. In the example, the class *location* has 3 events.
   c. Whether or not the events in the class are *mutually exclusive*. In a class with mutually exclusive events, only one event can be active at a time. Consequently, an event in such a class, when turned on, automatically turns off the previous event in the same class. This feature can be used to create simple on/off toggles (as present on most dedicated event recorders), as well as to implement multi-level switches. For example: the class *location* could contain the options *inside* vs. *outside*, or – as in our example – *in cage, in yard* and *near pool*. Mutually exclusive event keys are operated by only one keypress, in contrast to non-exclusive duration events, which have to be turned 'on' and 'off' by subsequent keypresses. Events in different classes always operate completely independently from each other.
9. For each *event*, one has to specify:
   a. The *name* of the event. Examples: *in cage, walk, groom.*
   b. The *key* which will be used to signal the event. This can be any alphanumeric key except for a few that have a reserved function. In the example, the *g* is used to signal *shout*, and the *h* is used for *scratch.*
   c. The *label* for the event (if appropriate, see 6).
   d. If the class to which the event belongs is non-exclusive (see 8c), one can label it as a *duration event* or a *frequency event.* The former type refers to events with a meaningful duration, while of the latter only frequency and rate matter. This distinction is similar to what has been referred to as 'states' and 'events' (Altmann, 1974) or 'duration meaningful' vs. 'momentary' behaviours (Sackett, 1978). *Duration events* have to be turned 'on' and 'off' during operation of the event recorder, while for *frequency events*, each keypress signals an occurrence. Mutually exclusive events, by definition, always have a duration. In the example, *beat, touch* and *shout* are frequency events, while all others are duration events.

e. The *number of modifiers* connected to the event. In a configuration with modifiers, any event in a non-exclusive event class can have 0, 1 or 2 modifiers. If a key representing an event with modifier(s) is pressed, the event recorder will prompt for the modifier(s) before being ready to process the next event key. In a group observation, certain events could have 2 modifiers, the first one indicating the initiator and the second one the recipient of the behaviour. In the example, all *social behaviours* have 1 modifier indicating the recipient of the interaction.

10. For each *modifier*, one has to specify:
    a. The *name* of the modifier. This could e.g. be the name of a subject in a group observation (in the example: *John*, *Rob*, etc.).
    b. The *key* for the modifier. See 9b.
    c. The *label* for the modifier.
11. *Contingent variables*. One can store the values of up to 40 contingent (independent) variables together with the observational record. The variables are entered in the form of 'questions' to be 'asked' by the event recorder during operation, either *before* or *after* observations. The answers are then stored in the data file. Examples: name, sex, age, stimulus, treatment, observer, environmental conditions.

Because of the large flexibility and the practically unlimited number of combinations of classes, events, modifiers and contingent variables, the user can tailor the event recorder configuration precisely to his/her experimental design. This approach also makes optimal use of the selected hardware, as the user can choose for desirable optimizations, e.g. with respect to the way data are stored. This is of particular relevance if one of the three supported non-IBM compatible computers is used as event recorder. For example, the use of mnemonic labels for keys makes event recording easier and helps to prevent errors, but it also slows down program execution on those computers, which may be unacceptable in experiments with events occurring in rapid succession. Execution speed is also affected by the number of keys and classes defined. Further, the use of many contingent variables allows the user to store a lot of additional information, but each extra question takes up memory, which may become a limiting factor. But, since all configuration options are facultative, the user can decide where to compromise and where not.

The Observer as a program generator

The *configuration file* contains all the information entered by the user, as described in the previous section, in a coded form. The contents of this file are sufficient to let the PC itself operate as an event recorder. However, non-IBM compatible portable com-

puters need a separate event-recording program. All three computers have a fairly complete BASIC-interpreter, and programs written by The Observer are thus in BASIC. However, the BASIC dialects used by the computers are quite different, which makes the choice of event recorder crucial: an event recording program written for the TRS-80 Model 100 will not run on an Epson PX-8 and vice versa. If one wishes to use either a TRS-80 Model 100, Tandy 102 or Epson PX-8 as event recorder, one can let The Observer *generate* an event-recording program for the selected computer, on the basis of the information entered by the user. Event-recording programs written by The Observer are specifically adapted to the machine and experimental design chosen by the user. They contain only essential program code, are thus very compact, and occupy a minimum amount of memory in the portable computer. For example, if one wishes to have a maximum time for observations, the necessary code will be included in the program; if not, there will not be any unnecessary code taking up memory and slowing down the program. The same applies to all other aspects of the event recorder configuration.

The Observer transfers generated event-recording programs from the PC to the portable computer through the RS-232 interface, using the XON/XOFF protocol for flow control. The user is guided through this process in a step-by-step manner.

Operation of the event recorder

Timing of events

Computer timing of events has received considerable attention in research fields where accurate timing is an absolute necessity, such as operand conditioning or stimulus-response studies. Several software timers with millisecond resolution are now available, for different types of computers (e.g. Perone, 1985; Coyne, 1987; Graves & Bradley, 1988). However, event recording in direct-observation studies does not require timing with such high resolution; the reaction time of the observer will usually make sub-0.1 second resolution useless. Therefore, The Observer stores times rounded to 0.1 s. On the PC this is easily accomplished with QuickBASIC's TIMER function, that returns real time accurate to hundreds of seconds. The BASIC interpreters on the non-IBM compatible portable computers, however, only have a TIME$ function that returns time in whole seconds, while events may have to be recorded in more rapid succession. Therefore, programs generated by The Observer for non-IBM compatible computers contain a simple program loop that counts the number of (calibrated) fractions of a second elapsed between subsequent keypresses (cf. Perera, 1979). With the processing time for each keypress taken into account, an accuracy of 0.1 s can be reached.

Event recording

On the PC, an event recording session takes place within the large program environment, and is started by loading a suitable configuration file. On the non-IBM compatibles, event recording programs are dedicated, i.e. generated for a particular experimental design. In spite of this structural difference, operation of the event recorder – from the user's standpoint – is practically independent of the type of hardware used.

Prior to the first observation, the user can enter comments that are stored together with the data. Further, initial values of contingent variables can be set. Next, the program will prompt the user to start the observation by pressing the first event key. At each valid keypress, the program calculates the time elapsed since the start of the observation, and stores this plus the label attached to the key (or the key itself if no labels are used). On the PC, data are written to a disk file (Appendix C). On the non-IBM compatible portable computers data are stored in a file in the non-volatile random-access memory which serves as a virtual disk.

If the user presses a key representing an event to which one or more modifiers are attached, the program prompts for the appropriate key(s), which are also stored in the data file. If a non-exclusive *duration* event key is pressed for the first time, the event is turned 'on' and it is stored together with a plus sign (+). At the next depression of the same key, it is stored with a minus sign (-), and thus turned 'off'.

After the observation has been ended by the user (or by the program), the program displays a table with additional contingent variables, if such were defined. Subsequently, one can proceed with the next observation or leave the program. In the first case, any of the initial conditions (contingencies) can be edited, if necessary, after which the next observation follows. After terminating the event recording session, the user has access to the data file produced by the program. As there is no online data reduction, the complete event record is available for subsequent analysis.

If a non-IBM compatible portable computer is used, any remaining errors can be corrected by editing the file with the computer's editor, although this can also be done on the PC. Data files can be stored temporarily on disk or tape. For permanent storage and analysis, The Observer uploads files to the PC via the RS-232 interface in a similar manner as described above. During file transfer, the user can simultaneously obtain a print-out of the file on paper.

Screen display and interrupt functions

An effort has been made to provide the user with optimal visual feedback during event recording sessions. During an observation, the computer's screen displays the following items:

— the current time;
— the number of the current observation;
— the time at which the current observation started;
— the amount of free disk space;
— the names of the exclusive-event classes, with for each class the active event and the time at which it started. By definition, there is always one active event for each exclusive-event class.
— the non-exclusive events (labels or keys). These scroll in a window on the screen. They can be followed by one or more modifiers and/or an on/off code (+ or -).

The bottom line of the screen displays a number of labels indicating the following function-key interrupts (depending on hardware and event recorder configuration):

— *Error marker.* This function is explained below.
— *Notepad.* With this function, the user has access to an electronic notepad which can be called at any time. As soon as the 'Notepad-key' is pressed, a memory-resident editor – part of the program – is invoked and any amount of text can be entered, which is stored together with the time at which the notepad was invoked. This feature can be used for any kind of irregular notes during observations. However, it can also be used as a checksheet for any form of time sampling, without interrupting the continuous real-time record.
— *Amount of free disk space.* This figure is not continuously updated, because that would require an extra step in the processing of each keypress. Instead, the user can press the function key to have the figure updated.
— *Status of duration events.* In contrast with mutually exclusive events, of which only one can be active per class, non-exclusive events can occur at any time. Those events scroll off the screen, so that after a while, the user may no longer know whether a certain event is 'on' or 'off'. For that purpose, a function key can be used to display a list of all duration events (in combination with modifiers) that are 'on'.
— *Event frequencies.* This function displays a list of events (in combination with modifiers) that have occurred plus their frequency of occurrence, up-to-date to the last keypress.
— *On-line help.* This function displays a explanatory text about the use of the various keys during event recording (only available during event recording on the PC).
— *End of observation.* With this function key, the user can terminate the observation.

On the PC, the information is displayed in 'pop-up' windows that are dynamic (i.e. with size depending upon the amount of information displayed), of different colour

(gray-scale on monochrome monitors), and overlapping. Underlying screens are restored after a window is closed. These functions use assembly-language routines which makes their execution sufficiently fast to avoid interference with the regular event recording. Of course, this type of functions will usually be invoked only at times during a session that the user can direct his/her attention away from the subject under observation.

Error handling

Two types of errors can occur during observations. The user may press a key that has not been defined as an event (or modifier, if the program is waiting for one). This is detected by the program, which displays an error message and prompts the user to press a valid key. As soon as a valid key has been pressed, the *initial* time (upon which the invalid key was pressed) together with the *valid* event are stored. This type of error can be prevented by using a (e.g. cardboard) keyboard overlay that restricts user access to the defined keys.

One may also erroneously press a valid key while wanting to press another one. This error cannot be trapped by the program. As soon as the user realizes the mistake, the 'Error-marker' function key should be pressed, upon which a marker is written to the data file. The correct key can then be pressed and the observation can continue normally. The error can later be retrieved and corrected.

Data analysis

Many high-quality general-purpose software packages for numerical and statistical analysis on personal computers are available. Therefore, The Observer has been designed to provide the user with an analysis to a moderate level of sophistication (which may be sufficient for many users), and with a suitable interface between raw data files and existing, more sophisticated analysis packages.

Data files produced with the event recorder contain the name of the *configuration file* that describes the event recorder configuration with which the data were collected (Appendices B and C). This file is 'linked' to the data file during analysis. In this way, The Observer can analyze any kind of data file, given that it has access to the appropriate configuration file. By reading the configuration file, the program knows how to interpret the event codes and other recorded information in the data file. After a data file has been loaded, the user can select the type of analysis and different output files. Statistics calculated by The Observer include frequency or occurrence and duration (total, mean, and standard deviation of the mean) (Appendix E).

These can be calculated for:

1. *Classes of events.* Example: 'total time spent on social behaviours'.
2. *Events* (in combination with modifiers, if appropriate). Examples: 'total time spent *grooming*', 'average duration of a *walking* bout', 'frequency of *shouting*', 'total time spent *grooming Pete*'.
3. *Combinations of events.* This refers to a *nested analysis,* applicable to configurations with one or more exclusive-event classes. The program then calculates concurrences up to seven levels of nesting: four exclusive-event classes, non-nested events, and two levels of modifiers can be included in the analysis. Examples: 'frequency of *walking* while *in cage*', 'frequency of *grooming Charlie* (event + modifier) while *sitting* (exclusive event) *in yard* (exclusive event)'. The user can further select:
   — the *number of nesting levels.* For example, if the configuration includes three classes of exclusive events, and a number of non-exclusive events, not all exclusive-event classes have to be included in a nested analysis. If one selects one class, the events of the other classes are treated together with the non-exclusive events as 'non-nested events'.
   — the *order of nesting.* In the example with classes *location* and *locomotion behaviour,* one can obtain an analysis of all events 'by location * locomotion' or 'by locomotion * location'. See Appendix E for the effect of the order of nesting on the arrangement of statistics.

   Thus one data file can be analysed in several different manners.

The user can choose any (or all) of the following output files:

1. *Sequence file.* This is a file containing the complete event protocol listing, for each observation, with exclusive events (if those were defined) sorted per class (Appendix D). This format is suitable for analysis of sequences of events. Because of the many different ways in which sequential analysis can be performed (e.g. Bakeman, 1978; Fagen & Young, 1978; van Hooff, 1982), this has not been included in the program. The sequence file can be transferred to other programs for further analysis.
2. *Report file.* This is a text file (Appendix E) containing
   — the configuration of the event recorder;
   — initial user comments;
   — a report for each observation in the data file, with contingent variables, notes made during the observation (with the time at which they were made), and calculated statistics, according to the type(s) of analysis selected by the user.
3. *Spreadsheet-import file.* This file contains contingent variables and calculated frequencies and durations in a compact tabular format suitable for direct import into an electronic worksheet (e.g. Lotus 1-2-3). Such a format facilitates the calculation of summary statistics pooled across observations, contingency analysis, etc. The

spreadsheet can also serve as an interface with graphics programs or more advanced statistical software.

During analysis, the data file is checked for possible errors (error markers or editing errors). If errors are detected, a relevant error message is displayed, analysis is abandoned, and the user has to edit the data file before it can be analyzed successfully. For this purpose any editor or word processing program of the user's choice can be called from inside The Observer, as long as the computer's memory is sufficient. After successful analysis, the user can use the program to browse through the output file(s) on the computer's screen, or to print the file(s).

Final Remarks

The system presented here has been in operation for more than two years and has been adopted as a useful tool for research and educational purposes by several research groups. Due to the flexibility of the system, most experimental designs can easily be handled. Also, the user can simply alter the experimental design or sampling method, or change to other hardware, while observational data remain compatible. So far, The Observer has been used for the study of the behaviour of, among others, many species of insects (including fruitflies, mosquitoes, leafminers, aphids, moths, dung-inhabiting insects, predatory sawflies and parasitic wasps), predatory mites, molluscs (snails), fish (guppies), primates (rhesus monkeys and other macaques) and other mammals (free-ranging cattle).

As indicated above, the IBM PC has been chosen as host computer for this system to guarantee hardware compatibility with current and future standards in office and laboratory automation and thus to prevent obsoleteness. However, trends in the computer hardware market are hard to predict, and it may be necessary to adapt The Observer to the Apple Macintosh or to UNIX-based systems in the near future. Apart from that, the program is currently being adapted to other small portable computers.

Acknowledgements

Many people contributed in one way or another to the development of this system. In particular, I am indebted to my colleagues and many students at the Department of Entomology, and to M.F. Wallis de Vries (Department of Nature Conservation, Wageningen Agricultural University), E.L.H.M. van de Loo (Department of Clinical and Health Psychology, University of Leiden) and P.H.A. Timmers (Philips Data Systems, Apeldoorn). K. Storey (College of Education, University of Oregon, Eugene, U.S.A.) kindly provided me with unpublised materials. Special thanks to M.L. Boccia (Department of Psychiatry, University of Colorado Health Science Center, Denver, U.S.A.).

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Appendix A

Social behaviour of monkeys:
A hypothetical case study illustrating the use of The Observer.

The study group consists of 4 monkeys (John, Rob, Pete and Charlie) in an enclosed area, consisting of a cage, a yard and an outdoor pool. Observations are 10-min focal samples of one monkey. Prior to an observation, record is made of name, sex, age and treatment code. During the 10-min period, location and behaviour of the focal subject are recorded according to the following classification:

1. **Location** (mutually exclusive duration events)
   a. *In the cage*
   b. *In the yard*
   c. *Near the pool*

2. **Locomotion behaviour** (mutually exclusive duration events)
   a. *Walk*
   b. *Stand still*
   c. *Sit*

3. **Social behaviour** (directed at another individual)
   a. *Beat* – frequency event
   b. *Touch* – frequency event
   c. *Play with* – duration event
   d. *Groom* – duration event

4. **Other behaviours**
   a. *Shout* – frequency event
   b. *Scratch* – duration event

Appendix B

Example of the type of information contained in a *configuration file* used by The Observer, based on the example of Appendix A (MONKEY.CNF). In a real configuration file, information is stored in a more condense, coded form.

Type of event recorder	: IBM PC or compatible
Maximum time per observation	: 10 minutes
Data storage optimization	: Speed
Auditory feedback	: Off
Modifiers	: Yes
Labels for events	: Yes
Label length	: 2

Class	Excl.	Event	Key	Label	Freq/Dur	Modifiers
Location	Yes	In cage	q	ca	dur	0
		In yard	w	ya	dur	0
		Near pool	e	po	dur	0
Locomotion behaviour	Yes	Walk	r	wa	dur	0
		Stand	t	st	dur	0
		Sit	y	si	dur	0
Social behaviour	No	Beat	a	be	freq	1
		Touch	s	co	freq	1
		Play	d	pl	dur	1
		Groom	f	gr	dur	1
Other behaviours	No	Shout	g	sh	freq	0
		Scratch	h	sc	dur	0

Modifier	Key	Label
John	z	jo
Rob	x	ro
Pete	c	pe
Charlie	v	ch

Contingent variables:
Observation no.
Name
Sex
Age
Treatment code

Appendix C

MONKEY.ODF: Example of a data file produced with The Observer. The file is the result of a 10-min focal sample. The comments in the lines (after the quote) are not included in real data files. For meaning of codes, see Appendix B.

```
@CF,MONKEY.CNF                      'name of configuration file
@CO,Obs. of monkey social behavior  'initial comment
  0.0 ya                            'start observation, in yard
  0.0 wa                            'starts walking
 22.5 sh                            'shouts
 43.7 ca                            'in cage
 74.2 st                            'stands
 76.3 sc,+                          'starts scratching
 78.2 sc,-                          'stops scratching
 85.1 sc,+
 90.3 sc,-
 99.1 wa                            'starts walking
110.8 ya                            'in yard
135.8 po                            'near pool
150.2 to,ro                         'touches Rob
155.8 st
162.8 be,ro                         'beats Rob
170.3 wa
178.5 to,pe
180.2 st
184.8 pl,pe,+                       'starts playing with Pete
191.1 si                            'sits
243.0 pl,pe,-                       'stops playing with Pete
250.1 sh
252.2 pl,pe,+
345.8 gr,pe,+                       'starts grooming Pete
349.0 sc,+
452.7 sh
482.9 sc,-
492.1 wa
565.4 sh
600.0 {eo}                          'end of observation
@DT,04-07-1989,11:45:05             'date and time
@QB,1,john,m,6,tr1                  'contingent variables (questions before obs.;
                                                obs. no. = 1, name = John, etc.)
@NO,11:47:06                        'note made at 11:47:06
@NO,falls
@NO,11:51:36
@NO,picks up nut                    'note made at 11:51:36
@NO,{en}                            'end of notes
```

Appendix D

MONKEY.SEQ: Example of a sequence file produced by The Observer. This file contains two columns for the exclusive-event classes 'location' and 'locomotion behaviour', followed by the other (non-exclusive) behaviours. The latter are followed by a modifier and/or an on/off-code (+/-) if appropriate. For meaning of codes, see Appendix B.

```
  0.0 ya, wa
 22.5 ya, wa, sh
 43.7 ca, wa
 74.2 ca, st
 76.3 ca, st, sc, +
 78.2 ca, st, sc, -
 85.1 ca, st, sc, +
 90.3 ca, st, sc, -
 99.1 ca, wa
110.8 ya, wa
135.8 po, wa
150.2 po, wa, to, ro
155.8 po, st
162.8 po, st, be, ro
170.3 po, wa
178.5 po, wa, to, pe
180.2 po, st
184.8 po, st, pl, pe, +
191.1 po, si
243.0 po, si, pl, pe, -
250.1 po, si, sh
252.2 po, si, pl, pe, +
345.8 po, si, gr, pe, +
349.0 po, si, sc, +
452.7 po, si, sh
482.9 po, si, sc, -
492.1 po, wa
565.4 po, wa, sh
600.0 {eo}
@DT,04-07-1989,11:45:05
@QB,1,john,m,6,tr1
@NO,11:47:06
@NO,falls
@NO,11:51:36
@NO,picks up nut
@NO,{en}
```

Appendix E

MONKEY.REP: Example of a report file produced with The Observer. The file is the result of analyzing the data file MONKEY.ODF (Appendix C). The section 'configuration of the event recorder' normally included in report files, has been omitted to save space. The configuration is represented in Appendix B.

```
*******************************************************************************

DATA FILE ANALYZED        : MONKEY.ODF
LINKED CONFIGURATION FILE : MONKEY.CNF
DATE OF ANALYSIS          : 06-13-1989

*******************************************************************************


INITIAL COMMENT

Obs. of monkey social behavior

*******************************************************************************


Date                          :  04-07-1989
Start of observation          :  11:45:05 h
Total duration of observation :  600 sec
Observation no.               :  1
Name                          :  john
Sex                           :  m
Age                           :  6
Treatment code                :  tr1

-------------------------------------------------------------------------------


NOTES MADE DURING OBSERVATION

TIME      NOTE
11:47:06  falls
11:51:36  picks up nut

-------------------------------------------------------------------------------
```

(continued on next page)

FREQUENCIES AND DURATIONS, LUMPED PER EVENT CLASS
(for classes with frequency > 0)

DURATION EVENTS

CLASS	N	DURATION (TOTAL)	DURATION (MEAN)	DURATION (SD)
Location	1	600.0	600.00	0.00
Locomotion behaviour	1	600.0	600.00	0.00
Social behaviour	2	406.0	203.00	204.78
Other behaviours	3	141.0	47.00	75.28

FREQUENCY EVENTS

CLASS	N	DURATION (TOTAL)	DURATION (MEAN)	DURATION (SD)
Social behaviour	3	-	-	-
Other behaviour	4	-	-	-

FREQUENCIES AND DURATIONS, LUMPED PER EVENT
(for events with frequency > 0)

EVENT	MODIFIER	N	DURATION (TOTAL)	DURATION (MEAN)	DURATION (SD)
(Location)					
In cage	-	1	67.1	67.10	0.00
In yard	-	2	68.7	34.35	13.22
Near pool	-	1	464.2	464.20	0.00
(Locomotion behaviour)					
Walk	-	4	248.7	62.17	40.82
Stand	-	3	50.3	16.77	7.27
Sit	-	1	301.0	301.00	0.00
(Social behaviour)					
Beat	Rob	1	-	-	-
Touch	Rob	1	-	-	-
	Pete	1	-	-	-
Play	Pete	2	406.0	203.00	204.78
Groom	Pete	1	254.2	254.20	0.00
(Other behaviours)					
Shout	-	4	-	-	-
Scratch	-	3	141.0	47.00	75.28

(continued on next page)

FREQUENCIES AND DURATIONS, FOR COMBINATIONS OF EVENTS
(for combinations with frequency > 0)

ORDER OF NESTING: Location * Locomotion behaviour

LEVEL 1 LOCATION	LEVEL 2 LOCOMOTION	NON-NESTED EVENT	MODIFIER	N	DURATION (TOTAL)	DURATION (MEAN)	DURATION (SD)
In cage	Walk	-	-	2	42.2	21.10	13.29
	Stand	-	-	1	24.9	24.90	0.00
		Scratch	-	2	7.1	3.55	2.33
In yard	Walk	-	-	2	68.7	34.35	13.22
		Shout	-	1	-	-	-
Near pool	Walk	-	-	3	137.8	45.93	53.90
		Touch	Rob	1	-	-	-
			Pete	1	-	-	-
		Play	Pete	1	107.9	107.90	0.00
		Groom	Pete	1	107.9	107.90	0.00
		Shout	-	1	-	-	-
	Stand	-	-	2	25.4	12.70	2.55
		Beat	Rob	1	-	-	-
		Play	Pete	1	6.3	6.30	0.00
	Sit	-	-	1	301.0	301.00	0.00
		Play	Pete	2	291.8	145.90	132.94
		Groom	Pete	1	146.3	146.30	0.00
		Shout	-	2	-	-	-
		Scratch	-	1	133.9	133.90	0.00

ORDER OF NESTING: Locomotion behaviour * Location

LEVEL 1 LOCOMOTION	LEVEL 2 LOCATION	NON-NESTED EVENT	MODIFIER	N	DURATION (TOTAL)	DURATION (MEAN)	DURATION (SD)
Walk	In cage	-	-	2	42.2	21.10	13.29
	In yard	-	-	2	68.7	34.35	13.22
		Shout	-	1	-	-	-
	Near pool	-	-	3	137.8	45.93	53.90
		Touch	Rob	1	-	-	-
			Pete	1	-	-	-
		Play	Pete	1	107.9	107.90	0.00
		Groom	Pete	1	107.9	107.90	0.00
		Shout	-	1	-	-	-
Stand	In cage	-	-	1	24.9	24.90	0.00
		Scratch	-	2	7.1	3.55	2.33
	Near pool	-	-	2	25.4	12.70	2.55
		Beat	Rob	1	-	-	-
		Play	Pete	1	6.3	6.30	0.00
Sit	Near pool	-	-	1	301.0	301.00	0.00
		Play	Pete	2	291.8	145.90	132.94
		Groom	Pete	1	146.3	146.30	0.00
		Shout	-	2	-	-	-
		Scratch	-	1	133.9	133.90	0.00

****

3 Calling Behaviour of *Mamestra brassicae*: Effect of Age and Photoperiod[1]

ABSTRACT – The calling posture, diel periodicity of calling and the effect of age and photoperiod on calling behaviour were studied in virgin females of the cabbage moth, *Mamestra brassicae* L. During calling, the legs were extended laterally and the whole body was raised at an angle of 0-45° with the substrate. The ovipositor was extruded and pointed downward at an angle of ca. 45° with the abdomen. The wings were raised above the abdomen in a "V" shape, with the upper surface at an angle of 0-45° with the substrate. The antennae were mostly directed hindward and held close to the body. No calling activity was observed during the first scotophase. Most females called for the first time during the 2nd or 3rd scotophase after emergence, regardless of the photoperiod. Moths maintained under 16L:8D started calling significantly later in the scotophase than those maintained under 18L:6D. With increasing age, moths initiated calling significantly earlier. Under the 16L:8D photoregime, the mean onset calling time decreased from scotophases 2 to 3, after which it stabilized around ca. 260 min after the start of the scotophase. With 18L:6D, the onset of calling decreased until scotophase 4, and subsequently stabilized around ca. 130 min after lights off.

Introduction

Mamestra brassicae L. (Lepidoptera: Noctuidae) is a noctuid moth with a wide host range including cabbage and various other crucifers. Due to the feeding damage caused by the caterpillars, it is considered an important pest in temperate zone cabbage and other vegetable crops (Hill, 1987). Its eggs and larval instars are attacked by various natural enemies, including egg parasitoids of the genus *Trichogramma*. In their search for host eggs, *Trichogramma* spp. respond to a variety of semiochemicals (for a review see Noldus, 1989*b*; *Chapter 9*). One of those cues is the sex pheromone of the host, which is thus used as a kairomone by the parasitoid. For instance, *T. pretiosum* Riley responds to the sex pheromone of its host *Heliothis zea* (Boddie) (Lewis *et al.*, 1982; Noldus, 1988*b*; *Chapter 5*). For a parasitoid of *M. brassicae* a similar effect has been found. Noldus & van Lenteren (1985*a*; *Chapter 4*) showed that *T. evanescens* Westwood responds to the odour of calling females in an olfactometer. We

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have recently initiated a more detailed investigation of the orientation behaviour of *Trichogramma* spp. in response to host sex pheromones. Essential for such studies is a reliable source of sex pheromone. For many moth species, the sex pheromone has been chemically identified (Arn *et al.*, 1986), so that synthetic attractants can be used in bioassay studies. Thus we could use synthetic sex pheromone blends in wind tunnel experiments of the response of *T. pretiosum* to the sex pheromones of *H. zea* and *Spodoptera frugiperda* (J.E. Smith) (Noldus *et al.*, 1988*a*; *Chapter 7*).

However, in spite of identification studies for more than a decade, the sex pheromone of *Mamestra brassicae* has not yet been identified unambiguously. While it is now generally accepted that the main component is (Z)-11-hexadecen-1-yl acetate (Z11-16:Ac), uncertainty still exists about the presence and significance of minor components (Szentesi *et al.*, 1975; Bestmann *et al.*, 1978; Descoins *et al.*, 1978; Hirai *et al.*, 1978; Kovalev *et al.*, 1979; Novak *et al.*, 1979; Struble *et al.*, 1980; Farine *et al.*, 1981; van de Veire & Dirinck, 1986; Attygalle *et al.*, 1987; Bestmann *et al.*, 1988). Therefore, the most reliable source of sex pheromone for use in experiments is still a calling female moth.

Several complicating factors are attached to the use of living moths as source of sex pheromone. First, one needs to obtain calling activity of moths under the conditions of an experimental set-up. In many species of Lepidoptera, female calling activity depends on endogenous factors (e.g. age, mating status, stage of egg development) as well as on exogenous factors (e.g photoperiod, temperature) (Shorey, 1974). In addition, calling activity does not necessarily lead to a constant release of sex pheromone: the absolute amount released as well as the composition may vary between individuals as well as within the individual (Pope *et al.*, 1982; Löfstedt *et al.*, 1985; Raina *et al.*, 1986; Attygalle *et al.*, 1987).

Several reports in the literature indicate that calling by *M. brassicae* occurs mainly in the second half of the scotophase (e.g. Attygalle *et al.*, 1987; Bestmann *et al.*, 1988; Birch *et al.*, 1989). The only detailed information on the calling behaviour of *M. brassicae* is available from the work of M.A. Subchev in Bulgaria, who described *M. brassicae*'s calling posture and studied the effect of age, temperature and photoperiod (Subchev, 1980, 1983, 1985). However, a species' pheromone communication system can vary between populations in different geographical regions. Examples of variation in pheromone composition are known from Lepidoptera (e.g. Klun *et al.*, 1975; Löfstedt *et al.*, 1986; Peña *et al.*, 1988), Coleoptera (Lanier *et al.*, 1980; Miller *et al.*, 1989) and Heteroptera (Aldrich *et al.*, 1987). Apart from *chemical* variation, adaptation to different environmental conditions might also have led to *behavioural* variation between populations in different areas. Therefore, the calling behaviour of Dutch *M. brassicae* was examined. The practical goal of this study was to establish the conditions in the laboratory under which the incidence of prolonged calling activity is highest, as needed for investigations on the role of *M. brassicae*'s sex pheromone in

the foraging behaviour of *Trichogramma* wasps. Here we report on the effect of two factors: photoperiod and moth age.

Materials and Methods

Moth rearing

A stock colony of *M. brassicae* was maintained on Brussels sprouts (*Brassica oleracea* var. *gemmifera* cv. Titurel) at 20 ± 2 °C, 50 ± 10 % r.h. and a 16L:8D photoperiod. The colony had been reared in the laboratory for ca. 80 generations. Pupae were collected from the stock colony 1-2 weeks prior to emergence and sexed. Female pupae were placed in a 20 x 20 x 8 cm plastic container with a layer of sawdust, and held at 20 ± 1 °C, 60 ± 10 % r.h. and the experimental photoperiod. The photoperiod was reversed to that in nature and was either 16L:8D (lights off at 9:00 h) or 18L:6D (lights off at 10:00 h). During the photophase, the climate box was illuminated by a white fluorescent tube, producing ca. 3.5 W/m^2 light intensity in the center of the box. Preliminary checks at the start of scotophase and photophase had indicated that most moths emerged during the photophase. During the experimental period, daily checks for adult emergence were made at the start of the photoperiod. Newly emerged moths were placed individually (or in groups of 2-4) in glass cylinders (12 x 7 cm) with a gauze cover, a piece of filter paper on the bottom, and a feeding container with cotton wool soaked in 10 % sugar solution. Keeping moths in small groups had no apparent effect their behaviour.

Observations

Observations were made during the scotophase, under a dimmed red light of 0.004 W/m^2 intensity in the center of the climate box. At 30-120 min intervals a scan sample was made of all adults, and for each moth we recorded in which of the three following activities it was engaged: *quiescence* (no locomotion; no body movement; no or slight vibration of wings), *locomotion* (walking or flight), or *calling*. Calling behaviour is described in detail below. In total almost 2700 observations of moth activity were made.

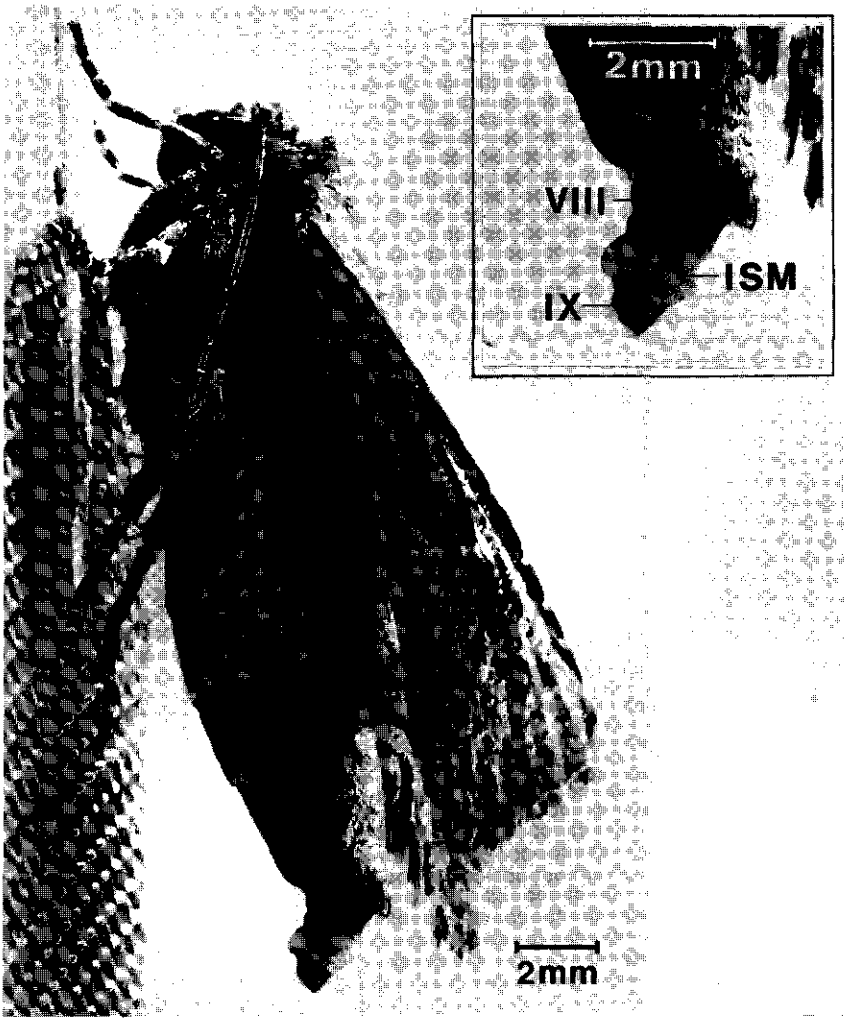


Figure 3-1. Female *Mamestra brassicae* moth in calling posture. Inset: close-up of extruded ovipositor, showing abdominal segments 8 (VIII) and 9 (IX) and the intersegmental membrane (ISM).

Data analysis

Observational data were classified per scotophase hour and sorted by photoperiod and moth age. Age was expressed as the scotophase number, starting with the first complete scotophase after emergence. Data were available for moths aged 1 to 9 scotophases. Photoperiod/age/hour combinations with fewer than 5 observations were not included in the analysis. As far as data were available, the proportion of females that called was determined for each photoperiod/age combination. The onset of calling was determined as the time (in min after lights off) halfway between the first moment that calling was observed and the previous observation. Differences in proportions of females calling were subjected to G-tests with Yates' correction (STSC, 1986). Onset calling times were analyzed using two-way ANOVA for unbalanced designs, followed by Duncan's multiple range test for comparison of means (STSC, 1986).

Results

Calling behaviour

Mamestra brassicae females initiated calling during the scotophase. Calling was usually preceded by a short period of walking and/or flight. After this, females settled for a long period, preferably on a vertical substrate, with the head oriented upward, or (if a suitable vertical substrate was not present) on the bottom of the container, or hanging from the cover. The start of calling activity was often indicated by a short period during which females rhythmically protuded and retracted the ovipositor. Occasionally, the ovipositor was pressed against the substrate. During most of the calling period, the moths assumed a typical posture. The legs were extended laterally and the whole body was raised at an angle of 0-45° with the substrate, i.e. with the posterior end of the abdomen elevated. The ovipositor was extruded and pointed downward at an angle of ca. 45° with the abdomen. The wings were raised above the abdomen in a "V" shape, with the upper surface at an angle of 0-45° with the substrate. The antennae were mostly directed hindward and held close to the body, but sometimes directed sideways.

The ovipositor consists of the 8^{th} and 9^{th} abdominal segments, which are invisible when the female is not calling. During extrusion, the intersegmental membrane is exposed to the air (Figure 3-1). This membrane, light-coloured and clearly distinguishable from the neighbouring segments, is the site of actual pheromone release (Otto *et al.*, 1976; Attygalle *et al.*, 1987).

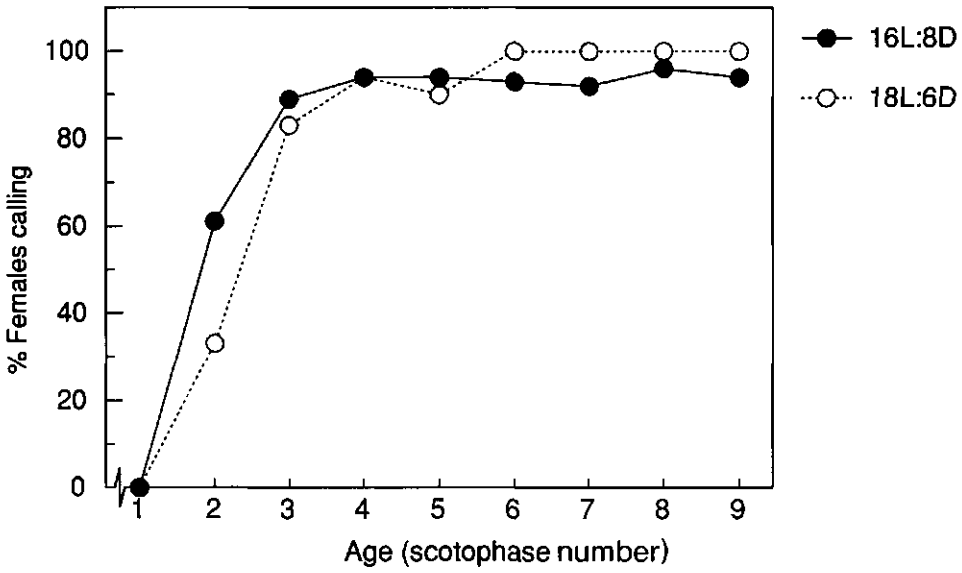


Figure 3-2. Proportions of female *Mamestra brassicae* moths calling at various ages, under two different photoregimes. Scotophase 0, 16L:D8: N = 20, 18L:6D: N = 19. For other numbers of observations, see Figure 3-3. Data per age class not significantly different at P = 0.05 (G-test with Yates' correction [STSC, 1986]).

Proportion of females calling

Figure 3-2 shows the relation between photoperiod, age and proportion of females calling. No calling activity was observed during the first scotophase. Most females called for the first time during the second or third scotophase after emergence. There was no significant effect of the photoperiod for any of the age classes.

Diel periodicity

The activity of females during the scotophase is presented in Figure 3-4. Most locomotion was observed in the first half of the scotophae. Under a 16L:8D photoregime, most calling occurred in the second half of the scotophase, and very little calling occurred prior to the 4^{th} hour in the scotophase. Under 18L:6D, calling occurred during a larger part of the scotophase, and calling was frequently observed in

the 2nd hour. Due to the sampling method used, these observations did not yield detailed information about the calling pattern of individual females. However, from continuous observations (Noldus, unpubl.) we know that once females initiate calling, they often continue without interruption until the end of the scotophase or even during a short part of the following photophase. Females were usually not disturbed by the dimmed red light used during observations.

Onset of calling

Photoperiod and age both had an effect on the mean onset calling time (Figure 3-3). Moths that were maintained under 16L:8D started calling significantly later in the scotophase than those maintained under 18L:6D ($F = 201.68$, $P < 0.0005$). With

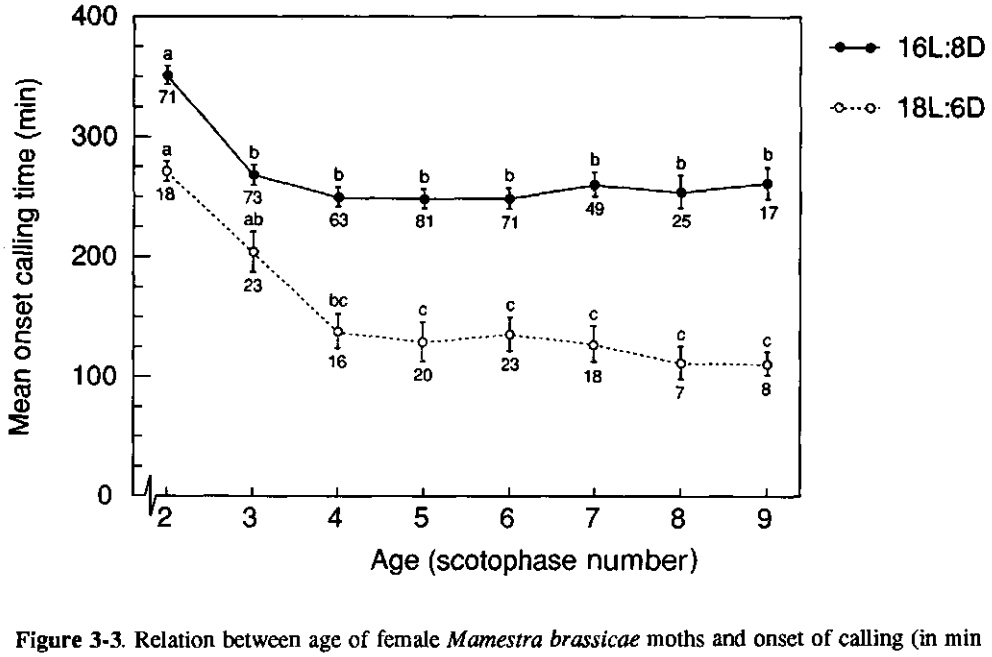
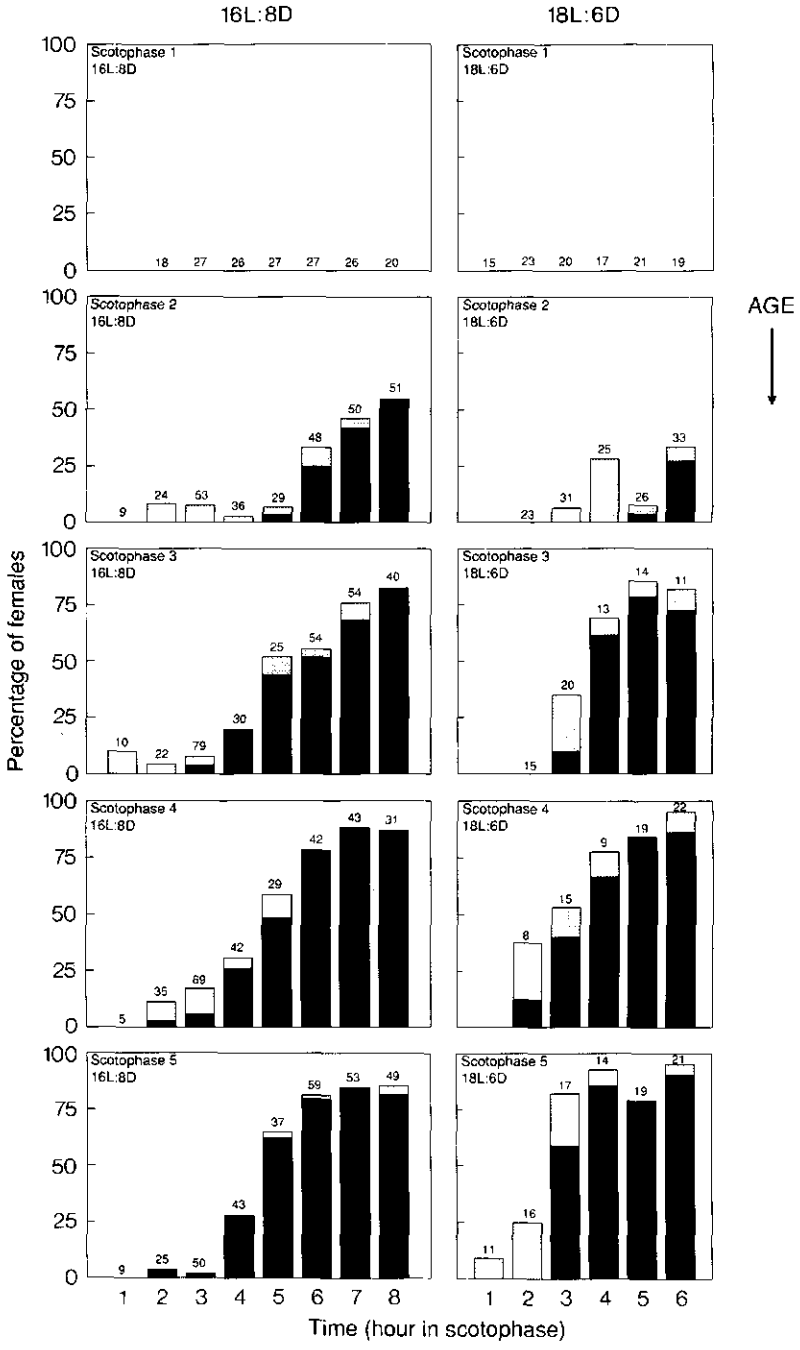


Figure 3-3. Relation between age of female *Mamestra brassicae* moths and onset of calling (in min since start of scotophase, mean ± s.e.), under two different photoregimes. Data include only moths that called. Numbers below bars: no. of observations. Different letters indicate significant differences between means within a photoperiod (Duncan's multiple range test, P = 0.05 [STSC, 1986]).


16L:8D
18L:6D
Scotophase 1
16L:8D
18 27 28 27 27 26 20
Scotophase 1
18L:6D
15 23 20 17 21 19
Scotophase 2
16L:8D
9 24 53 36 29 48 50 51
Scotophase 2
18L:6D
23 31 25 26 33
Scotophase 3
16L:8D
10 22 79 30 25 54 54 40
Scotophase 3
18L:6D
15 20 13 14 11
Scotophase 4
16L:8D
5 35 69 42 29 42 43 31
Scotophase 4
18L:6D
8 15 9 19 22
Scotophase 5
16L:8D
9 25 50 43 37 59 53 49
Scotophase 5
18L:6D
11 16 17 14 19 21
Percentage of females
0
25
50
75
100
1 2 3 4 5 6 7 8
1 2 3 4 5 6
Time (hour in scotophase)
AGE

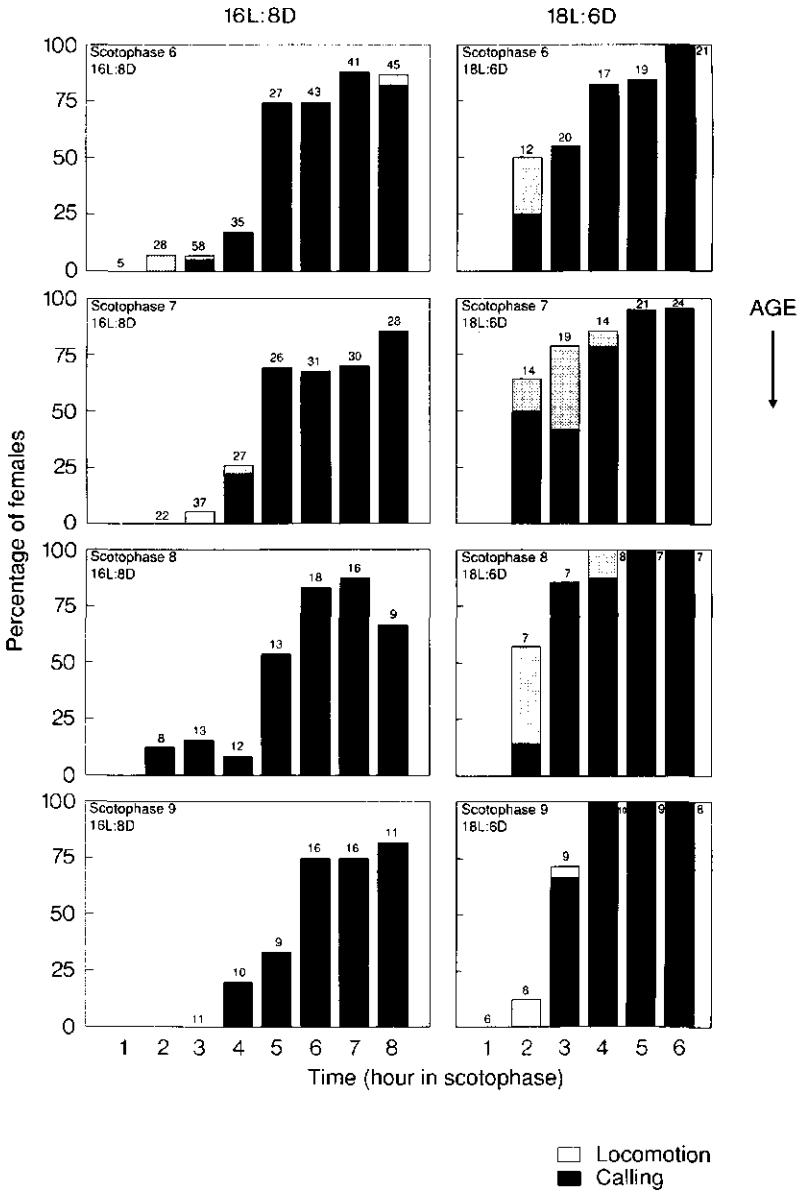


Figure 3-4. Activity of female *Mamestra brassicae* moths at various ages, under two different photoregimes. Each histogram represents a photoperiod/age combination. Age is expressed as scotophase number. Bars indicate proportion of females engaged in locomotion or calling; the remaining females are quiescent. Numbers above bars: no. of observations.

progressing age, calling started significantly earlier in the scotophase ($F = 13.15$, $P < 0.0005$). Under the 16L:8D photoregime, the mean onset calling time decreased from scotophases 2 to 3, after which it stabilized at 255 ± 4 min after the start of the scotophase (calculated for age ≥ 3, N = 300, mean ± s.e.). With 18L:6D, the onset of calling decreased until scotophase 4, and stabilized at 127 ± 7 min after lights off (for age ≥ 4, N = 73).

Discussion

Although most adult moths eclosed during the photophase, the experimental test groups included small proportions of scotophase-emerged females. This has obviously influenced the accuracy of the age determination in our study. Nevertheless, most age-related trends are statistically significant.

The calling behaviour of our Dutch population of *M. brassicae* is very similar to what has been reported for a Bulgarian population of this species. This applies to the calling posture (Subchev, 1980) as well as to the effect of age and photoperiod (Subchev 1983). The typical wings-up posture found in both populations is common among many species of moths. This posture has recently been shown to enhance pheromone release by increasing the wind speed near the pheromone gland in arctiids (Conner & Best, 1988). Subchev (1983) also recorded absence of calling in 1-day old moths and subsequent increase of the proportion of females calling and decrease of the mean onset calling time, leveling off after scotophase 3. He also found a shift toward later onset of calling with increasing length of scotophase, which corresponds with our data for 16L:8D and 18L:6D. Due to our experimental design, mean onset of calling occurred at about 12:00 h in real time under both photoregimes. Therefore, a shift in onset of calling with a change in photoperiod might reflect an endogenous rhythm adapting to real time. However, other studies have shown that the lights-off signal is the cue responsible for setting the diel timing of calling in *Mamestra* species. (Subchev, 1983; Howlader & Gerber, 1986*a*). In conclusion, the Dutch and Bulgarian populations of *M. brassicae* do not differ in any of the behavioural events measured, nor in the timing of those events.

The behaviour of *M. brassicae* is also similar to that of related *Mamestra* species. *Mamestra brassicae* shows changes in calling behaviour with increasing age similar to those in *M. configurata* Walker (Howlader & Gerber, 1986*b*). In this species also, the onset of calling advances in time with decreasing length of scotophase (Gerber & Howlader, 1987). This mechanism can be used by *Mamestra* moths to maintain a calling period sufficiently long for mating to occur independent of ambient light conditions (Gerber & Howlader, 1987).

Both *M. brassicae* and *M. configurata* occasionally press their ovipositor against

the substrate during calling, in particular in the beginning of the calling period. The function of this behaviour is not known. It may be an example of active substrate marking, as has been described for *Pectinophora gossypiella* (Saunders) (Colwell *et al.*, 1978; *Chapter 8*).

Mamestra brassicae has a pattern of continuous (uninterrupted) calling, like *M. suasa* (Den. & Schiff.) (Tóth, 1979) and *M. configurata* (Howlader & Gerber, 1986*a*). This makes it suitable for use as an *in vivo* source of sex pheromone. From the 3rd scotophase onwards, many females called for more than four hours on a stretch. In contrast, many other Noctuidae have a discontinuous calling pattern, with many short calling bouts, separated by interruptions of variable length. For instance, the calling behaviour of *Heliothis zea* consists of irregular bouts of extrusion of the abdominal tip (Agee, 1969), which makes this species rather impractical as an *in vivo* sex pheromone source (Noldus, 1988*b*). On the other extreme are certain arctiids, which release their sex pheromone as an aerosol instead of by passive evaporation (Krasnoff & Roelofs, 1988).

With reference to the original goal of this study – i.e. to establish the best conditions for use of *M. brassicae* as pheromone source – and within the range of situations tested, maximum reliability of sex pheromone release can be expected under a 16L:8D photoperiod, with virgin females that are at least 3 days old, after the 4th hour of the scotophase.

Acknowledgements

We are endebted to J.H.M. Buser and J. Dirven, who collected part of the data. B. Geerligs made the photograph, and F.J.J. von Planta and P.J. Kostense assisted with the preparation of the figures. Thanks are further due to R.T. Cardé, M. Dicke, J.C. van Lenteren, J.J.A. van Loon, O.P.J.M. Minkenberg, D.R. Papaj, M.A. Subchev, M. Tóth and J.H. Tumlinson for their constructive comments on an earlier version of the manuscript.

4 Response of *Trichogramma evanescens* to Host Odours in an Airflow Olfactometer[1]

ABSTRACT – In a four-armed airflow olfactometer *Trichogramma evanescens* Westwood females were attracted by volatiles released by virgin females of the great cabbage white butterfly, *Pieris brassicae* L. Males or recently mated females did not cause attraction. Furthermore, *T. evanescens* was also attracted by volatiles released by calling virgin cabbage moths, *Mamestra brassicae* L. However, the parasitoids did not respond to (Z)-11-hexadecen-1-yl acetate (the main component of the sex pheromone of *M. brassicae*), a crude hexane extract of the sex pheromone gland, or to males or recently mated females.

Introduction

Many parasitic Hymenoptera are guided by chemical stimuli in the process of host-habitat and host location (Vinson, 1981; Weseloh, 1981). From a distance, a parasitoid may be attracted by volatile chemicals emanating form the host habitat, the host, or both. The latter substances are called kairomones (for definition see Brown *et al.*, 1970; Nordlund & Lewis, 1976). Once it has arrived in the host habitat, the parasitoid may be arrested by contact kairomones. This paper deals with some of the volatile kairomones involved in the searching behaviour of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). Contact kairomones have been discussed elsewhere (Noldus & van Lenteren, 1985*b*).

Some volatile stimuli involved in the searching process of *Trichogramma* spp. have already been demonstrated. For example, plant odours may influence the rate of parasitisation by *Trichogramma* spp. in the field (Bar *et al.*, 1979; Altieri *et al.*, 1981). Recently, volatiles released by the host have been shown to function as a kairomone for the parasitoid: Lewis *et al.* (1982) observed increased rates of parasitism by wild *Trichogramma* spp., elicited by volatiles present in the abdominal tip and those in a pinkish excretion (supposed to be meconium) as well as by a synthetic sex pheromone blend of *Heliothis zea* (Boddie). The same phenomenon has been found

1 Published in slightly different form as: L.P.J.J. Noldus & J.C. van Lenteren (1985). Kairomones for the egg parasite *Trichogramma evanescens* Westwood. I. Effect of volatile substances released by two of its hosts, *Pieris brassicae* L. and *Mamestra brassicae* L. *Journal of Chemical Ecology* 11, 781-791.

with the egg parasitoid *Telenomus remus* Nixon, in reaction to synthetic sex pheromone components and to an extract from abdominal tips of its host *Spodoptera frugiperda* (J.E. Smith) (Nordlund *et al.*, 1983).

During the past few years efforts have been made in the Netherlands to develop biological control of five lepidopteran species in cabbage crops by means of inundative releases of *T. evanescens* (van Lenteren *et al.*, 1982; van der Schaaf *et al.*, 1984). The results described above led us to examine whether or not volatiles released by the adults of hosts of *T. evanescens*, and encountered by this parasitoid in Dutch cabbage fields, can serve as a kairomone and whether these substances might be used to manipulate parasitoid behaviour. The study was conducted with two host species: the great cabbage white butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae), and the cabbage moth, *Mamestra brassicae* L. (Lepidoptera: Noctuidae).

We examined the response of *T. evanescens* to virgin females, males, and mated females of *P. brassicae*. In the case of *M. brassicae*, we attempted to determine the reaction of the parasitoid to the sex pheromone. Therefore, experiments were carried out with the main component (Z)-11-hexadecen-1-yl acetate (Z11-16:Ac), with a crude extract of the sex pheromone gland, and with calling virgin moths. Additionally, responses to males and mated females of this species were tested.

Materials and Methods

Parasitoids

The *T. evanescens* strain used for the experiments was collected in the Netherlands in 1981 from eggs of *M. brassicae* in cabbage (strain no. 11 in Pak & van Heiningen, 1985). It has been reared in the laboratory since then on eggs of *Ephestia kuehniella* Zeller. Adult wasps were maintained on honey in glass tubes at 25 ± 1 °C. For the experiments, only female wasps with an age varying from 2 to 4 days were used. This age was chosen because wasps younger than 48 h are apparently not strongly motivated te react to host-seeking stimuli (Smits, 1982). Previous to an experiment, three eggs of the host species to be tested were offered to each wasp for approximately 1 h. Only wasps that were observed to have been parasitising during the hour of exposure were used in the experiment. Such 'experienced' wasps have been shown to commence searching more effectively than inexperienced wasps (Gross *et al.*, 1981*b*).

Host insects and other odour stimuli

Pupae of *P. brassicae* and *M. brassicae* were obtained from laboratory rearings of the Departments of Entomology and Animal Physiology, Agricultural University, Wageningen. Some of the pupae were sexed and isolated; after emergence, the adults were kept separate. The adults emerging from the remaining pupae were maintained in cages and were used as a source of eggs. *Pieris brassicae* was kept at 20 ± 1 °C. *Mamestra brassicae* was kept under a reversed photoregime of 18L:6D (photophase: 20 ± 1 °C; scotophase: 15 ± 1 °C), in order to make experiments possible during the day.

Synthetic Z11-16:Ac was formulated on rubber septa at a dosage of 2 mg/septum. Septa of this kind have been used in field traps for monitoring purposes for some years (Terytze & Adam, 1981). The crude extract of the sex pheromone gland of *M. brassicae* was dissolved in *n*-hexane to a concentration of 10 FE/ml (FE = female equivalent). The rubber septa as well as the gland extract solution were stored at -25 °C until use in experiments.

Experimental set-up and procedures

The experiments were carried out in a slightly altered version of the four-armed airflow olfactometer described in detail by Vet *et al.* (1983). Because their olfactometer was too large for *T. evanescens* – the wasps spent too much time walking from one flow field to another and were hardly visible on the video monitor – some modifications had to be introduced in order to make observations of this parasitoid possible. The size of the exposure chamber was halved (ray of crescents: 67.5 mm; inner height: 5 mm; inner diameter of stainless steel tubes: 3 mm), the bottom was made of white acrylate, and for the construction of the upper part (odourless) silicone glue was used. In order to create flow fields with sharp boundaries, the flow was reduced to 27 ml/min through each arm.

For the experiments with *P. brassicae* the olfactometer was supplied with a 2 l glass container in which the butterflies could move freely. This container could easily be connected to the apparatus without the flow rate being altered. One to three butterflies were put into the container, and the control arms of the olfactometer remained empty. The butterflies were replaced after every 10 replications.

In the first experiment concerning *M. brassicae*, we tested synthetic Z11-16:Ac by offering one rubber septum (loaded with 2 mg Z11-16:Ac) in one arm of the olfactometer; the others remained empty. The septum was replaced after every 10 observations.

For the experiments with the crude extract of the sex pheromone gland, four pieces of Whatman #1 filter paper (4 cm^2) were used, one of which was treated with

0.1 ml of the hexane extract (10 FE/ml) so that 1 FE was present on the paper. The three others were treated with 0.1 ml hexane only. A second series was conducted with a load of 10 FE by using 1 ml of the solution on the test filter paper and 1 ml of hexane on the remaining three filter papers. The experiments were started as soon as the solvent had evaporated. The filter papers were also replaced after every 10 observations.

The experiments with living moths of *M. brassicae* required more preparation. In order to obtain an airstream through the olfactometer containing sex pheromone molecules released by calling virgin females, some conditions had to be met. First of all, the experiments had to be conducted during the period of calling activity of moths in the field. This is known to be highest in the second half of the scotophase (*Chapter 3*). With the photoregime used, we could thus determine at what time during the day tests had to be done. Secondly, for calling activity of *M. brassicae*, a dark environment is necessary. For this reason some special adaptations were introduced to make experiments with moths in the brightly illuminated olfactometer set-up possible (Figure 4-1). In a light-tight compartment outside the set-up, a

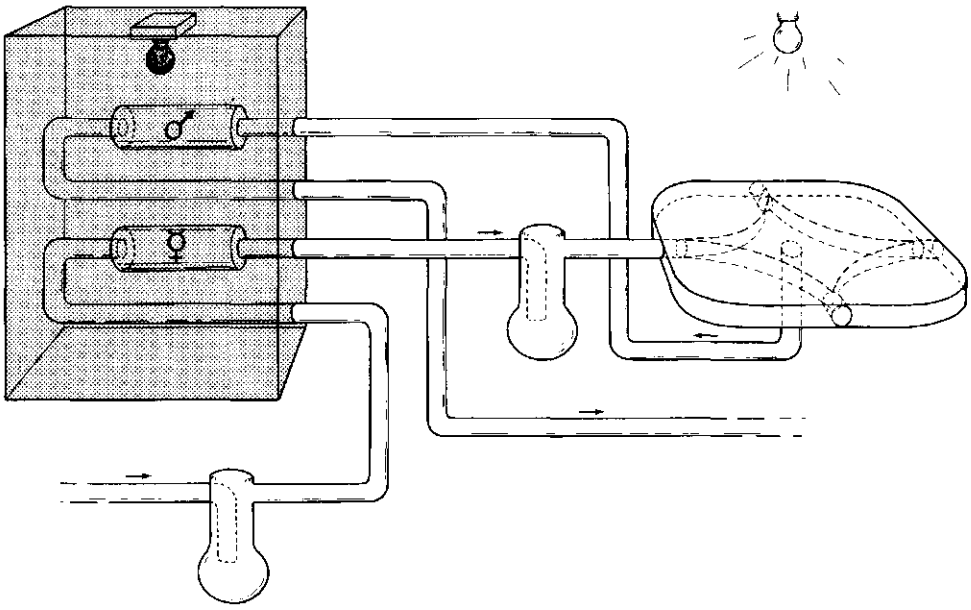


Figure 4-1. Schematic representation of the adaptations of the four-armed airflow olfactometer of Vet *et al.* (1983) to experiments with male and with calling female moths of *Mamestra brassicae*.

Plexiglas™ cylinder (11.5 x 4.5 cm) was situated, connected by (odourless) silicone tubing to the first and third vial of one of the olfactometer arms. A virgin moth was put into the cylinder; with the aid of a light-lock and a very weak red darkroom lamp, the moth could be observed without being disturbed by the fluorescent light. Thus we could determine whether the calling posture (protrusion of the abdominal tip) was assumed. But, since calling behaviour is no guarantee that sex pheromone release is actually taking place (Pope *et al.*, 1982), another verification was necessary. For this purpose we used the typical response of the male moth, consisting of wing fanning, walking, and flying (Novak *et al.*, 1979), as proof of the presence of sex pheromone molecules in the air stream. A second cylinder, containing a male moth that had not yet mated, was placed in the dark compartment. This cylinder was connected between the air outlet of the olfactometer and the vacuum compressor. The experiment was not started until both the female calling posture and the male wing fanning response had been observed. The moths were replaced after every 10 replications.

The experiments were conducted in a climate room at a temperature of 25 ± 1 °C and 60 ± 5 % r.h. Experiments consisted of series of at least 40 observations. They were carried out according to the procedure of Vet *et al.* (1983), except for the 'number of first choices'. This measurement was abandoned because, during preliminary observations, the wasps turned out to cross the 'first-choice-line' before the odour fields could have been formed. After an insect had entered one of the arms of the olfactometer and had stayed there for more than 2 min, it was said to have made a 'final choice'. Statistical analysis of the results was also done according to Vet *et al.* (1983).

Results

Pieris brassicae

The first experiment was performed with virgin females of *P. brassicae*. These were often observed in the rearing cage sitting in a typical position, in which the wings were spread out, the abdomen was raised slightly, and the lateral lobes of the genital apparatus were more or less turned aside (Figures 4-2 and 4-3). This posture stands somewhat halfway between the 'mate-refusal posture' and the 'mate-acceptance posture' which normally occur only during the mating sequence, preceded by male stimulation (Chovet, 1982). It was never adopted by males or recently mated females. Thinking of the calling position known of many moth species, we wondered whether a volatile substance was released at that moment, and what the effect on the parasitoid might be. The results are given in Table 4-1*a*. Attraction of the parasitoid

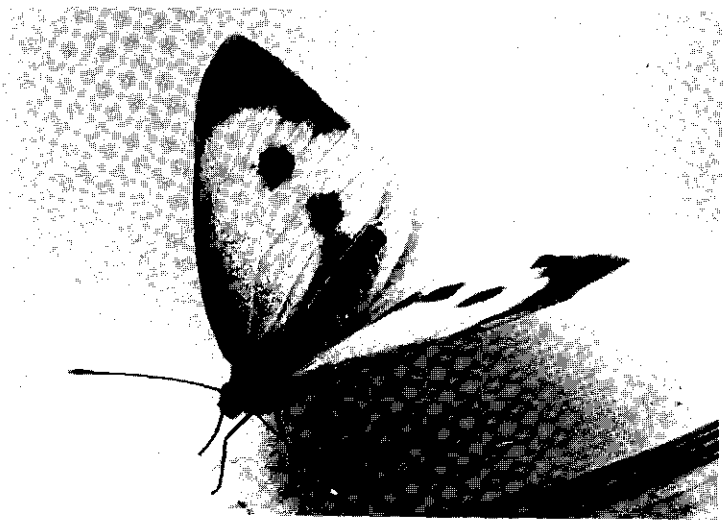


Figure 4-2. Virgin female *Pieris brassicae* in the typical posture during which the volatile kairomone was released. Note the slightly raised abdomen.

Figure 4-3. As Figure 4-2, dorsal view.

was distincly shown by the fact that all the final choices were made for the *P. brassicae* field and that the average percentage of time spent in this field was significantly longer than the time spent in the other odour fields.

In order to determine whether the attraction was caused by an odour specifically released by the virgin females, and not by an odour present in all adult butterflies of *P. brassicae* ('general body odour'), control experiments were carried out with males and females that had been mated the day before the experiment. It is very unlikely that such females release odours that are related to the mating behaviour, be-

Table 4-1. Response of female *Trichogramma evanescens* to odour of living butterflies of *Pieris brassicae* in an olfactometer.

Butterflies tested	Response	N	Odour field[1]				P[2]
			1	2	3	4	
A. Virgin females	No. final choices	17	17				***
	Mean % time spent/field	40	67.9	10.7	15.3	6.1	
	Friedman rank sum		135.5	82	94.5	88	***
B. Males	No. final choices	12	2	5	2	3	n.s.
	Mean % time spent/field	50	17.1	32.5	27.7	22.7	
	Friedman rank sum		112.5	142.5	116.5	128.5	n.s.
C. Mated females	No. final choices	14	4	4	4	2	n.s.
	Mean % time spent/field	50	26.1	27.4	24.8	21.7	
	Friedman rank sum		128.5	128	119.5	124	n.s.

1 1 = *Pieris brassicae*, 2 + 3 + 4 = clean air.

2 Friedman 2-way ANOVA by ranks; n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

cause *P. brassicae* females do not mate again until five days or more after the first copulation (David & Gardiner, 1961). The results of these experiments are given in Tables 4-1*b* and 4-1*c*. No preference was shown in this case: the numbers of final choices as well as the time allocation were randomly distributed over the four flow fields.

From these results it is concluded that virgin females of *P. brassicae* can release a volatile substance which acts as a kairomone for *T. evanescens* and which is not released by males or recently mated females.

Mamestra brassicae

Szentesi *et al.* (1975) demonstrated the presence of a sex pheromone in *M. brassicae*. Subsequently several investigators have tried to elucidate its chemical compostion. The main component has turned out to be (Z)-11-hexadecen-1-yl acetate (Z11-16:Ac), but uncertainty still exists about the presence and ratios of minor components (Bestmann *et al.*, 1978; Farine *et al.*, 1981; Novak *et al.*, 1979; Struble *et al.*, 1980; *Chapter 3*). We have examined the response of *T. evanescens* to synthetic Z11-16:Ac, to a crude extract of the sex pheromone gland, and to volatiles released by calling virgin females of *M. brassicae*.

In the first experiment, in which Z11-16:Ac was offered, no response of *T. evanescens* could be measured. The data in Table 4-2*a* show that the numbers of final choices as well as the average percentage of time spent in the different flow fields did not differ significantly. The same applied to both loads of the crude hexane extract of the sex pheromone gland (Tables 4-2*b* and 4-2*c*).

Table 4-2*d* however shows that as soon as the volatiles of *M. brassicae* released by calling virgin moths were offered, significant attraction of *T. evanescens* occurred. Both at the final-choice level and as far as the time allocation per odour field are concerned, the parasitoids demonstrated a distinct preference for the *M. brassicae* field.

That the attraction was indeed caused by the sex pheromone and not by some other volatile is strongly suggested by the fact that male *M. brassicae* moths responded in a typical way to the same volatiles and by the control experiments in which males or recently mated females were offered. Tables 4-2*e* and 4-2*f* show that *T. evanescens* responded neither to male moths nor to females mated the day before the experiment.

These results indicate that the sex pheromone of *M. brassicae*, in the form released by calling virgin moths, can act as a volatile kairomone for *T. evanescens*.

Table 4-2. Response of female *Trichogramma evanescens* to odour of various substances originating from *Mamestra brassicae* in an olfactometer.

Material tested		Response	N	Odour field[1]				P[2]
				1	2	3	4	
A.	Synthetic Z11-16:Ac	No. final choices	8	4	1	2	1	n.s.
		Mean % time spent/field	40	28.7	21.7	27.2	22.4	
		Fieldman rank sum		110	91	102	97	n.s.
B.	Crude extract of the sex pheromone gland (1FE)	No. final choices	4	3	1			n.s.
		Mean % time spent/field	40	28.1	23.6	28.4	19.9	
		Friedman rank sum		102	93	110	95	n.s.
C.	Crude extract of the sex pheromone gland (10 FE)	No. final choices	8	3	1	3	1	n.s.
		Mean % time spent/field	40	24.0	28.8	23.2	24.0	
		Friedman rank sum		103	104	93	100	n.s.
D.	Odour of calling female moth	No. final choices	9	7	1	1		**
		Mean % time spent/field	44	39.4	23.6	21.9	15.1	
		Friedman rank sum		140.5	116	96.5	87	***

Table 4-2, *continued.*

Material tested	Response	N	Odour field[1]				P[2]
			1	2	3	4	
E. Odour of male moth	No. final choices	1	1				
	Mean % time spent/field	40	26.8	20.9	23.3	29.0	
	Friedman rank sum		103	87	94	116	n.s.
F. Odour of mated female moth	No. final choices	2		1	1		
	Mean % time spent/field	40	16.9	31.1	34.1	17.9	
	Friedman rank sum		89.5	113	108	89.5	n.s.

1 1 = material tested, 2 + 3 + 4 = clean air.
2 Friedman 2-way ANOVA by ranks; n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Discussion

The first three experiments have shown that virgin females of *Pieris brassicae* can release a volatile kairomone for *T. evanescens*. With the results of *Heliothis zea* (Lewis *et al.*, 1982), *Spodoptera frugiperda* (Nordlund & Lewis, 1983), and *Mamestra brassicae* (this paper) in mind, one might think that *P. brassicae* females produce a sex pheromone. However, the mating behaviour of *P. brassicae* has been studied in detail by several authors, and none of them mentions the existence of a female sex pheromone (David & Gardiner, 1961; Chovet, 1982; Feltwell, 1982). In general, mate location in butterflies is assumed to occur mainly on the basis of visual stimuli (Myers, 1972; Scott, 1974; Boppré, 1984). The only chemical stimuli that are known to play a role in the mating sequence of *P. brassicae* are short-range volatiles originating from the androconial scales of the male (Bergström & Lundgren, 1973). It is not known whether the volatile substance released by the virgin females has an intraspecific function too.

The results of the experiments with *M. brassicae* showed that *T. evanescens* is attracted by volatiles which are probably the sex pheromone released by calling virgin females, but not by the main component (Z)-11-hexadecen-1-yl acetate, nor by a crude extract of the sex pheromone gland (at the two concentrations tested). This suggests that there are differences between the pheromone released into the air by the insect and the contents of the pheromone gland. This phenomenon has been demonstrated in other species too; Tumlinson *et al.* (1982) list some possible causes for its occurence. Our results affirm their statement that bioassays with sex pheromones should be based on materials collected from living calling females, since it is the only way to assure that one is testing the proper material.

Our results suggest that some component(s) other than only Z11-16:Ac is (are) necessary for the attraction of *T. evanescens* in the olfactometer, for the latter did not elicit a response of the parasitoid. In the future, blends of components should be tested separately to determine the exact cause of the attraction. This could be done after analysis of a filter collection of the pheromone, such as might be done with a Porapak device (Byrne *et al.*, 1975; Tumlinson *et al.*, 1982). We must note that we have tested only one concentration of Z11-16:Ac. Before discarding Z11-16:Ac as a kairomone for *T. evanescens*, other dosages should be tested as well.

Throughout this paper, the olfactory response of *T. evanescens* to host odours has been referred to as *attraction*. However, especially in the case of *M. brassicae*, numbers of final choices were quite low (7 out of 44). The four-arm airflow olfactometer used in this study may not be the optimal apparatus to analyse olfactory orientation in *Trichogramma*. Experiments especially designed for that purpose are reported elsewhere (*Chapter 6*).

Finally some remarks may be made on the possible function of the kairomones in the searching behaviour of the parasitoid. Volatiles released by virgin females do not necessarily have a direct temporal or spatial correlation with the moment or site of oviposition. Therefore, we do not expect the egg parasitoid to be guided directly towards the place of release. The kairomone might rather lead the parasitoid to an area where mating is in progress and where oviposition is thus likely to take place or to have taken place. At the moment a single female host is calling, eggs of conspecifics may already be present in the surroundings. Hence, responding to the sex pheromone of the host seems adaptively favorable for an egg parasitoid. This also applies to the sex pheromone of *M. brassicae*, which is released at night. It may yet be detectable for *T. evanescens*, which is active during the day, if pheromone molecules are retained in the surroundings of the calling moths (e.g. by adsorption to plants) and are re-released very slowly (Farkas & Shorey, 1974). However, this hypothesis remains to be tested for *Trichogramma* (*Chapter 8*). Responding to the sex pheromone of the host might also serve to avoid competition with predators. Thus, it may be important for an egg parasitoid to find the host eggs shortly after oviposition and

parasitise them as soon as possible, as some predators are known to dislike eggs that have turned black as the result of a developing parasitoid larva (Lewis *et al.*, 1982). Actual finding of the host egg may occur only after an intense search stimulated by contact kairomones left by ovipositing hosts. These substances are discussed elsewhere (Noldus & van Lenteren, 1985*b*).

The volatile effects described above may promise exciting possibilities for the use of sex pheromones in insect pest control, as has recently been stressed by Greenblatt & Lewis (1983): disruption of mating of the pest may be attended by enhancement of parasitisation by the parasitoid.

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5 Response of *Trichogramma pretiosum* to the Sex Pheromone of *Heliothis zea* in an Airflow Olfactometer[1]

ABSTRACT – This chapter contains results of olfactometer experiments with the egg parasitoid *Trichogramma pretiosum* Riley and its host the corn earworm moth, *Heliothis zea* (Boddie). The sex pheromone of the host significantly reduced the total number of border crossings between odour fields in the olfactometer. Also, female parasitoids made significantly more visits to the calling moth odour field than to the opposite control field in the olfactometer. Further, the wasps spent significantly more time in the olfactometer field containing the sex pheromone released by calling virgin moths, than in control fields. If non-calling virgin moths were used as odour source, the response was reversed and wasps were repelled by the odour of the moths, and the numbers of visits were evenly distributed over the four flow fields. These results are discussed in relation to the foraging ecology of egg parasitoids.

Introduction

Parasitoid wasps of the genus *Trichogramma* employ a vast array of chemical cues in the search and successful attack of host eggs. Considerable knowledge has accumulated about the short range kairomones involved in host location and host recognition. Moth scales contain a contact kairomone that stimulates searching behaviour in *Trichogramma* spp. (Lewis *et al.*, 1972; Smits, 1982; Noldus & van Lenteren, 1985*b*; Zaborski *et al.*, 1987). Host accessory gland secretions, present on and around lepidopteran eggs, cause arrestment and induce host recognition by the parasitoids (Noldus & van Lenteren, 1985*b*; Nordlund *et al.*, 1987; Pak & de Jong, 1987). Much less is known about long distance search for hosts. Plant odours seem to play a role in host-habitat location by *Trichogramma* (Altieri *et al.*, 1981, 1982; Nordlund *et al.*, 1985*a*). The present study deals with the means by which *Trichogramma* detect host communities (here defined as more or less spatially distinct host infestations) within a habitat. Recent research evidence indicates that the sex pheromone of the host may

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play a significant role in this respect. Lewis *et al.* (1982) found that eggs of the cotton bollworm, *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae), were more heavily parasitised by naturally occurring *Trichogramma* spp. in cotton plots treated with synthetic sex pheromone of *H. zea* than in control plots. This finding aroused our interest about the behavioural mechanism underlying the observed effect. Increased parasitisation in treated plots could be due to attraction of parasitoids from a distance, arrestment and enhanced searching activity of local wasps, or both. Noldus & van Lenteren (1985*a*; *Chapter 4*) studied this problem in a comparable host-parasitoid system: the cabbage moth, *Mamestra brassicae* L. (Noctuidae) and *T. evanescens* Westwood. In olfactometer experiments *T. evanescens* responded positively to the odour of calling virgin *M. brassicae* females, and not to that of non-calling mated females nor to that of male moths. Besides that, *T. evanescens* made significantly more visits to the calling moth odour field than to the opposite control field (Noldus, unpubl.). Here we present the results of olfactometer experiments designed to determine whether *T. pretiosum* Riley shows a similar response to its host *H. zea*.

Materials and Methods

Parasitoids

The *Trichogramma pretiosum* wasps used in these experiments were from a laboratory culture originally obtained from Hermosilla, Mexico. The same stock was used by Lewis *et al.* (1982). The parasitoids were reared, following the procedure of Lewis & Redlinger (1969), on *H. zea* eggs at ca. 26 °C and 70 % r.h., under a 14L:10D photoperiod (light on at 6.00 h). For the experiments we used female wasps on the second day after emergence.

Prior to a test, female parasitoids were given an oviposition experience with *H. zea* eggs, according to the following procedure. Individual wasps were exposed for 30-60 min to 3-5 *H. zea* eggs that had been glued on a 5 x 25 mm strip of cardboard with rubber cement, in a 1 x 5 cm glass vial. The *H. zea* eggs had been obtained from a laboratory culture and had been washed with sodium hypochlorite as described by Burton (1969), irradiated with 25 krad (^{60}Co source) when 8-36 hours old, and stored at ca. 10 °C for no longer than 24 hours. The parasitoids were observed to insure that they parasitised at least one egg. The females were then isolated in empty glass vials and stored at 26 ± 1 °C for ca. 30 min prior to their use in experiments.

Host insects

Heliothis zea moths were reared according to the procedure of Burton (1969). Pupae were sexed and separated, and allowed to emerge under reversed photoperiod (14L:10D, lights off at 9.00 h) to facilitate experiments during daytime. Under these conditions, onset of the scotophase for the moths coincided with the start of the 3rd h of the photophase for the parasitoids. Adults were maintained on 10 % sugar water at ca. 25 °C and 70 % r.h. Virgin female moths were used between the 2nd and 8th hour of the scotophase, on the 2nd or 3rd night after emergence, when frequency of calling and pheromone release are highest (Pope *et al.*, 1984; Raina *et al.*, 1986). Male moths were used during the 2nd-4th scotophase.

Experimental set-up and procedure

Although the sex pheromone of *H. zea* has been chemically identified (Klun *et al.*, 1980) we used calling virgin moths as odour source, as we preferred to work with an odour blend as natural as possible, in order not to exclude any components that might be of relevance to the parasitoids. However, as the calling behaviour of *H. zea* consists of irregular bouts of extrusion of the abdominal tip (Agee, 1969), in contrast to female *Mamestra brassicae* moths, which often call for two hours or more, during one or two bouts (Subchev, 1980, 1983; Tóth, 1982; Attygalle *et al.*, 1987; *Chapter 3*), it is difficult to obtain long periods of continuous calling in a laboratory set-up. Therefore, we constructed an experimental set-up, based on the one used by Noldus & van Lenteren (1985*a*; *Chapter 4*), that allowed simultaneous observation of the activities of the wasp and monitoring of the behaviour of the moths (Figure 5-1). The basic set-up consisted of a four-armed airflow olfactometer, modified after Vet *et al.* (1983). The olfactometer as described by Vet *et al.* is made of Plexiglas™, which has a low resistance to strong organic solvents and high temperatures, which makes thorough cleaning and removal of pheromone traces impossible. Instead of that, the central exposure chamber in our set-up was made of a piece of white acetal (Delrin™), milled to form the bottom and side walls of the chamber to which all connections were made, and a glass cover plate that could be screwed onto the bottompiece with four acetal bolts. The upper surface of the bottompiece was polished so smoothly that no measures were necessary to prevent air leaks between bottompiece and cover. Further, no glass containers were used between the odour sources and the exposure chamber of the olfactometer, to keep the distance between odour source and exposure chamber as small as possible.

In experiment 1, three to five virgin moths, used as odour source, were placed in a glass cylinder. This cylinder was constructed from a modified ground ball and

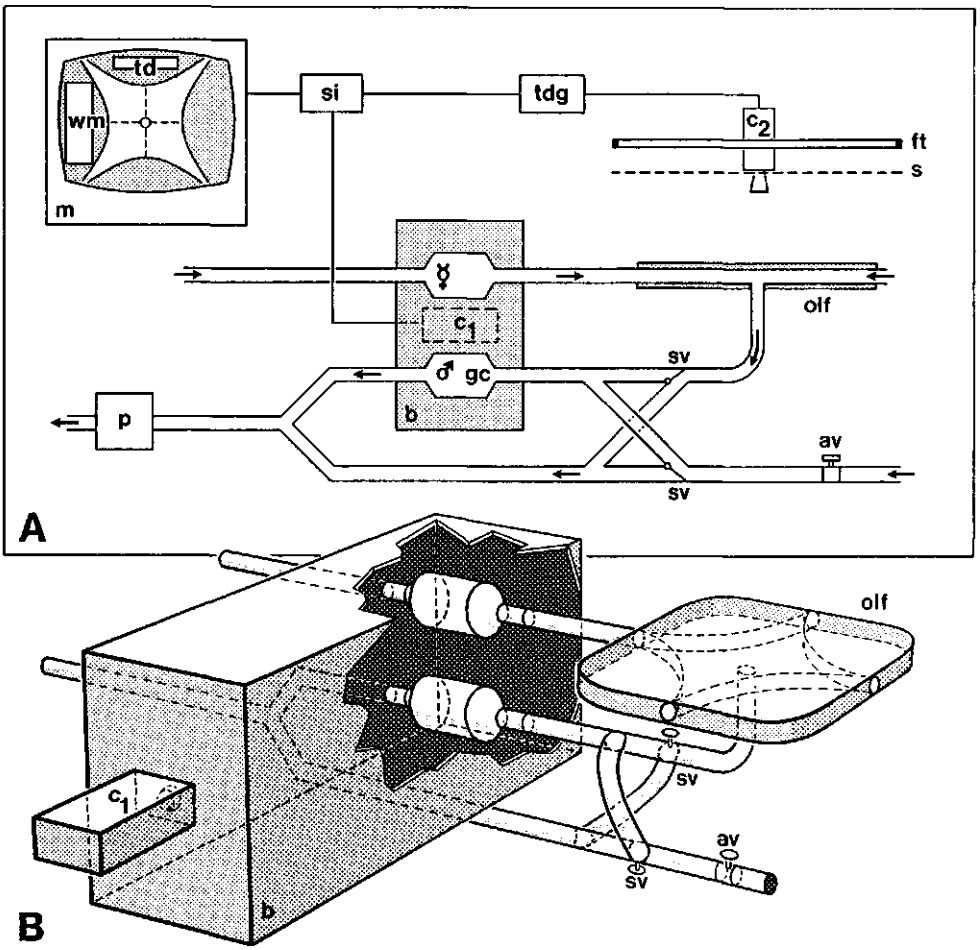

Figure 5-1. A. Schematic representation of the olfactometer set-up and peripheral equipment. B. Detail of part of the set-up. *av*: adjustable valve; *b*: dark box; *c1*: camera 1; *c2*: camera 2; *ft*: fluorescent tube; *gc*: glass chamber with moths; *m*: video monitor; *olf*: olfactometer; *p*: membrane pump; *s*: diffusing screen; *si*: video splitter/inserter; *sv*: switching valve; *td*: time/date on monitor; *tdg*: time/date generator; *wm*: window showing moths. Arrows indicate the direction of the air flow.

socket joint held together by a metal clamp, 90 mm long x 25 mm Ø, with a fritted glass filter (max. pore diameter 175 µm) – for improved uniformity of the air flow inside the cylinder – on the upwind end, and was connected to one arm of the olfactometer. A second glass cylinder (with a fritted filter on the downwind end) containing 3-5 male moths was attached to the exhaust air stream, and their behaviour was used as a check for the actual presence of sex pheromone in the air stream. In order to prevent habituation of the males to the pheromone, air to this cylinder was controlled by an arrangement of switching valves that provided a selection of either clean air or olfactometer exhaust air to pass over the males. Most of the time, clean air was passed over them. The airstream flowing through the olfactometer was directed over the males at ca. 2 min intervals by simultaneously turning the two switching valves. The switching did not alter the speed of the air flow over either the females or the males (effectuated by an adjustable valve) in order to avoid disturbance of their behaviour. The cylinders holding both male and female moths were contained in a single light-controlled box, measuring 39 x 18 x 27 cm (Figure 5-1*b*).

The activity of the moths was monitored, under the light of a 4 watt red lamp, with the aid of a video camera (RCA TC 2000, 8 mm/1.4 lens, camera 1). The light intensity inside the cylinders holding the moths was less than 0.007 W/m^2. The olfactometer was illuminated by two fluorescent tubes, through a frosted glass plate to diffuse the light uniformly. The behaviour of the wasps was observed with a second video camera (RCA TC 2000, 25 mm/1.4 lens, camera 2), with time/date added to it (Panasonic WJ-810 time/date generator), on a monitor (Panasonic WV-5470). To make simultaneous observation of the behaviour of parasitoids and moths possible, we used a video splitter/inserter (RCA TC 1470) with which the image from camera 1 was projected in a window on the image from camera 2 on the monitor. This was possible because of the shape of the olfactometer: the four concave sidewalls left four zones on the monitor empty for projection. Time/date were projected in the upper one, the window with the image of the moths in the left one. However, the maximum size of this window was too narrow to allow projection of the two complete glass cylinders in which the moths were present at a scale that calling behaviour of the females could still be established clearly. This was solved by slightly desynchronising the video image of camera 1, so that it slowly 'moved' sideways within the window, and thus the complete cylinders were 'scanned'. In this way, each individual passed along at a sufficiently slow speed and large scale, so that female calling activity and male response could easily be observed. Female moth calling activity was indicated by the extension of the terminal abdominal segments. Male moths responded to pheromone-loaded air with antennal movement, wing vibration and ambulatory activity. Further movement was limited by the enclosure of the glass container. A parasitoid was released into the olfactometer chamber only after calling activity of at least two of the female moths as well as a clear reponse of at least two of

the male moths had been observed. For an observation to be classified as valid, the same had to apply to the checks made during and at the end of the 10-min observation. Other observations were discarded.

Experiment 2 was a control experiment to test whether the reponse of the parasitoids to the calling moths was indeed due to odours associated with calling activity and not to other cues ('general body odour'). We used the same method as in experiment 1, but here only the observations counted where no calling activity of any of the females, nor any response of the males was observed during any of the checks pertaining to an observation.

After every observation the olfactometer bottompiece and cover were thoroughly cleaned with hexane. The next observation was not started until the solvent traces had evaporated.

Recording of the parasitoids' behaviour was done with the aid of a TRS-80 Model 100 computer (Tandy Corp., Fort Worth, Texas, U.S.A.) programmed as an event recorder (cf. *Chapter* 2). Parameters measured for each observation were: the number of visits to the various odour fields; the proportion of time spent by the parasitoid in the four odour fields during the 10-min interval; whether or not an insect entered one of the arms; if so, whether or not it stayed there longer than 2 min ('final choice' *sensu* Vet *et al.*, 1983). All experiments were carried out at 26 ± 1 ° C.

Results

Experiment 1: Response to calling moths

During the 10 min observations, the parasitoids made a significantly higher average number of visits to the flow field containing the odour of the moths than to the opposite control field. The adjacent control fields received intermediate numbers of visits (Table 5-1). Further, the insects stayed significantly longer in the flow field containing the odour of the calling moths, compared with the control fields (Table 5-2). Although 4 females entered the arm connected to the test field, none stayed there longer than 2 min.

Experiment 2: Response to non-calling moths

In the control experiment with non-calling virgin moths the total number of quadrants visited was significantly larger than in experiment 1 and the visits were evenly distributed over the four flow fields (Table 5-1). Further, a preference for the odour of the moths was not exhibited. Instead, the parasitoids spent a significantly smaller

Table 5-1. Average numbers of visits made by female *Trichogramma pretiosum* wasps to flow fields of the olfactometer.

Experiment	Odour source in test field	N	Total[1]	Flow field				P[2]
				Test	Left	Opposite	Right	
1	Calling moths[3]	15	23.0	7.8	5.9	4.1	5.2	< 0.01
2	Non-calling moths	39	31.4	7.7	7.7	8.0	8.0	> 0.05

1 Numbers in this column are significantly different (Mann-Whitney U test, $P < 0.01$).
2 Test of difference between numbers in test field vs. opposite field within one row (Mann-Whitney U test).
3 In 7 cases, only one moth was used, of which the behaviour was only monitored before and after the observations.

Table 5-2. Average percentage of time spent by female *Trichogramma pretiosum* wasps in flow fields of the olfactometer.

Experiment	Odour source in test field	N	Flow field				P[1]
			Test	Left	Opposite	Right	
1	Calling moths[2]	15	58.3	14.2	13.5	14.0	< 0.001
2	Non-calling moths	39	20.1	26.7	21.7	30.5	< 0.01

1 Friedman two-way analysis of variance by ranks.
2 In 7 cases, only one moth was used, of which the behaviour was only monitored before and after the observations.

proportion of their time in the test flow field compared with the adjacent control fields (Table 5-2). Also, only 5 out of the 39 females tested entered one of the arms, and no preference for any arm was shown.

Discussion

This study demonstrates that *Trichogramma pretiosum* can show an overt behavioural response to the odour emitted by calling *Heliothis zea* moths. The odour led to a decrease in the total number of border crossings between fields and a higher number of visits to the odour permeated field compared with the opposite field. Further, the proportion of time spent in the calling moth odour field versus control air fields was significantly higher. The fact that a reaction of the wasps was elicited only by female moths during calling activity and concurrent with male moth responses, strongly suggests that the parasitoids were indeed responding to the sex pheromone of the moth. The reaction of *T. pretiosum* to its host's sex pheromone may be illustrative of a more general phenomenon among egg parasitoids of Noctuidae, as similar effects have also been found for the palearctic species *T. evanescens*, parasitoid of *Mamestra brassicae* (Noldus & van Lenteren, 1985*a*; *Chapter* 4), as well as for *Telenomus remus*, parasitoid of *Spodoptera frugiperda* (Nordlund *et al.*, 1983).

These experiments confirm that the sex pheromone of *H. zea* serves as a kairomone for *T. pretiosum*. It seems adaptive indeed for an egg parasitoid to use host sex pheromone as an indicator for the probable presence of host eggs, because as far as known no other long distance cues more directly connected to the eggs themselves are available for *Trichogramma* spp. (*Chapter* 9). However, the results do not provide much information about the mechanism causing higher parasitisation rates in the field, as found by Lewis *et al.* (1982). A better understanding of the behavioural mechanisms governing the responses to these semiochemicals is essential for an interpretation of the present data in terms of searching efficiency and the eventual ability to manipulate such responses in the scope of biological pest control (Lewis & Nordlund, 1985; Jones, 1986). Attraction of parasitoids to infested areas has been proposed as an explanation (e.g., Wall, 1984; Vinson, 1986). However, in the present olfactometer experiments the wasps were not *attracted* by the odour. As attraction should be reflected in a directed movement towards the odour source, one would expect that in this set-up wasps would walk into the test arms and make a final choice for the odour of the calling moths. However, the majority of the 10 min interval was spent in the exposure chamber. In experiment 1, only 4 wasps ever walked upwind into the test arm, but none stayed there longer than 2 min. Comparably, in the experiment of Noldus & van Lenteren (1985*a*; *Chapter* 4), only 17 out of 44 insects walked upwind into the test arm, and only 7 made a final choice for the sex pheromone of *M. brassicae*. This is in contrast to the attraction and high numbers of

final choices found with larval parasitoids in olfactometer experiments (Vet, 1985). These small numbers may be due to physical characteristics of the olfactometer set-up (e.g., higher wind speeds around openings of arms, amount of light inside arms). Alternatively, a behavioural response leading to *arrestment* by the host's sex pheromone rather than attraction may be the mechanism causing higher rates of parasitisation in the field. Experiments to test this hypothesis are described in the next chapter (*Chapter 6*).

Other aspects of the odour of *H. zea* moths, in addition to the sex pheromone, apparently influence the searching behaviour of *T. pretiosum* as indicated by the repellent effect of the odour of non-calling virgin females in experiment 2. Noldus & van Lenteren (1985*a*; *Chapter 4*) did not find significant repulsion by the odour of non-calling *M. brassicae* females (although the average proportion of time spent in this odour field was considerably shorter compared with control fields), but they did find smaller number of visits to the moth odour field (Noldus, unpubl.), which was not found in the present experiments. There is not yet an interpretation for these effects.

One final aspect cannot be left unaddressed here. In the experiments described here, we have brought the parasitoids into direct contact with the odour of calling moths by rearing the latter under reversed photoperiod. However, in the field there is a gap in time between the release of sex pheromone by the host moths and searching activity of *Trichogramma* wasps. Calling activity by *H. zea* occurs during the night (Pope *et al.*, 1984; Raina *et al.*, 1986) and, although *Trichogramma* spp. will parasitise host eggs in darkness (Quednau, 1958; Klink, 1964), searching and parasitisation activity in the field appears to be restricted to daytime (Ashley *et al.*, 1973). Evidence from the studies of C. Wall and colleagues (Wall *et al.*, 1981; Wall & Perry, 1983) suggests that adsorption of pheromone to vegetation might be the factor that makes this material available as a kairomone for diurnal parasitoids. Experiments to test this hypothesis are described elsewhere (*Chapter 8*).

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USA) and the Department of Entomology, Agricultural University (Wageningen, The Netherlands).

6

How *Trichogramma* Parasitoids Use Moth Sex Pheromones as Kairomones: Orientation Behaviour in a Wind Tunnel[1]

ABSTRACT – The orientation behaviour of *Trichogramma* egg parasitoids (*T. evanescens* Westwood and *T. pretiosum* Riley) in response to the sex pheromone of their noctuid hosts (*Mamestra brassicae* L. and *Heliothis zea* (Boddie)) was investigated in a wind tunnel. Wasps were released on platforms which served as models of leaves, and were exposed to overhead light and an air stream that was either clean or loaded with host sex pheromone. The wasps exhibited (1) upwind anemotaxis which was not affected by odour, (2) odour-modulated positive phototaxis, and (3) odour-induced inverse orthokinesis. Compared to clean air, residence times, walking times and path lengths on a platform were higher in pheromone-loaded air than in clean air. In pheromone-loaded air, walking velocity was reduced. During locomotion on a horizontal platform, net movement was upwind, regardless of the presence or absence of pheromone in the air. On a platform inclined 45°, anemotaxis appeared offset by positive phototaxis. If wasps were released on top of a glass rod above a platform, host sex pheromone caused wasps to land shortly after take-off. These results are used to explain higher rates of parasitism of moth eggs in pheromone-treated plots in earlier field experiments.

Introduction

The role of semiochemicals in the foraging behaviour of parasitoids has received considerable attention during the last decade (for reviews see Vinson, 1984*b*, 1986; Nordlund *et al.*, 1988), in particular in view of possible application of semiochemicals to enhance the efficacy of parasitoids in biological control programs (for review see Lewis & Nordlund, 1985). Initially most studies focused on presence or absence of responses, often using indirect parameters. However, a sound assessment of the extent to which semiochemicals can be applied for behavioural modification requires a throrough understanding of behavioural responses following perception of chemical cues by foraging insects. This notion applies to herbivorous insects (Kennedy, 1977,

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1978) as well as to parasitoids (Jones, 1986). Understanding behavioural mechanisms requires the development of suitable bioassays for observing and analysing responses of parasitoids to semiochemicals. Only recently have such studies been undertaken (Drost *et al.*, 1986; Shu & Jones, 1988; Zanen *et al.*, 1989).

Semiochemicals play a role in various phases of the process of host finding and selection by egg parasitoids of the genus *Trichogramma* (for a review see Noldus, 1989*b*; *Chapter 9*). Sex pheromones of hosts appear to function as kairomones in the detection of host aggregations by these wasps. Lewis *et al.* (1982) found an increase in the rate of parasitisation of eggs of *Heliothis zea* (Boddie) on cotton by *Trichogramma* spp. when plots were treated with synthetic sex pheromone of *H. zea*. This finding provoked speculation that parasitoids could be *attracted* from a distance to infested areas (Wall, 1984; Vinson, 1986)[2]. However, alternatively, behavioural responses leading to *arrestment* by host sex pheromone and enhancement of local searching activity might be the mechanism causing higher rates of parasitisation in the field.

Experiments in a 4-way airflow olfactometer (Vet *et al.*, 1983) showed that two species of *Trichogramma* responded positively to the odour of calling moths: *T. pretiosum* Riley responded to its host *H. zea* (Noldus, 1988*b*; *Chapter 5*) and *T. evanescens* Westwood responded to *Mamestra brassicae* L. (Noldus & van Lenteren, 1985*a*; *Chapter 4*). However, from these results the behavioural mechanism of the response of parasitoids to the odour of calling moths could not be elucidated. Most airflow olfactometers are unsuitable for studies of orientation (Kennedy, 1977), and this is also true for the one used in our previous studies: the small exposure chamber allows only walking; flight is impossible. Furthermore, the insect cannot leave the experimental arena. Here we present a wind tunnel assay in which the orientation behaviour of *Trichogramma* can be studied in more detail, e.g. to discrimate between behaviours leading to attraction vs. arrestment. Experiments were designed to find out how wasps respond to host sex pheromone during locomotory activity on a substrate and at the onset of flight. Two different parasitoid-host systems were used: a Dutch system with *T. evanescens* and its host *M. brassicae*, and an American system including *T. pretiosum* and its host *H. zea*.

2 The terms *attraction* and *arrestment* are used with recognition that they refer to the end result of a behavioural response and do not imply a specific orientation mechanism (Kennedy, 1978).

Materials and Methods

Parasitoids

Trichogramma evanescens wasps were taken from a laboratory culture, originating from material collected on *Chilo* sp. in Egypt in 1981 (strain no. 57 in Pak & van Heiningen, 1985). Parasitoids were reared on eggs of the mediterranean flour moth, *Ephestia kuehniella* Zeller, at 25 ± 1 °C and 60 ± 5 % r.h. Adults were maintained at 15 ± 1 °C, 50 ± 5 % r.h. and 16L:8D.

Trichogramma pretiosum wasps came from a laboratory culture originally obtained from Hermosilla, Mexico. Parasitoids were reared, following the procedure of Lewis & Redlinger (1969), on eggs of *Heliothis zea* at ca. 26 °C and 70 % r.h., under a 14L:10D photoperiod.

In all experiments, 2-3 day old female wasps were used.

Moths

Mamestra brassicae was reared on Brussels sprouts, *Brassica oleracea* L. var. *gemmifera* cv. Titurel, at 21-22 °C, 60 ± 10 % r.h. and 16L:8D. The colony had been reared in the laboratory for ca. 80 generations. Pupae were collected from the colony, sexed and separated, and emerged under reversed photoperiod (16L:8D, lights off at 9:00 h) to facilitate experiments during daytime. Adults were fed a 10 % sugar solution. *Heliothis zea* was reared on artificial diet according to the procedure of Burton (1969).

Odour sources

Since the chemical composition of the sex pheromone of *M. brassicae* has still not been established unambiguously (*Chapter 3*), we used the most reliable source of sex pheromone, i.e. calling virgin moths. Moths were used during the 3rd-6th scotophase after emergence, during the last 4 h of the scotophase, when calling activity occurs consistently. During these periods, extrusion of the abdominal tip often continues for several hours (*Chapter 3*).

The calling behaviour of *H. zea* females consists of irregular bouts of abdominal tip extrusion rather than long periods of continuous calling, which complicates their use as odour source in a laboratory set-up (Noldus, 1988*b*; *Chapter 5*). For this species a synthetic sex pheromone blend is available, which was used as the odour source. The blend was identified by Klun *et al.* (1980) as the most effective sex attractant in the field and was also used by Lewis *et al.* (1982). It consisted of a 87:3:2:8 mixture of (Z)-11-hexadecenal, (Z)-9-hexadecenal, (Z)-7-hexadecenal and hexadecanal, loaded

on rubber septa at a dosage of 1 mg. With an airflow of 500 ml/min over a septum, this resulted in a release rate of ca. 55 ng/h, as determined by collection of the volatiles and analysis by gas chromatography using the method of Heath *et al.* (1986). This is close to the rate at which the major component Z11-16:Al is released during peak emission (Pope *et al.*, 1984).

Wind tunnel and general experimental procedures

Design principle

Insect responses to chemical cues cannot be investigated meaningfully apart from their total sensory ecology (Cardé, 1986; Prokopy, 1986). For instance, odour may trigger orientation to visual stimuli (Baker, 1985). It has long been known that *Trichogramma* spp. respond strongly to light. Although they are also negatively geotactic, upward flight to an overhead light source is primarily phototactic. This conclusion follows from various *Trichogramma* studies in the field (Schread, 1932; van Steenburgh, 1934) and in the laboratory (Quednau, 1958; Klink, 1961; Coulson *et al.*, 1982; Brower & Cline, 1984). Therefore, we used a combination between light and odour as the basic stimuli in the present study. To test if the response of *Trichogramma* wasps to host sex pheromone results in arrestment, we used light to stimulate a wasp to move away from a release point, and exposed the insect to a chemical cue. A chemical was considered to be an arrestant if phototaxis was suppressed by presentation of the chemical.

Wasps were released on differently shaped platforms which served as simple models of leaves – with distinct physical boundaries – and provided convenient substrates for behavioural observation.

Inhibition of flight initiation occurs in *Trichogramma* at air speeds higher than 30 cm/s (Keller, 1985). Therefore, we used an odour supply system that created a *wide* odour plume – completely enveloping the platform – at a *low* wind speed.

Description of the apparatus

Figure 6-1 shows the wind tunnel apparatus. A centrifugal ventilator (Fischbach CE 340/E 30-4) drew ambient air into the tunnel. Before the air enters the test section, it was cleaned by a 2 cm thick layer of activated charcoal and a glasswool air filter (A.A.F. Amer-glass Blue) between perforated galvanized steel plates (9.52 holes/cm^2, 2 mm Ø, 30 % transmittance). An empty section with a similar perforated steel plate and a 40-mesh brass screen (0.2 mm wire, 47 % transmittance) reduced the eddy size of the stream so that it entered the test section as a uniform air stream. At the

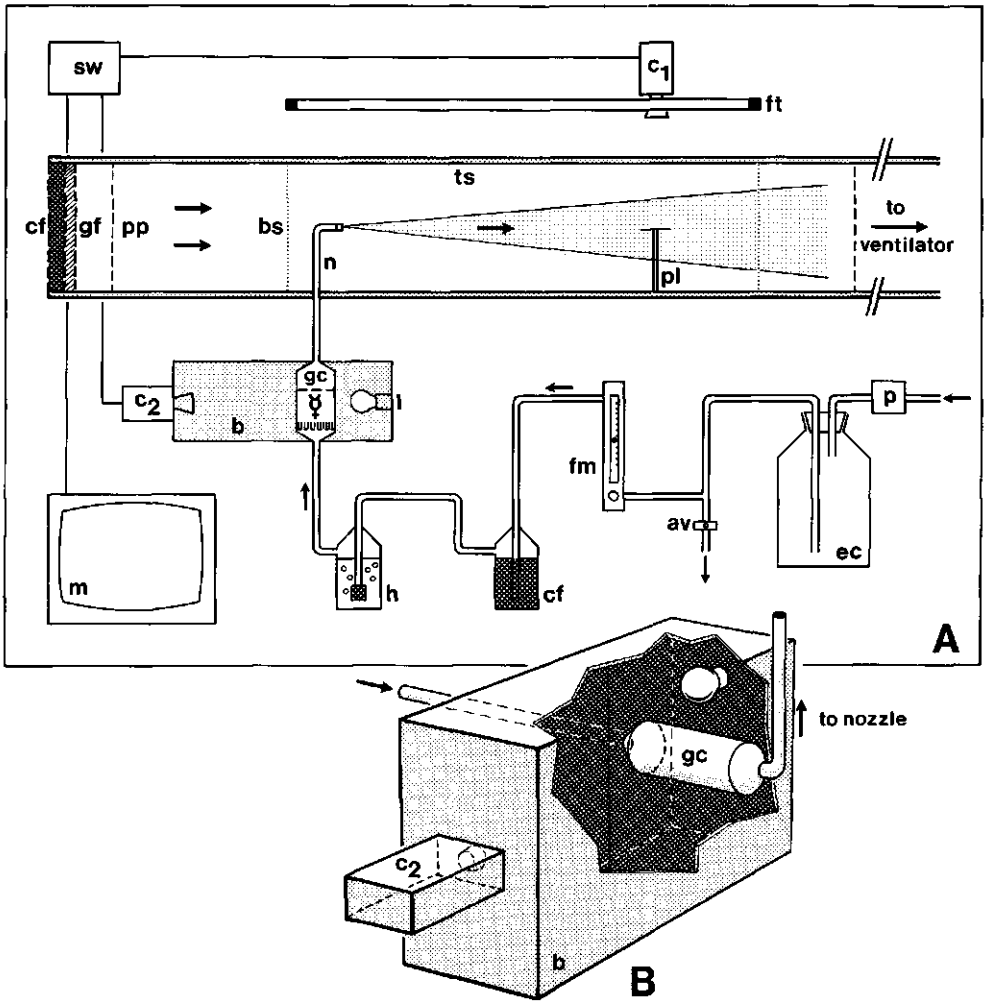

Figure 6-1. **A.** Schematic representation of the wind tunnel system. **B.** Detail of part of the set-up, present when calling moths were used as odour source. *av*: adjustable valve; *b*: dark box; *bs*: brass screen; *c1*: camera 1; *c2*: camera 2; *cf*: charcoal filter; *ec*: empty container; *fm*: flow meter; *ft*: fluorescent tube; *gc*: glass container with moths; *gf*: glasswool filter; *h*: humidifier; *l*: red lamp; *m*: video monitor; *n*: nozzle; *p*: membrane pump; *pl*: platform; *pp*: perforated plate; *sw*: switch; *ts*: test section. Arrows indicate the direction of the air flow.

downwind end of the test section, a hygrometer was mounted in the side wall. In the ceiling, five holes were available for introduction of a hot-wire anemometer probe; these holes were closed during operation of the tunnel. Behind the test section, a thermometer was mounted in the ceiling. Between the test section and the ventilator were another brass screen and perforated steel plate which, together with the narrow slit through which the air was drawn, guaranteed an even underpressure across the height and width of the downwind end of the tunnel. Eventually, the air was exhausted out of the building to prevent contamination of the room with test odours. To minimize disturbance of the low wind speed in the test section of the tunnel by changes in air pressure outside the building, there was no direct connection between the tunnel and the ventilator, nor between the ventilator and the outer wall. By leaving a 3 cm gap between tunnel and ventilator, the air displacement inside the tunnel was only ca. 25 % of the total air displacement of the ventilator, which allowed a high and stable number of r.p.m. Further, air was blown via the exhaust hose of the ventilator at a distance of 50 cm into the inlet of another ventilator (Itho Indolator 25 K) mounted in the wall, which produced a continuous air stream out of the building, greater than the amount of air exhausted out of the wind tunnel.

The test section (100 x 30 x 30 cm) originated from a tunnel described by Visser (1976) and was modified for the present study. The frame and floor were made of zinc, had streamlined corners, and were coated on the interior with white, ethanol-resistant paint. Glass doors in sidewall and ceiling, sealed with silicon tubing, permitted access to the interior. Wasp locomotory behaviour was observed with a video camera (Sony AVC-3250 CE, 12.5-75 mm/1.8 lens, camera 1) mounted on a rack on top of the tunnel, connected to a monitor. Observations of flight behaviour were made directly through a window. The test section was surrounded by a white curtain to ensure a uniform light distribution inside the tunnel, and to prevent disturbance of the insects by moving objects.

The odour plume in the tunnel was produced by blowing the odour-laden air into the tunnel through a nozzle – modified after Zanen *et al.* (1989). A membrane pump blew air sequentially through an empty 10 l glass container – that reduced the variations in air pressure caused by the pump membrane, a combination of an adjustable valve and a flow meter – that allowed a variable leak and accurate setting of the flow rate (Brooks Instrument, Sho-Rate 1355 Purgemeter, R2-15-D tube), a charcoal filter, and a container with distilled water (humidifier). The air then passed over the odour source to be tested and into the nozzle in the test section.

In control experiments with clean air, there was no odour source, so the humidifier was connected directly to the nozzle. When calling female moths were used as odour source, a wooden box (black on the inside) underneath the tunnel held a glass container with the moths (Figure 6-1*b*). This container measured 13 x 3.5 cm and consisted of two pieces with 38/40 ground ends sealed with a Teflon™ sleeve.

The upwind end contained a fritted glass filter (porosity 2, 80 mesh), to ensure air displacement - and thus odour collection - over the whole transverse profile of the container. By means of a video camera (Sony AVC-3250 CE, 28 mm/2.8 lens, camera 2) and a dimmed red lamp (producing ca. 0.015 W/m^2 in the center of the container) one could observe the behaviour of the moths without disturbance (e.g. to check whether moths were calling). The red lamp was only on while the moths were observed, to prevent a temperature rise. In experiments 2 and 3, where the synthetic sex pheromone of *H. zea* was used, a Swagelok™ brass container (Crawford Fitting Co., Solon, Ohio, U.S.A.) holding the pheromone-loaded septum was connected to the nozzle. Of the 500 ml/min blown over the septum, 70 % was discarded via a 3-way stopcock directly downwind of the container.

Air was blown at a rate of 150 ml/min through the nozzle mounted in the central axis of the tunnel, 7 cm from the upwind end. This was a brass pipe (6 mm ID), bent at a 90° angle, with a flat hexagonal brass cap screwed into it (sealed with a rubber O-ring) which had an opening of 0.3 mm. With the prevailing ventilator speed (which produced an ambient stream of ca. 5 cm/s), this resulted in a turbulent jet plume with a diameter of ca. 15 cm (determined by visualising the plume with ethylene diamino-tetraacetate smoke) and an air speed of ca. 15 cm/s in the center (determined with a hot-wire anemometer), measured at 60 cm downwind from the opening of the nozzle. The experimental platform (see below) was placed at this location. Overhead illumination was provided by four fluorescent tubes, producing a light intensity of 3.8 W/m^2 at the center of the platform. An air speed of 15 cm/s was chosen because it is not inhibitory for *T. exiguum*, a species similar in size to *T. evanescens* and *T. pretiosum* (Keller, 1985).

General procedures

In experiment 1, 3-4 virgin *M. brassicae* moths were transferred in a glass container into the dark box and were allowed to acclimatise for 1 h. The airflow was started as soon as continuous calling activity of at least two moths was observed. In experiments 2 and 3, a septum loaded with sex pheromone of *H. zea* was placed in the container, which was subsequently connected to the nozzle, after which the airflow was started.

Observations were only started after the membrane pump had run for ca. 20 min to allow build-up of an equilibrium between pheromone release and adsorption to container walls and tubing and thus a more or less stable pheromone concentration in the plume. After a day of testing, the wind tunnel was rinsed with hexane (except for the painted parts, which were cleaned with ethanol), and clean air was drawn through at 50 cm/s until the next day.

The behaviour of the insects was recorded on a Tandy 102 microcomputer, programmed as an event recorder with the software package The Observer (Noldus, 1988*a*; *Chapter* 2). Events were timed in 0.1 s.

In experiment 1, a switch allowed us to alternate between the image of camera 1 (the wasp) and camera 2 (the moths), to check the behaviour of the moths at regular intervals. An observation was classified as valid if calling activity of at least two moths had been observed before and after the observation.

Experimental procedures

Experiment 1: Locomotory behaviour on a horizontal plane

In the first experiment, the experimental arena consisted of a 5 x 5 cm horizontal platform positioned at a height of 15 cm (Figure 6-2*a*). It was covered with filter paper that was folded over the edges and attached to the underside with adhesive tape. A thin streak of odourless petroleum jelly along the edge on the underside prevented wasps from walking underneath the platform. The paper was replaced after every 10 trials. Wasps were released individually in the center of the platform and were observed until they flew from the platform, with a time limit of 15 min.

Due to the minute size of the wasps, behaviour observed on the video monitor was classified as *walking* or *not walking*. To record the position of the insect, a grid of 16 square sectors (1.25 x 1.25 cm) was superimposed on the image of the platform on the monitor, and behavioural events were recorded separately for each sector. During a number of observations, walking tracks were recorded on video tape. During replay, the tracks were traced onto acetate sheets with tickmarks indicating 10-s intervals, after which the coordinates were stored in a computer file via a digitizing tablet. The following parameters were determined:

— Total residence time on the platform.
— Time spent walking: absolute and as percentage of the total time.
— Percentage of time spent in the upwind half of the platform.
— Path length.
— Tortuosity of the walking track. For this parameter, we used the fractal dimension of the path, i.e. the slope of the linear regression of log(estimated total path length) on log(step length) (Dicke & Burrough, 1988). This index (D) falls between 1 (completely straight) and 2 (extremely tortuous). Maximum step length (λ_{max}) was 2.5 mm.
— Walking velocity: total path length divided by absolute walking time.
— Number of different sectors visited.

Experiment 1 was carried out with *T. evanescens* and *M. brassicae*. Wasps were naive with respect to oviposition and contact with plants or *M. brassicae*-related stimuli.

Preliminary experiments indicated that there was no effect of the time of day on the behaviour of *Trichogramma* in the wind tunnel. Therefore, controls were done in the morning and the pheromone treatment in the afternoon. Prior to observations wasps were isolated in gelatin capsules for 30-60 min. The experiment was conducted at 24 ± 1 °C and 60 ± 10 % r.h.

Experiment 2: Locomotory behaviour on an inclined plane

In experiment 2 behaviour was studied on a platform inclined at 45° with the lower edge directed to the wind (Figure 6-2*b*). Wasps were released individually in the center of the platform and were observed until they flew away, with a time limit of 10 min. The same parameters were measured as in experiment 1.

This experiment was carried out in Tifton (Georgia, U.S.A.), in a wind tunnel set-up similar to the one described above, with *T. pretiosum* and the synthetic sex

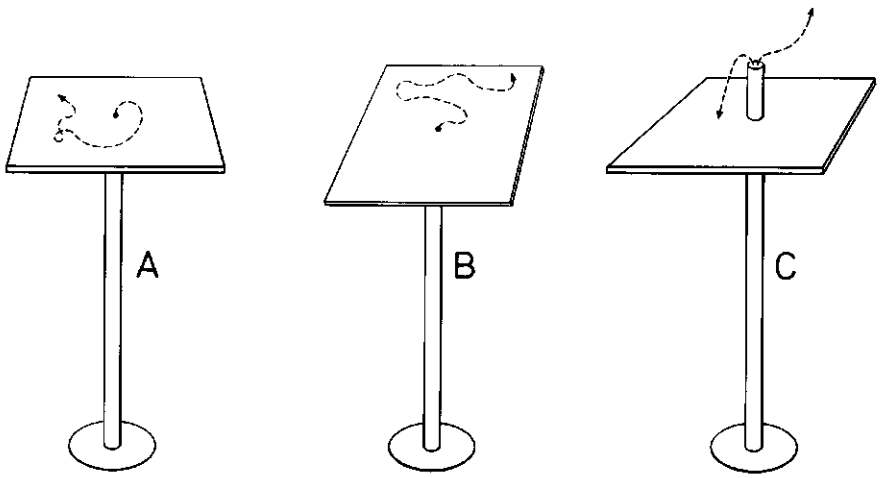

Figure 6-2. Schematic representation of platforms used in wind tunnel experiments. **A.** Horizontal platform (experiment 1). **B.** Inclined platform (experiment 2). **C.** Platform with central glass rod (experiment 3).

pheromone of *H. zea*. Treatments were alternated. Between treatments the wind tunnel was rinsed with solvents (see above) and clean air was drawn through at 50 cm/s for at least 2 h. Wasps were allowed to oviposit in a *H. zea* egg ca. 1 h prior to the experiment and were then isolated for ca. 30 min as described by Noldus (1988*b*; *Chapter 5*). The experiment was done at 29 ± 1 °C and 60 ± 10 % r.h.

Experiment 3: Flight behaviour

This experiment was designed to investigate the influence of host sex pheromone on *Trichogramma*'s behaviour at take-off. Wasps were released individually on top of a glass rod (4 mm Ø), extending 2 cm above the center of a green horizontal platform (6 x 6 cm), with a 2 mm wide ring of odourless petroleum jelly around the rod (1 mm under the top) preventing wasps from walking down (Figure 6-2*c*). Preliminary observations showed that flights could be classified as upward flights towards the overhead light, or flights resulting in a landing on the platform. This provided an assay to test the effect of odour on:
— Latency to take-off.
— Percentage of individuals landing on the platform.
— Distribution of landings over the upwind and downwind half of the platform.
Animals that did not take off from the rod within 5 min were discarded (ca. 1 % of the trials). After every observation the rod was cleaned with 96 % ethanol to prevent accumulation of odour molecules or traces of the wasps. Insects species and other experimental conditions were the same as in experiment 2.

Statistical analysis

Frequencies and durations of behaviours were computed with The Observer. All data were tested for normality, using either the Shapiro-Wilk test (if $N \leq 50$) or the Kolmogorov test (SAS, 1985). Most parameters showed significant deviation from a normal distribution and were thus analysed with non-parametric statistical tests. Otherwise parametric tests were used. Further details are given as footnotes in the tables.

Results

Experiment 1: Locomotory behaviour on a horizontal plane

The presence of the sex pheromone of *M. brassicae* in the air led to a significant increase in total time spent on the platform, time spent walking and path length of *T. evanescens* (Table 6-1, Figure 6-3). From these parameters, proportion of time spent walking (= walking time / total time) and walking velocity (= path length / walking time) can be calculated. However, since both total time and walking time differed between clean air and sex pheromone, a comparison across treatments could be confounded by a change of these parameters with time. Therefore we randomly sampled 20 observations (which lasted at least 5 min each) and calculated proportion walking and velocity for subsequent 1-min intervals, and plotted both against time. There was no correlation between either parameter and time, for both stimuli (Spearman rank-correlation test, $P > 0.05$). This justified a comparison of mean proportion of time spent walking and velocity across treatments. The walking velocity was significantly lower in pheromone-loaded air, while the proportion of time spent walk-

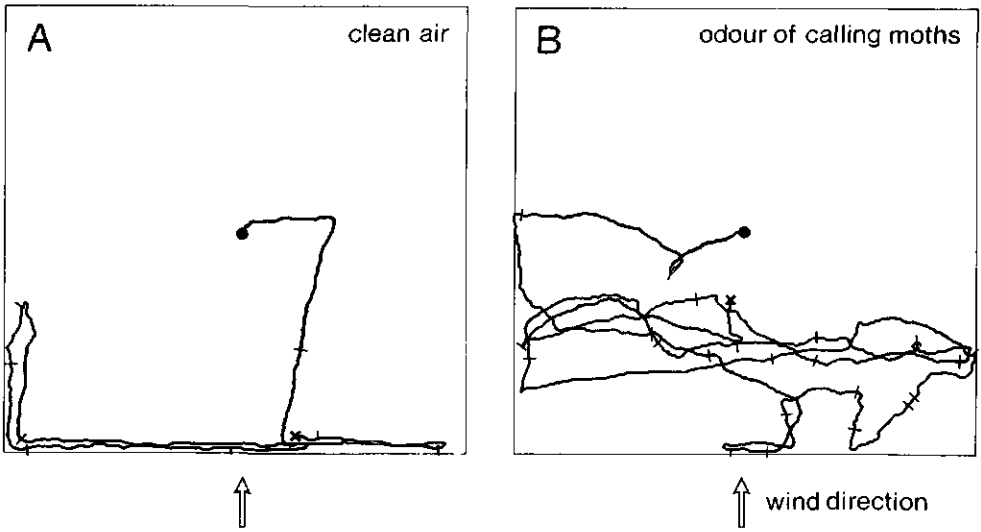

Figure 6-3. Walking tracks of *Trichogramma evanescens* wasps on a platform in the wind tunnel. **A.** Clean air. **B.** Air loaded with the sex pheromone of *Mamestra brassicae* by passing it over calling moths.

Table 6-1. Behaviour of individual *Trichogramma evanescens* wasps on a horizontal platform in a wind tunnel, when exposed to clean air or air loaded with the sex pheromone of *Mamestra brassicae*.

Parameter	Clean air	Pheromone-loaded air	P[1]
Total time on platform (s)	180.2 ± 7.1 (127)[2]	345.8 ± 25.3 (122)	****
Time spent walking (s)	79.4 ± 9.8 (103)	176.6 ± 16.1 (103)	****
Percentage of time spent walking (%)	48.1 ± 2.3 (103)	52.9 ± 2.5 (97)	n.s.
Path length (mm)	173.0 ± 43.0 (27)	297.6 ± 49.1 (26)	*
Tortuosity (D)	1.055 ± 0.007 (27)	1.048 ± 0.004 (26)	n.s.
Walking velocity (mm/s)	2.4 ± 0.1 (27)	1.3 ± 0.1 (26)	***
Percentage of time in upwind half (%)	69.5 ± 4.8 (49)	71.6 ± 4.5 (43)	n.s.
Percentage of wasps spending more than 50 % time in upwind half (%)	73.5 (49) [**][3]	76.7 (43) [***][3]	
No. of wasps reaching edge	24 (27)[4]	22 (24)[4]	n.s.[5]
Fraction of time in 2 mm edge zone[6] (%)	42.7 ± 5.5 (24)	59. 8 ± 6.0 (22)	n.s.
Percentage of wasps spending more than 15.4 % time in edge zone (%)	79.2 (24) [**][3]	86.4 (22) [***][3]	
Angle between wind direction and line from point of release to point of reaching edge or flight (°)	61.8 ± 9.4 (27)	63.2 ± 8.8 (24)	n.s.
Percentage of wasps spending more time upwind than downwind before reaching edge or flight (%)	81.5 (27) [**][3]	79.2 (24) [**][3]	n.s.[5]
Proportion of platform traversed (number of different 1.25 x 1.25 cm sectors traversed / 16)	0.47 ± 0.03 (53)	0.59 ± 0.05 (45)	n.s.
Proportion of platform traversed (number of different 1 x 1 cm sectors traversed / 25)	0.39 ± 0.08 (27)	0.57 ± 0.06 (24)	*

Table 6-1, *continued.*

1 Mann-Whitney U test (SAS, 1985), unless indicated otherwise. n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$.
2 Mean ± s.e. (number of observations), unless indicated otherwise.
3 In parentheses: number of observations. In brackets: 2-tailed significance level of difference from 50 % (sign test) (Siegel, 1956).
4 Number measured (number of observations).
5 Chi-square test, on numbers (Sokal & Rohlf, 1981).
6 Edge zone comprised 15.4 % of total surface area.

ing remained unaffected (Table 6-1).

We examined if *Trichogramma* wasps oriented to the direction of the air flow and if so, whether or not this was affected by odour. In clean air overall ca. 70 % of the time was spent in the upwind half of the platform, and most insects spent more than 50 % of the time upwind, which suggests a positive anemotaxis (Table 6-1). The distribution of time over the two halves was not altered by host pheromone. However, the percentage time in the upwind half is a very coarse measure of anemotactic orientation, as it appeared to be confounded by a strong edge effect: most wasps reached the edge of the platform and many of them subsequently spent a considerable part of their time walking along the edge around the platform. We calculated the time spent in the 2 mm wide zone along the edge (15.4 % of the surface area) – which corresponds with the estimated limit of visual perception of *Trichogramma* wasps (Laing, 1938; Glas *et al.*, 1981) – and compared this with the time expected if walking was random. The observed time distribution was clearly non-random (Table 6-1). Thus, an edge effect could have obscured a chemically modulated directed movement. It also explains the low measured path tortuosities: the calculated D-values were only slightly larger than 1, indicating a straight path (cf. Dicke & Burrough, 1988), and appeared not to be affected by the odour. To avoid the influence of the edge effect, we examined the part of the track between the point of release and the point where the animals first reached the edge or left the platform. For this part we determined: (1) the heading, i.e. the angle between the wind direction and the line connecting both points, and (2) whether the majority of the track was upwind or downwind of the point of release. The outcome of these calculations corresponded closely with the previous result: in both test situations, (1) the net heading was upwind and there was no significant difference between the angles in clean vs. pheromone-loaded air, and (2) most wasps made their initial track predominantly upwind from the release point (Table 6-1). We conclude that *Trichogramma*, when walking on a horizontal substrate, exhibits upwind anemotaxis,

which is not affected by host sex pheromone.

To estimate the proportion of the substrate that was searched, we counted the number of different sectors traversed by the wasps and divided that by the total number of sectors. The event recorder data yielded these numbers for 1.25 x 1.25 cm sectors. There was no significant difference between behaviour in clean air vs. odour-loaded air (Table 6-1). However, the size of these sectors was rather large compared to the size of *Trichogramma* and the bends in its walking path, which negatively affects the accuracy of a grid method (Reddingius *et al.*, 1983). For the video-recorded walking tracks we could also determine this parameter with a smaller sector size by superimposing a different grid. Table 6-1 shows that with sectors of 1 x 1 cm the difference between treatments is statistically significant.

Experiment 2: Locomotory behaviour on an inclined plane

In experiment 1 we used a horizontal platform. Thus light was uniformly distributed over the substrate. However, in nature leaves are rarely horizontal and illumination is rarely uniform. In the following experiment we studied the walking behaviour of *Trichogramma* on an inclined platform in the wind tunnel. These experiments were carried out with *T. pretiosum* and the synthetic sex pheromone of *H. zea*.

As in the previous experiment, total time and walking time were higher in pheromone-contaminated air than in clean air (Table 6-2). In this experiment, the behaviour was not recorded on video tape and complete walking tracks were thus not available. Path length was estimated by counting the total number of 1 x 1 cm platform sectors traversed (cf. Reddingius *et al.*, 1983). As this parameter is only used for comparative purposes, no conversion factor was applied to estimate the actual distance covered. Velocity was estimated by computing the average number of 1 x 1 cm sectors traversed per unit of walking time. Results indicated a significantly longer path and a lower velocity in pheromone-loaded air vs. clean air, similar to the results of experiment 1.

There were two obvious differences with experiment 1. First, the percentage time spent walking was higher in odorous air. Second, wasps allocated time to upwind and downwind halves of the platform differently. In experiment 1, wasps spent sigificantly more time upwind than downwind, regardless of treatment. Here insects spent equal amounts of time in upwind and downwind halves in clean air, while in pheromone-laden air the number of insects spending most time upwind was even significantly less than 50 % (Table 6-2). This suggests that on an inclined plane, upwind anemotaxis was offset by a positive phototaxis attracting the wasps to the higher part of the platform. However, time distribution over upwind and downwind part was not significantly different between the treatments, as in experiment 1.

Table 6-2. Behaviour of individual *Trichogramma pretiosum* wasps on an inclined platform in a wind tunnel, when exposed to clean air or air loaded with the sex pheromone of *Heliothis zea*.

Parameter	N[1]	Clean air	Pheromone-loaded air	P[2]
Total time on platform (s)	170	109.3 ± 10.3[3]	162.6 ± 13.2	****
Time spent walking (s)	170	62.3 ± 5.8	120.5 ± 9.2	****
Percentage of time spent walking (%)	170	68.2 ± 1.7	84.1 ± 1.5	****
Number of 1 x 1 cm sectors visited	170	16.8 ± 1.3	25.8 ± 2.0	****
Walking velocity (number of sectors visited/s)	170	0.36 ± 0.02	0.25 ± 0.01	****
Proportion of platform traversed (number of different 1 x 1 cm sectors traversed / 25)	170	0.43 ± 0.02	0.56 ± 0.02	****
Percentage of time in upwind half (%)	70	49.0 ± 3.8	40.9 ± 2.8	n.s.
Percentage of wasps spending more than 50 % time in upwind half (%)	70	51.4 (n.s.)[4]	31.4 (**)	

1 Equal numbers of observations for both treatments.
2 Mann-Whitney U test (SAS, 1985). n.s.: P > 0.05; *: P < 0.05; **: P < 0.01; ***: P 0.001; ****: P < 0.0001.
3 Mean ± s.e.
4 In parentheses: 2-tailed significance level of difference from 50 % (sign test).

Experiment 3: Flight behaviour

In experiments 1 and 2, wasps were constrained to movement on a 5 x 5 cm platform, and observations ended as soon as wasps flew off the platform. The following experiment examined the effect of host sex pheromone on the wasps' orientation at the onset of flight.

A narrow glass rod in the center of a platform provided a take-off point for *T. pretiosum* wasps. The preflight behaviour was similar to that described for *T. exiguum* (M.A. Keller, pers. comm.). After a short period of 'walking' on the surface of the

rod, a wasp usually assumed a 'pointing posture' with its antennae pointed upwind, followed by a 'wings erect posture' with its wings extended upwards, before it flew from the rod (see also *Chapter 8*). None of the 700 insects tested showed a sustained upwind flight. Flights that ended in landing on the platform rarely went higher than a few cm above the release point, and often the description 'downward hop' was more adequate than 'flight'.

Latency to take-off was not affected by the presence or absence of host sex pheromone in the air (Table 6-3). The tendency to land shortly after take-off was, however, affected by odour. In clean air, ca. 15 % of individuals landed on the platform; the majority flew upwards to the ceiling of the tunnel towards the light. In contrast, when the air plume contained the sex pheromone of *H. zea*, the percentage of animals landing on the platform almost tripled, indicating a suppression of the phototactic response in a significantly higher percentage of the insects. Landings on the platform were distributed equally over upwind and downwind half, regardless of the presence or absence of pheromone. This indicates that *Trichogramma* compensated for drift in the 15 cm/s air stream, but that host sex pheromone did not cause upwind flight.

Table 6-3. Flight behaviour of *Trichogramma pretiosum* wasps in a wind tunnel, when exposed to clean air or air loaded with the sex pheromone of *Heliothis zea*.

Parameter	Clean air	Pheromone-loaded air	P[1]
Number of observations	350	350	
Latency to take-off (s)	37.6 ± 2.7 [2]	38.4 ± 2.7	n.s.
Percentage of individuals landing on platform	14.9 ± 7.4 [3]	40.8 ± 13.1	****
Percentage of individuals landing on upwind half of platform	46.5 ± 7.5[2] (34)[4] [n.s.][5]	48.1 ± 3.7 (99) [n.s.]	n.s.

1 n.s.: P > 0.05; *: P < 0.05; **: P < 0.01; ***: P < 0.001; ****: P < 0.0001.
2 Mean ± s.e. Difference tested with Mann-Whitney U test (STSC, 1986).
3 Mean ± s.d. Difference tested with t-test (STSC, 1986).
4 In parentheses: number of landings of which position was recorded.
5 In brackets: 2-tailed significance level of difference from 50 % (sign test).

Discussion

The behaviour of *Trichogramma* in the wind tunnel was characterised by upwind anemotaxis, unaffected by host sex pheromone, and positive phototaxis, modulated by the odour. This was expressed as prolonged locomotion on a substrate and delayed initiation of flight, and induction of landing shortly after take-off. The result is arrestment of *Trichogramma* in areas where it perceives the stimulus. We did not find attraction to the source of the odour. The increased rates of parasitisation in treated plots found by Lewis *et al.* (1982) may thus be explained by the interception and extended retention of parasitoids already present or moving through the plots, rather than directed movement from a distance. Aggregation of *Trichogramma* wasps in host-infested areas may thus be based on a simple mechanism of passive downwind drift, followed by landing and locomotion, with subsequent take-off dependent on local wind velocity and chemical cues. This mechanism resembles the searching and dispersal behaviour of other small arthropods, such as aphids (Kennedy & Stroyan, 1959) and predatory mites (Sabelis & Dicke, 1985). It has further been observed in lacewings, which are also considered to be weak fliers (Duelli, 1984). Free flight by *Trichogramma* probably only occurs under the plant canopy. Upwind flight in open air is usually impossible under field conditions with high and unpredictable wind speeds (Keller, 1985) and net dispersal in field experiments is indeed mostly downwind (Keller *et al.*, 1985).

It need not be surprising that we did not find attraction of *Trichogramma* by its host's sex pheromone. In larval parasitoids, the flying insect orients directly to the source of semiochemicals – a feeding host – which is also the target of its searching effort (Drost *et al.*, 1986, 1988; Elzen *et al.*, 1987; Hérard *et al.*, 1988*a*; Zanen *et al.*, 1989). This behaviour is similar to that of male moths locating a calling female (Cardé, 1984). For an egg parasitoid, the sex pheromone of the host (kairomone for the parasitoid) may be an indication of reproducing adults and therefore of the presence of eggs in the area. However, the pheromone is not an indication of the precise location of the host eggs, and thus it does not seem adaptive for the wasp to locate spots where pheromone has been released. Prolonged searching in an area where host pheromone is perceived will increase the probability that the wasp contacts other cues with a closer spatial correlation with oviposition sites (e.g. host scales; see *Chapter 9*). To date, host eggs themselves are not known to release odours that attract *Trichogramma* from a distance (Laing, 1937; Noldus, 1989*a*; *Chapter 9*).

The volatile kairomone also reduced the walking velocity of *Trichogramma*. This inverse orthokinesis is a common component of odour-induced arrestment (Baker, 1985) and is also part of *Trichogramma*'s response to contact kairomones (Gardner & van Lenteren 1986; Shu & Jones, 1988). It may increase the chance that searching wasps contact host scales or eggs. In the whitefly parasitoid *Encarsia formosa* Gahan,

for example, the chance of encountering hosts is negatively correlated with walking velocity (Li *et al.*, 1987).

Wind and light can apparently be competing stimuli for walking *Trichogramma* wasps. In experiment 1 wasps spent most of their time in the upwind half of a horizontal plane, while in experiment 2 where an inclined plane was used this anemotaxis appeared to be offset by phototactic upward (and thus downwind) movement. Obviously, an inclined plane also facilitates geotactic orientation but, as mentioned above, the literature indicates that light is a stronger stimulus for *Trichogramma* than gravity.

The lack of attraction found in the wind tunnel may also explain why insects rarely walked into odour-delivering arms of an olfactometer while showing a clear tendency to remain in a field associated with that arm (Noldus & van Lenteren, 1985*a*; Noldus, 1988*b*; *Chapter 4, 5*). This underscores that results of airflow olfactometer experiments have to be interpreted very carefully in terms of orientation behaviour.

The wind tunnel assay described in this paper has proven to be suitable for the study of orientation behaviour in *Trichogramma*. Obviously, the apparatus is a trade-off between the ideal environment in which wasps can move freely and naturally, and the practical constraints of making direct observations on insects of not more than 0.5 mm in size. For instance, in order to make wasps visible in the tunnel and on the monitor, we were forced to maximize contrast by using a bright white background and platform cover, which obviously resulted in an environment with spectral properties different from what insects experience in the field.

In our study locomotory behaviour was studied on small platforms, which mimiced the shape of a leaf. The results confirmed casual observations that these insects are strongly thigmotactic[3]. Thus what seems like an artefact of the set-up may actually be true to natural conditions, since leaves also represent restricted areas with boundaries. Like many species of arthropods, *Trichogramma* spp. tend to move along physical structures on leaves such as veins and edges (G.A. Pak, pers. comm.; *Chapter 8*). Walking patterns like these can not be described meaningfully by means of a tortuosity index, as such an index is only useful in an environment where movement is unrestricted. In our experiments, the tortuosity index was clearly distorted by an edge effect, causing values close to 1. Of most tracks, the 'unconstrained part', i.e. the part between the release point and the point where the edge was reached for the first time, was too short to be analysed. Thus conclusions about a possible klinokinetic component of *Trichogramma*'s response to the kairomone cannot be drawn from the present data.

3 Thigmotaxis = a directed response to continuous contact with a solid surface (Lincoln *et al.*, 1982)

This study was carried out at two localities, using different but very comparable host-parasitoid systems. The experimental conditions differed somewhat between the two localities. However, as we focused on general mechanisms rather than on species characteristics, we think the results can be regarded as complementary. One aspect needs further attention, however. The comparison between experiments 1 and 2 is complicated by the fact that in experiment 2 the wasps had an oviposition experience prior to the test. It is not certain to what extent this has influenced the results. Oviposition can have an effect on behaviour of *Trichogramma* in a wind tunnel: in another study, *T. evanescens* showed increased residence times, walking times and path lengths, and equal time distribution over a horizontal platform after an oviposition. In addition, however, the oviposition effect completely overruled the effect of host sex pheromone, which was no longer expressed (Noldus *et al.*, 1988*b*). The latter was not the case with *T. pretiosum* in the present experiments (there was a strong effect of *H. zea* pheromone), and therefore the oviposition experience is not likely to have been a significant factor.

Several authors have speculated about the possible application of semiochemicals to enhance the efficacy of parasitoids in biological control programs (Lewis & Nordlund, 1985; Coppel, 1986; Powell, 1986; van Lenteren, 1987). However, in only few studies have behaviour-modifying chemicals been tested outside the laboratory (Gross *et al.*, 1975; Lewis *et al.*, 1979, 1982; Roland *et al.*, 1989) and application has not been incorporated in control programs to date (Noldus, 1989*b*; *Chapter 9*). There are potential applications of *Trichogramma*'s response to host kairomones in inundative release. Manipulation of wasp behaviour in the field could be part of a multi-tactic use of sex pheromones (Lewis & Nordlund, 1985). Apart from this, the response to a given concentration of host sex pheromone could be used as a criterion in the selection of candidate species or strains for inundative release programs (Noldus, 1989*b*; *Chapter 9*). Before this is done, the relevance of responses in the laboratory to searching efficiency in the field must be firmly established. Also, we need to know more about how host odours are distributed in the field relative to the distribution of host eggs. The spatial pattern of pheromone and eggs is crucial to characterising the adaptiveness of responses by egg parasitoids to host sex pheromones.

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7

Differential Response of *Trichogramma pretiosum*, an Egg Parasitoid of *Heliothis zea*, to Various Olfactory Cues[1]

ABSTRACT – The behaviour of *Trichogramma pretiosum* Nixon wasps when exposed to different olfactory cues was studied in a wind tunnel. Compared to clean air, the sex pheromone of its host *Heliothis zea* (Boddie) increased wasp residence times, walking times and path lengths on a platform, and decreased walking velocity. If wasps were released on top of a glass rod above a platform, the odour caused the wasps to land shortly after take-off. In addition, a clear dose effect with regard to total residence and walking times was found. These responses were not elicited by three dosages of the sex pheromone of *Spodoptera frugiperda* (J.E. Smith), nor by a blend of saturated acetates. These results correspond with the observation that *H. zea* is a common field host of *T. pretiosum*, whereas eggs of *S. frugiperda* are rarely attacked by this parasitoid.

Introduction

Olfactory receptor systems of insects are shaped continuously by natural selection to perceive chemical cues (i.e. semiochemicals) in an ever-changing environment. Of particular siginificance for foraging parasitic wasps are host-derived kairomones, the function of which has now been demonstrated for many species of parasitoids in a variety of taxonomic groups (Vinson, 1984*a*, 1985). Here we focus on cases where the kairomone used by a parasitoid is part of the intraspecific communication system of the host, so that the parasitoid can be labeled as a 'chemical eavesdropper' or 'chemical spy'. Examples hereof are known from several parasitoids of bark beetles which respond to their host's aggregation pheromone (Wood, 1982). A similar phenomenon has recently been demonstrated for egg parasitoids of the genus *Trichogramma*. These insects have received – and continue to receive – considerable research attention, due mainly to their potential as biological control agents against lepidopteran crop pests (Voegelé *et al.*, 1988). *Trichogramma pretiosum* Riley, for ex-

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ample, is a candidate for inundative releases against *Heliothis* spp. (King *et al.*, 1986; Ridgway *et al.*, 1988; King & Coleman, 1989).

Initial field and greenhouse observations (Lewis *et al.*, 1982) and subsequent olfactometer experiments (Noldus, 1988*b*; *Chapter 5*) indicated that female *T. pretiosum* utilize the sex pheromone of *H. zea* as a kairomone in their search for host eggs. We have recently analysed the orientation behaviour of two *Trichogramma* spp. (*T. evanescens* Westwood and *T. pretiosum*) in response to the sex pheromone of their noctuid hosts (*Mamestra brassicae* L. and *Heliothis zea* (Boddie)) in a wind tunnel (*Chapter 6*). Orientation by wasps to host sex pheromone was achieved by a combination of odour-modulated phototaxis and odour-induced inverse orthokinesis. Compared to clean air, kairomone-loaded air decreased walking velocity and increased residence times, walking times and path lengths on a platform. During locomotion on a horizontal platform upwind anemotaxis was evident but remained unaffected by odour. On a platform inclined 45°, the lower edge facing upwind, anemotaxis appeared to be offset by positive phototaxis. If wasps were released on top of a glass rod above a platform, the kairomone caused wasps to land shortly after take-off.

The sex pheromone of *H. zea* is a mixture of four straight-chain even-carbon-numbered aldehydes. The synthetic blend identified as the most effective sex attractant in the field consisted of (Z)-11-hexadecenal (Z11-16:Al), (Z)-9-hexadecenal (Z9-16:Al), (Z)-7-hexadecenal (Z7-16:Al) and hexadecanal (S-16:Al), in a 87:3:2:8 ratio (Klun *et al.*, 1980). This blend was also used in the previous experiments with *Trichogramma* (Lewis *et al.*, 1982; *Chapter 6*). Given the fact that *T. pretiosum* is found on several species of Lepidoptera in the field (Pinto *et al.*, 1986), one may wonder if *T. pretiosum* will respond to any randomly chosen blend of straight-chain aliphatic compounds.

Further, the responses of *T. pretiosum* to the sex pheromone of *H. zea* recorded so far were obtained in laboratory set-ups, either an four-arm airflow olfactometer (Noldus, 1988*b*; *Chapter 5*) or a wind tunnel (*Chapter 6*), in which host pheromone was tested against clean air. Due to the artificial environment, responses to stimuli recorded in such assays might represent a response to *something* vs. *nothing*, rather than a host-directed response (Jones, 1986).

To investigate these two hypotheses, comparative wind tunnel experiments were carried out in which *T. pretiosum* was exposed to the sex pheromone of *H. zea* and to (1) a biologically active sex pheromone blend of another moth species, but non-host of *T. pretiosum*, and (2) an artifical blend of acetates.

As first test substance we used the sex pheromone of the fall armyworm moth, *Spodoptera frugiperda* (J.E. Smith). This was chosen because both *S. frugiperda* and *H. zea* are noctuids, with similar geographical distributions and substantial overlap in host-plant ranges (Harding, 1976; Sparks, 1979; Hill, 1987). However, *S. frugiperda* is not a host of *T. pretiosum*. Calling female *S. frugiperda* release a 5-component blend,

consisting of (Z)-7-dodecen-1-yl acetate (Z7-12:Ac) (3.2 %), 11-dodecen-1-yl acetate (11-12:Ac) (2.2 %), dodecan-1-yl acetate (S-12:Ac) (1.9 %), (Z)-9-tetradecen-1-yl acetate (Z9-14:Ac) (90.1 %) and (Z)-11-hexadecen-1-yl acetate (Z11-16:Ac) (2.6 %) (Tumlinson *et al.*, 1986).

The second test material was a mixture that was chemically related to lepidopteran sex pheromones but that *T. pretiosum* would not encounter in the field. We used a blend of three saturated acetates – dodecanyl acetate (S-12:Ac), tetradecanyl acetate (S-14:Ac) and pentadecanyl acetate (S-15:Ac) – that has no known function as a semiochemical.

Materials and Methods

Parasitoid rearing

Trichogramma pretiosum wasps were reared on eggs of *H. zea* as described by Noldus (1988*b*; *Chapter 5*). *Heliothis* eggs were obtained from a laboratory culture; they had been treated with a solution of sodium hypochlorite for removal of adhesives and for sterilization (Burton, 1969). Only 2-day old female wasps were used. They were allowed to oviposit in a *H. zea* egg ca. 1 h prior to the experiment and were then isolated for ca. 30 min (Noldus, 1988*b*; *Chapter 5*).

Odour sources

The following odour sources were used in the experiments:

1. The synthetic sex pheromone of *H. zea*. The blend consisted of a 87:3:2:8 mixture of Z11-16:Al, Z9-16:Al, Z7-16:Al and S-16:Al. It was loaded on rubber septa at dosages of 0.1, 1 and 10 mg. These high dosages were necessary to obtain release rates in a range covering that of a calling female, at an airflow of 500 ml/min over the septum. A septum loaded with 1 mg released ca. 55 ng/h, as determined by collection of volatiles and analysis by gas chromatography using the method of Heath *et al.* (1986). This is close to the rate at which the major component Z11-16:Al is released during peak emission (Pope *et al.*, 1984).
2. The synthetic sex pheromone of *S. frugiperda*. The blend tested was loaded on rubber septa in such a ratio as to release the five compounds in the approximate ratio as they are released by calling females. It consisted of Z7-12:Ac (0.6 %), 11-12:Ac (0.7 %), S-12:Ac (0.4 %), Z9-14:Ac (87.0 %) and Z11-16:Ac (11.3 %), loaded on septa at dosages of 0.1, 1 and 3 mg.
3. A blend of three saturated acetates (S-12:Ac, S-14:Ac and S-15:Ac) in a 33:33:34

mixture. This blend was tested at a dosage of 1 mg/septum.
The desired amount of a blend in 200 µl hexane was pipetted into the large well of a rubber septum and the solution was allowed to soak into the septum. Then the septum was aired under a laboratory hood for 48 h to remove the solvent. Before a test, septa were aired for 1 h, and between tests they were stored at room temperature.

Experimental procedures

Wind tunnel

We used a horizontal, low-speed wind tunnel similar to the one described previously (*Chapter 6*), with minor modifications. Air was blown over the septum at a rate of 500 ml/min, of which 70 % was discarded via a 3-way stopcock directly downwind of the container holding the septum. Odour was injected into the main stream by blowing the odour-laden air through a glass nozzle (made from a Pasteur pipet, cf. Zanen *et al.*, 1989) at a rate of 150 ml/min. With the prevailing ventilator speed (which produced a main stream of ca. 5 cm/s), this resulted in a turbulent jet plume with a diameter of ca. 10 cm and an airspeed of ca. 15 cm/s in the center at 45 cm downwind from the opening of the nozzle (measured with a hot-wire anemometer). At this point a platform was located (see below), which was thus completely enveloped by the plume. The design of experiments was based on a combination of overhead light and airborne chemicals as competing stimuli for *Trichogramma* wasps (see *Chapter 6*). Overhead illumination was provided by four fluorescent tubes, which gave a light intensity of ca. 5.9 W/m^2 at the center of the platform.

At the start of a test, a septum loaded with test chemicals was placed in a brass container, which was connected to the nozzle, after which the airflow was started. Observations were begun after the pump had run for at least 20 min, to allow the odour concentration to stabilize. Treatments were alternated in a systematic fashion. Between treatments the wind tunnel was rinsed with ethanol and clean air was drawn through at 50 cm/s for at least 2 h. After a day of testing, the wind tunnel was rinsed with ethanol and ventilated with clean air until the following day. The behaviour of the insects was recorded on a TRS-80 Model 100 microcomputer, programmed as an event recorder (cf. *Chapter 2*). All experiments were performed at 29 ± 1 °C and 60 ± 10 % r.h.

Walking experiments

Two types of experiments were conducted. In both, platforms were used as experimental arena in order to mimic leaves. In the first, referred to hereafter as

'walking experiments', the experimental arena consisted of a platform, inclined at 45°, with the lower edge facing the wind (see *Chapter 6*, Figure 6-2*b*). The center of the platform was 15 cm above the floor of the wind tunnel. It was made of white cardboard covered with translucent plastic and had a surface area of 5 x 5 cm. A thin streak of odourless petroleum jelly along the edge underneath the platform prevented the wasps from walking to the lower surface. The platform was cleaned with ethanol after every observation. Wasps were released individually in the center of the platform and were observed until they flew away, or until 10 min had elapsed. The behaviour of the wasps was classified as either *walking* or *not walking*. In order to record the position of the insect, a grid of 25 square sectors (1 x 1 cm) was drawn on the platform, and behavioural events were recorded separately for each sector. The following parameters were measured:

— Total time on the platform.
— Time spent walking: both absolute and as percentage of the total time.
— Proportion of time spent in the upwind half of the platform.
— Path length: estimated by counting the total number of platform sectors traversed (cf. Reddingius *et al.*, 1983). As this parameter was only used for comparative purposes, no conversion factor was applied to estimate the distance actually covered.
— Walking velocity: estimated total path length divided by absolute walking time.
— Proportion of the platform traversed: estimated by counting the number of different sectors visited and dividing this by the total number of sectors (i.e. 25).

Flight experiments

The second type of experiments, referred to as 'flight experiments', were designed to test the effect of various odours on *Trichogramma*'s behaviour at the onset of flight. Wasps were released individually on top of a glass rod (4 mm Ø), extending 2 cm above the center of a green horizontal platform (6 x 6 cm), with a 2 mm wide ring of odourless petroleum jelly around the rod (1 mm from the top) preventing wasps from walking down the rod (*Chapter 6*, Figure 6-2*c*). Flights could be categorized into upward flights towards the overhead light, and flights resulting in a landing on the platform, providing an assay to test the effect of odour on:

— Latency to take-off.
— Percentage of individuals landing on the platform.
— Frequency of landings on the upwind and downwind half of the platform.

Animals that did not take off from the rod within 5 min were discarded (ca. 1 % of the trials). After every observation the rod was cleaned with ethanol to prevent accumulation of odour molecules or traces of the wasps.

Statistical analysis

Frequencies and durations of behaviours were computed with The Observer, a software package for behavioural research (Noldus, 1988*a*; *Chapter 2*). All data were tested for normality, using the Shapiro-Wilk test (if $N \leq 50$) or else the Kolmogorov test (SAS, 1985). The distribution of most parameters deviated significantly from a normal distribution. Those parameters were analysed with non-parametric statistical tests. Otherwise parametric tests were used. In the dose-response experiments, the dosages were compared to test for a dose effect; in a number of cases values of a single dosage were tested against those obtained with clean air. Further details are given as footnotes in the tables.

Results

Response to various olfactory cues

In the first walking experiment, parasitoids were exposed to three different odours and clean air. All odours were loaded on septa at a dosage of 1 mg. As shown in Table 7-1 and Figure 7-1*a*, only the sex pheromone of *H. zea* elicited responses. These were typical of those found previously (*Chapter 6*): increased total residence and walking times, path length and proportion of platform traversed, and reduced walking velocity. The only significant effect of the sex pheromone of *S. frugiperda* was a tendency for wasps to spend proportionally more time in the downwind half of the platform, compared to clean air.

Similar results were obtained in the first flight experiment (Table 7-2, Figure 7-1*b*): unlike the sex pheromone of *H. zea*, the other odours did not alter the percentage of wasps landing on the platform. Latency and the frequency of landings on upwind and downwind halves of the platform remained unaffected by the odour offered. In no treatment did the percentage of individuals landing on a given side differ significantly from 50 %.

Dose-response relationships

In the previous experiments, *T. pretiosum* responded to the sex pheromone of *H. zea* but not to that of *S. frugiperda*. However, both stimuli were compared at a single dosage, and a response to the fall armyworm sex pheromone might be obscured by a different dose-response relationship. The lack of response to the sex pheromone of *S. frugiperda* might thus have been due to the dosage tested. In addition, the sex pheromone of *H. zea* had been tested at ca. 30 % of the concentration released by in-

Table 7-1. Behaviour of individual *Trichogramma pretiosum* wasps on a platform in a wind tunnel, when exposed to various stimuli.

Parameter	Stimulus				P[1]
	Clean air	Sex pheromone *Heliothis zea*	Sex pheromone *Spodoptera frugiperda*	Acetate blend	
Number of observations	50	50	50	50	
Total time on platform (s)	99.9 ± 15.6[2] a	186.3 ± 22.8 b	101.0 ± 15.3 a	73.7 ± 16.9 a	**
Time spent walking (s)	66.6 ± 10.9 a	148.6 ± 17.8 b	62.5 ± 8.4 a	42.7 ± 6.7 a	***
Percentage of time spent walking (%)	71.5 ± 3.1 a	86.3 ± 2.6 b	72.5 ± 2.9 a	78.2 ± 4.7 a	**
Number of sectors traversed	17.9 ± 2.8 a	30.3 ± 3.4 b	15.6 ± 1.6 a	12.8 ± 1.4 a	**
Walking velocity (number of sectors traversed/s)	0.33 ± 0.02 a	0.24 ± 0.02 b	0.35 ± 0.02 a	0.41 ± 0.02 a	**
Number of different sectors traversed	11.2 ± 0.9 a	15.7 ± 1.0 b	10.9 ± 0.8 a	9.0 ± 0.7 a	**
Proportion of platform traversed (no. different sectors/total no. sectors)	0.45 ± 0.03 a	0.63 ± 0.04 b	0.44 ± 0.03 a	0.36 ± 0.03 a	**
Percentage of time spent in upwind half (%)	53.3 ± 4.4 a	44.3 ± 3.5 ab	35.4 ± 3.8 b	45.9 ± 4.4 ab	*
Percentage of wasps spending more than 50 % time in upwind half (%)	58.0 (n.s.)[3]	38.0 (n.s.)	30.0 (**)	32.0 (*)	

1 Kruskal-Wallis test (SAS, 1985), followed by distribution-free multiple comparison (Hollander & Wolfe, 1973). Different letters in a row indicate significant differences. n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

2 Mean ± s.e.

3 In parentheses: 2-tailed significance level of difference from 50 % (sign test) (Siegel, 1956).

Table 7-2. Flight behaviour of *Trichogramma pretiosum* wasps in a wind tunnel, when exposed to various stimuli.

Parameter	Stimulus				P[1]
	Clean air	Sex pheromone *Heliothis zea*	Sex pheromone *Spodoptera frugiperda*	Acetate blend	
Number of observations	100	100	100	100	
Latency to take-off (s)	42.9 ± 5.8[2]	29.1 ± 4.1	35.3 ± 5.2	39.0 ± 5.4	n.s.
Percentage of individuals landing on platform	17.0 ± 11.2[3] a	46.0 ± 18.5 b	13.0 ± 5.1 a	13.0 ± 5.1 a	**
Percentage of individuals landing on upwind half of platform	38.4 ± 7.4[2] (17)[4] [n.s.][5]	50.9 ± 5.8 (46) [n.s.]	48.3 ± 15.7 (13) [n.s.]	33.3 ± 16.3 (13) [n.s.]	n.s.

1 n.s.: P > 0.05; *: P < 0.05; **: P < 0.01.
2 Mean ± s.e. Differences tested with Kruskal-Wallis test (STSC, 1986).
3 Mean ± s.d. of 5 blocks of 20 observations. Different letters indicate significant differences (95 % LSD intervals) (STSC, 1986).
4 In parentheses: number of landings of which position was recorded.
5 In brackets: 2-tailed significance level of difference from 50 % (sign test).

dividual calling females. However, *T. pretiosum* is likely to be exposed to the kairomone in the field at a different concentration. Therefore, we examined the behaviour of *T. pretiosum* when exposed to clean air vs. various concentrations of the pheromones of *H. zea* and *S. frugiperda*, respectively. Tables 7-3 and 7-4 present the results of walking experiments with the sex pheromone of *H. zea* and *S. frugiperda*, respectively. With increasing concentrations of the sex pheromone of *H. zea* we observed a significant increase in total residence and walking times (Table 7-3, Figure 7-1c). Other parameters (estimated path length, proportion of platform traversed) also showed an upward trend, but means were not significantly different. Furthermore, all parameters differed significantly from values obtained in clean air, as followed from paired tests with the lowest dosage. In contrast, dose-response relationships with *S. frugiperda* were insignificant for all parameters, except for walking velocity, which was higher with the highest dosage tested (Table 7-4). This value was also

Table 7-3. Behaviour of individual *Trichogramma pretiosum* wasps on a platform in a wind tunnel, when exposed to different dosages of the synthetic sex pheromone of *Heliothis zea*.

Parameter	Clean air	Sex pheromone dosage (mg)			P[1]
		0.1	1	10	
Number of observations	100	100	100	100	
Total time on platform (s)	97.2 ± 13.9[2]	117.2 ± 13.4 (***)[3] a	132.0 ± 16.6 a	174.3 ± 17.5 b	*
Time spent walking (s)	54.3 ± 7.5	95.7 ± 10.5 (***) a	99.4 ± 11.4 a	138.6 ± 13.9 b	*
Percentage of time spent walking (%)	68.7 ± 2.3	87.4 ± 1.5	86.1 ± 1.8	85.8 ± 1.8	n.s.
Path length (number of sectors traversed	15.0 ± 1.7	21.4 ± 2.0	22.9 ± 2.8	31.8 ± 3.3	n.s.
Walking velocity (number of sectors traversed/s)	0.40 ± 0.03	0.26 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	n.s.
Number of different sectors traversed	9.8 ± 0.6	12.6 ± 0.6	12.6 ± 0.6	14.2 ± 0.6	n.s.
Proportion of platform traversed (no. different sectors/total no. sectors)	0.39 ± 0.02	0.50 ± 0.02	0.50 ± 0.02	0.57 ± 0.03	n.s.

1 Kruskal-Wallis test for differences between dosages (i.e. clean air not included), followed by distribution-free multiple comparison. Different letters in a row indicate significant differences. n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

2 Mean ± s.e.

3 In parentheses: 2-tailed significance level for difference with clean air (Mann-Whitney U test).

higher than the value obtained in clean air.

Similarly, in flight experiments we also found a significant dose response with the sex pheromone of *H. zea* (Table 7-5, Figure 7-1*d*) and not with that of *S. frugiperda* (Table 7-6). With the pheromone of *H. zea*, increasing dosages yielded increasing percentages of animals landing on the platform, and the value obtained with the lowest dosage (0.1 mg) was significantly different from that found in clean air. In contrast, the concentration of pheromone of *S. frugiperda* did not affect the percentage of animals landing. With both odours, time until take-off and the distribution of land-

Table 7-4. Behaviour of individual *Trichogramma pretiosum* wasps on a platform in a wind tunnel, when exposed to different dosages of the synthetic sex pheromone of *Spodoptera frugiperda*.*

Parameter	Clean air	Sex pheromone dosage (mg)			P[1]
		0.1	1	3	
Number of observations	50	50	50	50	
Total time on platform (s)	99.9 ± 15.6[2]	85.6 ± 14.1	101.0 ± 15.3	108.7 ± 17.9	n.s.
Time spent walking (s)	66.6 ± 10.9	45.7 ± 7.2	62.5 ± 8.4	62.3 ± 10.5	n.s.
Percentage of time spent walking (%)	71.5 ± 3.1	61.6 ± 3.3	72.5 ± 2.9	64.4 ± 3.5	n.s.
Path length (number of sectors traversed	17.9 ± 2.8	14.5 ± 2.3	15.6 ± 1.6	21.2 ± 3.1	n.s.
Walking velocity (number of sectors traversed/s)	0.33 ± 0.02	0.40 ± 0.04 (n.s.)[3] ab	0.35 ± 0.02 (n.s.) a	0.40 ± 0.02 (***) b	*
Number of different sectors traversed	11.2 ± 0.9	9.4 ± 0.8	10.9 ± 0.8	12.1 ± 0.9	n.s.
Proportion of platform traversed (no. different sectors/total no. sectors)	0.45 ± 0.03	0.38 ± 0.03	0.44 ± 0.03	0.48 ± 0.04	n.s.

* See Table 7-3 for explanation of superscript numbers.

ings over upwind and downwind halves of the platform were not affected by odour concentration, and the percentage of animals that landed on the upwind side did not differ significantly from 50 %.

Discussion

The two questions posed at the onset of this study – (1) does the response of *T. pretiosum* to *H. zea* sex pheromone represent a general response to any blend of aliphatic compounds, and (2) could it be a general response due to the artificial environment rather than a host-directed response – can both been answered negatively. *Trichogramma pretiosum* apparently discriminated between different odours, since it did not respond to the sex pheromone of *S. frugiperda* nor to a blend of saturated

Table 7-5. Flight behaviour of *Trichogramma pretiosum* wasps in a wind tunnel, when exposed to different dosages of the synthetic sex pheromone of *Heliothis zea*.

Parameter	Clean air	Sex pheromone dosage (mg)			P[1]
		0.1	1	10	
Number of observations	150	150	150	100	
Latency to take-off (s)	40.7 ± 5.2	35.2 ± 4.3	36.6 ± 2.6	36.5 ± 5.5	n.s.[2]
Percentage of individuals landing on platform	16.3 ± 4.8	38.9 ± 3.9 a (****)[4]	43.4 ± 6.8 a	64.0 ± 9.7 b	**[3]
Percentage of individuals landing on upwind half of platform	37.5 ± 26.5 (6)[5] [n.s.][8]	56.3 ± 1.5 (18) [n.s.]	58.2 ± 3.1 (21) [n.s.]	–[6]	n.s.[7]

1 n.s.: P > 0.05; *: P < 0.05; **: P < 0.01; ***: P < 0.001; ****: P < 0.0001.
2 This row: mean ± s.e. Kruskal-Wallis test of differences between pheromone dosages (i.e. clean air not included) (STSC, 1986).
3 This row: mean ± s.d. of blocks of 20-25 observations. Different letters indicate significant differences between pheromone treatments (95 % LSD intervals).
4 In parentheses: two-tailed significance level of difference with clean air, t-test (STSC, 1986).
5 In parentheses (this row): number of landings of which position was recorded.
6 Not recorded.
7 As 2, but Mann-Whitney U test.
8 In brackets: 2-tailed significance level of difference from 50 % (sign test).

acetates. This result was probably not due to the dosage tested, as we found a clear dose effect with regard to total residence and walking times with *H. zea* and not with *S. frugiperda*. These results correspond with the fact that *H. zea* is a common field host of *T. pretiosum*, while eggs of *S. frugiperda* are very rarely parasitised by this species (Pinto *et al.*, 1986). That *T. pretiosum* does not use fall armyworm sex pheromone as a kairomone seems adaptive because eggs of this moth are difficult to parasitise: they are laid in batches covered by a thick layer of scales (Sparks, 1979), which seem to obstruct the parasitoid (Vickery, 1929; W.J. Lewis, pers. obs.). The scelionid *Telenomus remus* Nixon does attack *S. frugiperda* in the field (Wojcik *et al.*, 1976). It is more robust than *T. pretiosum*, and has been reported to dig successfully through the layer of scales of *Spodoptera litura* (F.) to reach the eggs (Braune, 1982). The same tactic may be used to attack *S. frugiperda*. Alternatively, *T. remus* may be aided by its dis-

Table 7-6. Flight behaviour of *Trichogramma pretiosum* wasps in a wind tunnel, when exposed to different dosages of the synthetic sex pheromone of *Spodoptera frugiperda*.*

Parameter	Clean air	Sex pheromone dosage (mg)			P[1]
		0.1	1	3	
Number of observations	200	100	100	100	
Latency to take-off (s)	39.3 ± 3.7	52.4 ± 7.6	35.3 ± 5.2	36.7 ± 4.8	n.s.[2]
Percentage of individuals landing on platform	14.0 ± 8.6	14.0 ± 7.3 a	13.0 ± 5.1 a	13.0 ± 2.4 a	n.s.[3]
Percentage of wasps landing on upwind half of platform	48.5 ± 7.0 (28)[5] [n.s.][8]	44.0 ± 20.7 (14) [n.s.]	48.3 ± 15.7 (13) [n.s.]	20.0 ± 7.3 (13) [n.s.]	n.s.

* See Table 7-5 for explanation of superscript numbers.

tally positioned ovipositor (typical for scelionids) which facilitates probing through the layer of scales, while *T. pretiosum* cannot do so because (like in most chalcidoids) its ovipositor is ventrally located (M.R. Strand, pers. comm.). *Telenomus remus* has shown to respond to an extract of abdominal tips of *S. frugiperda*, as well as to Z9-14:Ac and Z9-12:Ac, reported components of its sex pheromone (Nordlund *et al.*, 1983).

Trichogramma spp. have traditionally been considered generalists in their choice of hosts (Hase, 1925; Salt, 1935; Sweetman, 1958; Thomson & Stinner, 1989*a*). It has been suggested that plant-generalist natural enemies use more general chemical cues than plant specialists (Vinson, 1976; Sheehan, 1986). By this reasoning one might expect host-generalist parasitoids to use a wider range of chemical cues in their search for hosts than host specialists (Jones, 1986). Our study shows that *T. pretiosum* discriminated between blends of organic chemicals which – although they differed in several aspects – were all straight-chain even-carbon-numbered aliphatic compounds. Similarly, *T. minutum* responded to a hexane extract of scales of *Choristoneura fumiferana* (Clemens) and not to an extract of *Sitotroga cerealella* (Oliver) scales (Zaborski *et al.*, 1987). Although labeling organisms as 'generalist' or chemical compounds as 'general' seems rather arbitrary, these findings corroborate recent findings that species and/or strains of *Trichogramma* may be much more specific in their search for hosts and their use of semiochemicals than previously thought (van Dijken

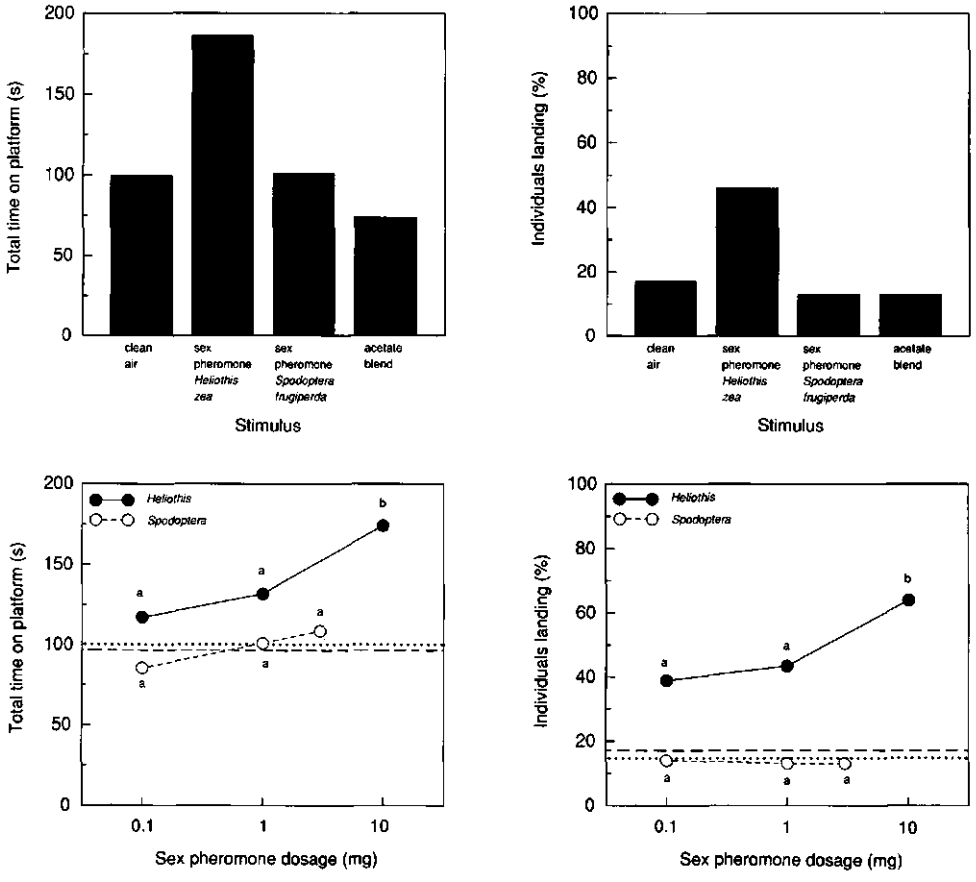

Figure 7-1. Response of *Trichogramma pretiosum* wasps to various olfactory cues in a wind tunnel: summary of results. **A.** Total time spent on a platform when exposed to various stimuli (data from Table 7-1). **B.** Percentage individuals landing on a platform when exposed to various stimuli (data from Table 7-2). **C.** Total time spent on a platform when exposed to different dosages of the synthetic sex pheromone of *Heliothis zea* or *Spodoptera frugiperda* (data from Tables 7-3 and 7-4). **D.** Percentage individuals landing on a platform when exposed to different dosages of the synthetic sex pheromone of *H. zea* or *S. frugiperda* (data from Tables 7-5 and 7-6). – – – –: value obtained in clean air, experiments with sex pheromone of *H. zea*; ·····: idem for experiments with sex pheromone of *S. frugiperda*. For more details, see the tables.

et al., 1986; Pak, 1988).

From the present results we cannot conclude what caused *T. pretiosum* to discriminate between the three odours. The blends differed in ratio of components, which in turn varied in chain length, presence of double bonds and functional group. As far as the pheromonal function of these substances is concerned, each of these aspects can confer response specificity (Tumlinson & Teal, 1987). With regard to kairomonal effects we can only speculate, for instance that *T. pretiosum* might respond specifically to aldehydes and *T. remus* to acetates. The question of specificity can also be applied to parts of pheromone blends. Is the whole blend essential for a response, or is one component sufficient? Noldus & van Lenteren (1985*a*) found that *T. evanescens* did not respond to Z11-16:Ac, the major component of the sex pheromone of *M. brassicae*, in olfactometer experiments with material tested at a single dosage. The concentration may have been incorrect, or a minor component (or combination of components) may be responsible for *Trichogramma*'s response to the odour of calling female moths. Obviously, additional studies are necessary to answer these questions.

Not uncommon among studies of insect behaviour, our wind tunnel experiments with *Trichogramma* were characterized by a large amount of behavioural variation among individuals. Large numbers of observations were required to show significant effects of treatments and almost all measured parameters had highly skewed frequency distributions (see also *Chapter 6*). To reduce this variation somewhat we gave parasitoids an oviposition experience with a host egg prior to a test, since this is known to enhance searching activity in *T. pretiosum* (Gross *et al.*, 1981*b*). This may have been a confounding factor in the present experiments on odour specificity. Recent research has shown that contact with host chemicals alone (even without oviposition) can be sufficient to increase responses to host odours (Vet, 1988). However, the host eggs used in our study had been washed with sodium hypochlorite prior to being offered to wasps, which probably removed any volatiles with which oviposition might have been associated. Furthermore, although experimental data are limited, no effect of oviposition in one host species on the response to kairomones from different hosts has ever been found for *Trichogramma* spp. (Gardner & van Lenteren, 1986; Thomson & Stinner, 1989*b*).

Another possibly confounding factor needs consideration here. The wasps used in the experiments originated from a laboratory culture of *T. pretiosum* that had been reared on *H. zea* for 15 years. It has been found for *Trichogramma* that prolonged rearing on a factitious host can increase acceptance of that host species (Taylor & Stern, 1971; Kaiser *et al.*, 1989; Noldus, 1989*b*; *Chapter 9*). However, *H. zea* is a natural host of *T. pretiosum*, preferred over other lepidopteran hosts, e.g. *Trichoplusia ni* Hübner (Ashley *et al.*, 1974*b*). In addition, as far as it has been investigated, the rearing host does not appear to affect preference for host chemicals in *Trichogramma* (Zaborski *et*

al., 1987; Noldus, 1989*b*; *Chapter 9*). Finally, since the rearing procedure included washing host eggs prior to parasitism by *T. pretiosum*, wasps were never exposed to *H. zea* sex pheromone. Therefore, the response of *T. pretiosum* to the sex pheromone of the corn earworm and the lack of response to that of the fall armyworm were probably not a result of artificial selection in the course of prolonged rearing on *H. zea*. A response to *H. zea* sex pheromone similar to that of wasps from the laboratory colony has been found in a strain of *T. pretiosum* shortly after collection from the field (Noldus, 1989*b*; *Chapter 9*).

As mentioned previously, testing odours against clean air in a wind tunnel may not be the optimal assay for understanding how wasps use chemical cues during foraging in a chemically variable environment. Jones (1986) therefore pleads for experiments in more natural settings where the significance of stimuli is studied by substracting them from a 'chemically complete' environment rather than adding them to a 'chemically deplete' environment. However, the minute size of *Trichogramma* complicates direct observations of behaviour under natural conditions. As a compromise, responses to kairomones can be studied in the laboratory in the presence of plants and other host-related cues. Results of recent experiments following this approach are reported elsewhere (*Chapter 8*).

Acknowledgements

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8 Moth Sex Pheromone Adsorption to Leaf Surface: Bridge in Time for *Trichogramma*[1]

ABSTRACT - When Brussels sprouts leaves were exposed to air passed over a single calling *Mamestra brassicae* moth, sex pheromone was adsorbed onto the leaf surface to such an extent that it subsequently elicited behavioural responses in conspecific male moths, as well as in female *Trichogramma evanescens*, egg parasitoids of *M. brassicae*. Male moths showed significant responses to odour-treated leaves at close distance (ca. 5 cm) in a wind tunnel and upon contact, but were not attracted at a distance of 1 m. In contrast, sex pheromone adsorbed to and re-released from the glass wall of a wind tunnel compartment attracted male moths from 1 m. *Trichogramma*'s locomotory behaviour on leaves is described. On leaves previously exposed to air passed over a calling female moth, *Trichogramma* wasps allocated significantly longer total residence time than on leaves treated with clean air or air passed over a non-calling female moth. In addition, wasps spent relatively more time along the margin and on the leaf underside on treated leaves compared to control leaves. Proportional time allocation to different behavioural components was not affected by leaf treatment. All effects persisted for at least 4 h, and after 24 h, treated leaves still increased wasp residence times. These results are the first example of responses of male moths to adsorbed airborne sex pheromone originating from a single female moth. They further suggest how sex pheromone released by nocturnal moths may function as a kairomone for diurnally foraging parasitoids.

Introduction

Interactions between organisms involving semiochemicals (or 'infochemicals' *sensu* Dicke & Sabelis, 1988) can best be studied in a multi-trophic context. That appears the best approach for a thorough analysis of evolutionary costs and benefits, and selective forces moulding intraspecific and interspecific communication systems. This notion is not new; it has repeatedly been stressed (e.g. Price *et al.*, 1980; Price, 1981, 1986), but is only recently being translated into integrated studies of the interactions between plants, herbivores and their natural enemies (Boethel & Eikenbary, 1986; Dicke, 1988*a*). Plants do not have to be passive victims of herbivory: evidence is accumulating that plants cannot only actively defend themselves against attack

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(Rhoades, 1985; Schultz, 1988), but can also play an important role as an intermediary between phytophages and their predators and parasitoids (Nadel & van Alphen, 1987; Dicke & Sabelis, 1989).

Brussels sprouts plants (*Brassica oleracea* L.) are attacked by various herbivorous insects, including the caterpillars of the cabbage moth, *Mamestra brassicae* L. (Lepidoptera: Noctuidae). The eggs of this species are in turn attacked by the parasitoid wasp *Trichogramma evanescens* Westwood. *Trichogramma* spp. use various semiochemicals in their search for host eggs, including plant synomones and host kairomones (for a review see Noldus, 1989*b*; *Chapter 9*). One of the cues to which foraging *Trichogramma* wasps respond is the sex pheromone of their host, which is thus used as a kairomone by the parasitoids. This was first implicated in field experiments by Lewis *et al.* (1982) with the noctuid *Heliothis zea* (Boddie). Laboratory olfactometer experiments confirmed a behavioural response of *T. pretiosum* Riley to the odour of calling *H. zea* moths (Noldus, 1988*b*; *Chapter 5*). Similarly, *T. evanescens* responded to the sex pheromone of *M. brassicae* in an olfactometer (Noldus & van Lenteren, 1985*a*; *Chapter 4*). A wind tunnel study with *T. evanescens* and *T. pretiosum* showed that host sex pheromone elicits orientation behaviours (inverse orthokinesis and suppression of phototaxis) that result in arrestment in the area where the odour is perceived (*Chapter 6*).

The 'chemical espionage' by *Trichogramma* wasps has so far been investigated in laboratory bioassays where stimulus emitter (the calling moth) and recipient (the wasp) were artificially synchronized. However, this does not reflect natural conditions. Both *M. brassicae* and *H. zea* release their sex pheromone during the scotophase (Subchev, 1983; Pope *et al.*, 1984; *Chapter 3*), while *Trichogramma* spp. search only during daylight (Ashley *et al.*, 1973). This raises the question: how can parasitoids perceive moth sex pheromones in the field? The aim of the present study was to investigate the possible role of the plant as a bridge in time between pheromone release by moths and kairomone perception by parasitoids. Brussels sprouts leaves are covered by an epicuticular wax consisting of long-chain aliphatic compounds, crystallized in a structure of fine tubules and/or platelets (Baker, 1982). Such a large apolar surface should favour adsorption of moth sex pheromone molecules, which are typically apolar straight-chain compounds (Tumlinson & Teal, 1987). (Z)-11-hexadecen-1-yl acetate (Z11-16:Ac), the main component of the sex pheromone of *M. brassicae* (*Chapter 3*), belongs to this class of chemicals.

Previous studies have shown that lepidopteran sex pheromones can be adsorbed onto plant surfaces and subsequently be re-released. Cotton leaves scent-marked by a female *Pectinophora gossypiella* (Saunders) – by touching the surface with the extruded abdominal tip – attracted male moths (Colwell *et al.*, 1978). Extensive studies with the pea moth, *Cydia nigricana* (F.), showed that wheat plants exposed to synthetic sex attractant adsorbed the chemical and subsequently attracted male

moths for several hours after exposure was terminated (Wall *et al.*, 1981; Wall & Perry, 1982, 1983).

In these studies the adsorption/re-release effect was obtained with rather high dosages of pheromone. During scent marking, *P. gossypiella* deposit a considerable quantity on the substrate. In their experiments with *C. nigricana*, Wall *et al.* used a synthetic sex attractant at a dosage that may have been higher than what is released by calling females. Here we show that airborne sex pheromone, released by an individual calling *M. brassicae* moth, is adsorbed onto Brussels sprouts leaves and affects the behaviour of conspecific male moths as well as that of *T. evanescens* wasps.

Materials and Methods

Plant and insect rearing

Host plants

Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* cv. Titurel) was used as host plant. Plants were reared in a greenhouse at 20 ± 3 °C, ambient humidity and with at least 16 h light/day.

Mamestra brassicae

A stock colony of *M. brassicae* was maintained on Brussels sprouts at 20 ± 2 °C, 50 ± 10 % r.h. and a 16L:8D photoperiod. It had been reared in the laboratory for ca. 80 generations. Pupae were obtained from the stock colony and sexed. They were placed in a 20 x 20 x 8 cm plastic container with a layer of saw dust, and incubated in a climate box, located in a dark room, at 20 ± 1 °C, 60 ± 10 % r.h. and a 16L:8D photoperiod. The photoperiod was reversed to that in nature to facilitate experiments during daytime. In order to have calling female moths and responsive males available throughout the day, two parallel sets of climate boxes were operated, with lights off at 5:00 h and 9:00 h, respectively. Male and female *M. brassicae* were kept in separate climate boxes, in different locations in the building, to prevent perception of female sex pheromone by male moths. During the photophase, the climate box was illuminated by a white fluorescent tube, that produced ca. 3.5 W/m^2 light intensity in the center of the box. Newly emerged female moths were placed individually (or in groups of 2-4) in glass cylinders (12 x 7 cm). Male moths were kept individually in 6 x 6 cm glass cylinders. The containers had a gauze cover, a piece of filter paper on the bottom, and a feeding container with 10 % sugar solution. Males were used during the 2^{nd}-9^{th} scotophase after emergence.

Trichogramma evanescens

The *T. evanescens* wasps used in this study were taken from a laboratory culture, based on material collected on *Chilo* sp. in Egypt in 1981 (strain no. 57 in Pak & van Heiningen, 1985). Parasitoids were reared on eggs of the mediterranean flour moth, *Ephestia kuehniella* Zeller, at 25 ± 1° C. Adults were maintained at 15 ± 1 °C and 16L:8D photoperiod. In all experiments, 3-day old honey-fed female wasps were used that were naive with respect to oviposition or contact with *M. brassicae*-related cues.

Preliminary tests revealed that considerable variation existed with respect to locomotory capacity among insects in the colony. Such variation reduces the discriminative ability of a bioassay based on residence times on a substrate (see below). Therefore, wasps were selected for locomotory capacity by exposing a rearing tube with wasps to an overhead light source. Only those insects that flew up into a glass container between rearing tube and light source within 3 min were used. This procedure effectively excluded individuals with limited walking and/or flight ability (Noldus, unpubl.). Wasps were isolated in gelatine capsules and stored in the experimental room for 30-90 min prior to testing.

Experimental procedures

Response of male moths to odour sources from a distance

Wind tunnel. The first experiments were carried out in a low-speed wind tunnel (Figure 8-1*a*). The system consisted of a series of compartments made of 4.5 cm ID glass tubing. A membrane pump blew air sequentially through a combination of an adjustable valve and a flow meter (Brooks Instrument, Sho-Rate 1355 Purgemeter, R6-15-A tube), allowing a variable leak and accurate setting of the flow rate, a charcoal filter and a container with water (humidifier), all connected with silicone tubing. Subsequently, the air passed through a funnel-shaped glass tube before it entered the wind tunnel. This connecting piece (*cp*) contained a fritted glass filter (porosity 2, 80 mesh) to ensure air displacement – and thus odour collection – over the whole transverse profile of the next compartment that contained the odour source to be tested.

The arrangement of compartments between the funnel-shaped connector and the test section of the tunnel varied with different experiments. In the first experiment, where male moths were exposed to a calling female moth or to clean air, it consisted of a single compartment (10 cm) that either contained a female moth or was left empty (Figure 8-1*a*, *mc*); wind speed was ca. 15 cm/s. In the next experiments, male moths were exposed to a leaf treated with either the odour of a calling female

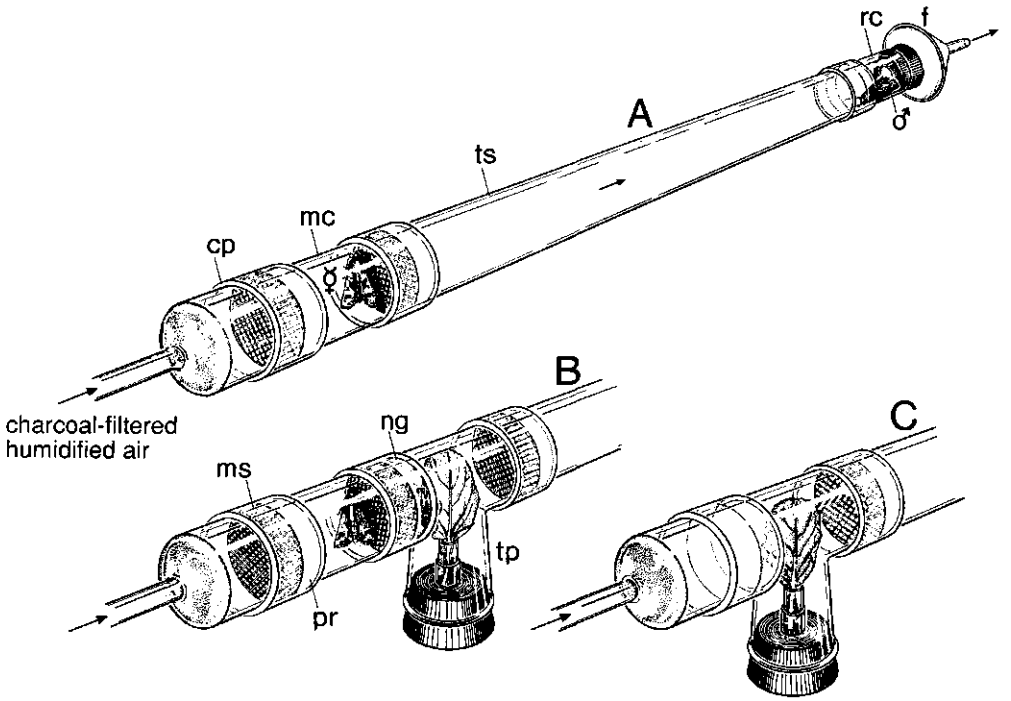

Figure 8-1. **A**. Schematic representation of the wind tunnel system. **B**. Arrangement of compartments during treatment of a leaf with the odour of a calling moth. **C**. Idem, during bioassay testing of a treated leaf. *cp*: connecting piece with glass filter; *f*: funnel through which air is exhausted; *mc*: compartment holding calling moth (left empty during treatment with clean air); *ms*: metal screen; *ng*: nylon gauze; *pr*: Plexiglas ring; *rc*: release compartment holding male moth; *tp*: T-piece holding cabbage leaf; *ts*: test section. Arrows indicate the direction of the air flow.

M. brassicae or with clean air. These experiments were carried out in two steps. During treatment of a leaf, the system contained a 10 cm compartment that either held a calling female moth or was left empty (*mc*), followed downwind by a compartment that held the leaf to be treated (Figure 8-1*b*, *tp*). The latter compartment was a 10 cm T-shaped piece of which the side arm had a 40/45 ground end, closed by a cork stopper. During subsequent testing of male responses to treated or control leaves, the system contained only a T-piece with a leaf (Figure 8-1*c*, *tp*). The presence of a leaf in the system reduced wind speed to ca. 11 cm/s.

After air had passed over the odour source it entered the test section of the wind tunnel, consisting of a 1 m long tube (*ts*), followed by a 10 cm compartment from which male moths were released (*rc*). The test section was separated from the compartment holding the odour source by a stainless steel screen (16 mesh, 66 % transmittance, *ms*). A 2.5 cm wide strip of stainless steel screen on the bottom of the test section provided grip for moths and facilitated walking. All glass compartments were connected by means of Plexiglas™ rings (*pr*), sealed air-tight with Teflon™ tape, which also facilitated the insertion of various types of screens between compartments. To prevent contamination of the room with test odours, air that left the downwind end of the tunnel was exhausted out of the room via a funnel (*f*) (placed at 5 cm from the downwind end) and hose connected to the laboratory vacuum system.

After a day of testing, the tunnel was dismantled and all components were cleaned with water and detergent. Glass and steel parts were subsequently rinsed with acetone and all parts except the 1 m tube were heated in an oven at 200 °C for 3 h. Plexiglas parts were rinsed with 96 % ethanol. The wind tunnel experiments were carried out in a controlled environment (20 ± 1 °C, 80 ± 10 % r.h.). Illumination was provided by dimmed red lamps which created a light intensity of 0.0007 W/m^2 near the tunnel.

Odour source. Since we wanted to use natural release rates, a calling virgin *M. brassicae* moth was used as source of sex pheromone in all experiments. Moths were used during the 3^{rd}-5^{th} scotophase after emergence, during the last 4 h of the scotophase, when calling activity occurs consistently (Subchev, 1983; *Chapter 3*). During the photophase (when *M. brassicae* females are quiescent) preceding a test, a surplus of females of proper age were placed individually in 10 cm glass tubes, closed at both ends with stainless steel screen. At least 15 min prior to use in the wind tunnel, 2 calling females were selected and transferred (in the glass tubes) to the experimental room in a light- and air-tight box. At the start of a test, a tube with a female was inserted into the wind tunnel system, as described above.

In experiments where non-calling female moths were used as control treatment, we used 4-10 day old moths that had mated 1-3 days before the experiment. We have

never observed renewed calling activity by mated *M. brassicae* until at least 4 days after mating.

Treatment of leaves with odours. For experiments we used leaves from the 10-12th visible node (counted from the apical meristem) of ca. 2-month old Brussels sprouts plants. These leaves had a surface area of 30 ± 2 cm^2. Leaves were picked ca. 24 h before tests and placed in 1 x 5 cm plastic vials with water. Only completely turgescent leaves were used. A leaf was introduced into the air stream, with the adaxial side facing upwind, by placing the vial holding the leaf into a hole drilled in the cork which fitted tightly into the ground end of the T-piece. Subsequently, (pheromone-loaded or clean) air was blown over the upperside of the leaf for a period of 3-4 h. The variation in length of treatment occurred near the end of the scotophase. During this period pheromone release rate is only a fraction of that during peak release (Bestmann *et al.*, 1988). The effective treatment duration was thus ca. 3 h in all experiments.

If a calling female was used as odour source, visual checks were made for calling activity of the moth at 30-min intervals. In the rare event that extrusion of the abdominal tip was not observed, the moth was immediately replaced by a calling female. In addition, the presence of sex pheromone in the air was verified by two tests with a male moth during the treatment period, carried out as described below. These tests invariably resulted in strong wing fanning responses and almost invariably in upwind flight up to the end of the test section, confirming release of sex pheromone.

Behavioural observations. At least 2 h prior to a test, male moths were placed individually in 10 cm glass tubes that served as release compartment (see above). These tubes were closed at both ends with a stopper. The latter were covered with a plastic sleeve to prevent contamination with test odours, had a central hole to permit undisturbed air flow, and had a circular piece of stainless steel screen attached to them to provide grip for the moth. Sleeve and screen were replaced after each test. At least 1 h prior to a test, tubes with males were transferred to the experimental room to allow them to become quiescent under ambient light and temperature conditions.

During quiescence, moths usually sat on the screen on one end of the compartment. An observation was started by removing the stopper at the other end and connecting the tube to the downwind end of the test section of the wind tunnel. From then on, the following parameters were recorded:

1. Distance from odour source: for this purpose, the test section of the tunnel had been divided into 10 cm sections, marked on the glass tube. All transitions between sections were recorded.

2. Locomotion behaviours:
   a. *Quiescent*: completely motionless.
   b. *Wing movement*: light wing vibration, no body movement.
   c. *Walking*: walking, no wing movement.
   d. *Walking + wing fanning*: walking and simultaneous wing vibration, much more intense than (b).
   e. *Flight*: flight; this was obviously hampered by the limited dimensions of the test section, but yet clearly distinct from walking behaviour.
3. Other behaviours:
   a. *Extension of claspers*: extension of the genital claspers.
   b. *Other*: all other behaviours, e.g. preening.

In the first experiment an observation lasted until extension of genital claspers occurred, while the moth was in contact with the upwind screen, or until 5 min had elapsed. In the second and third experiment all observations lasted 5 min. Males were only tested once per day, except for a few occasions where males were first used in a control test and next exposed to the treatment. Because of the low light conditions, data were recorded on audio tape. Later, data were entered into a Tandy 102 microcomputer, programmed as an event recorder with The Observer, a software package for behavioural research (Noldus, 1989*a*; *Chapter* 2).

Data analysis. Frequencies of occurrence and durations of behaviours, nested by location, were computed with The Observer. In addition, the following latencies (from the start of the observation) were determined:
— *Locomotion*: first occurrence of walking, walking + wing fanning, or flight.
— *Departure*: first departure from the release compartment.
— *Contact with screen*: first contact with the screen at the upwind end of the test section.
— *Extension of claspers*: first occurrence of clasper extension.

Frequency distributions of durations and latencies were significantly skewed (Shapiro-Wilk test, $P < 0.05$; STSC, 1986). Therefore, differences between means were tested with Mann-Whitney U tests (STSC, 1986). Frequency of occurrence of parameters among males across treatments were compared with the G-test with Williams correction (Sokal & Rohlf, 1981).

Response of male moths to contact with odour-treated leaves

This experiment was designed to measure the response of male *M. brassicae* moths to contact with leaves treated with the odour of a calling female. In order to have a treated leaf as well as a control leaf available during a bioassay, two similar wind

tunnels – each with one treatment – were operated simultaneously. A Brussels sprouts leaf was treated as described above by exposing the upperside to air passed over a calling *M. brassicae*. Subsequently, the leaf was laid flat on the bottom of a glass container (12 x 7 cm) and transferred to a different room for the bioassay. Male moths were brought into the experimental room at least 2 h prior to tests and were used only when quiescent. An observation was begun by gently placing a male moth on the leaf in the container, which was then closed. From then on, record was made of the occurrence of the following behaviours: *walking, walking + wing fanning* and *flight*. As we were mainly interested in whether or not moths would respond to treated leaves, we recorded the presence or absence of these behaviours during the first and second 60 s period ('one-zero sampling'; Altmann, 1974). Control tests were carried out with leaves treated with clean air. Differences in frequencies of occurrence of behaviours across treatments were tested with G-tests with Williams correction.

Behaviour of parasitoids on odour-treated leaves

The following experiments were designed to measure the response of *T. evanescens* wasps to leaves that had been exposed to the odour of a calling *M. brassicae* moth. Experiments consisted of two parts: treatment of leaves and bioassay.

Leaves were treated as described above in two separate wind tunnels. A number of experiments were carried out to test the persistence in time of a possible effect of contamination of leaves with host sex pheromone on the behaviour of wasps. In those experiments leaves that had been treated with odour just prior to the bioassay were tested against leaves that had been treated 4 or 24 h earlier. The latter leaves were stored between treatment and bioassay at 24 ± 1 °C and 60 ± 10 % r.h. in a gentle air stream (ca. 4 cm/s) caused by a wall-ventilator that exhausted air directly out of the building, with differently treated leaves located crosswind (at least 1 m distance) from each other.

Previous studies have shown that *Trichogramma* wasps do not orient in a directional fashion towards a source of host sex pheromone (*Chapter 6*). Therefore, the bioassay in the present study consisted of an observation cage in which the behaviour of parasitoids was observed on odour-treated leaves vs. control leaves (cf. Noldus & van Lenteren, 1985*b*). The cage measured 30 x 30 x 50 cm (l,w,h). The interior walls (except for the front side, which was left open) were covered with white paper to provide a uniform light distribution; a mirror against the hind wall facilitated observations. Overhead illumination was provided by two fluorescent tubes which resulted in 6-6.5 W/m^2 on the substrate. Temperature was 24 ± 1 °C and relative humidity 60 ± 10 %. Wasps were released individually on the upperside of a

Brussels sprouts leaf, placed in a vial with water and supported by a cotton wool plug. An observation started as soon as a wasp (after release on the leaf) moved and was terminated as soon as the wasp took flight. A complete record was made of the following parameters:

1. Location:
   a. *Upperside*: upperside of the leaf.
   b. *Underside*: underside of the leaf.
   c. *Margin*: a wasp was said to be walking along the margin if at least one leg was in contact with the margin. Thus defined, the margin comprised a ca. 0.5 mm wide zone along the edge on upper- and underside. Since wasps walking along the margin frequently change location between the upper- and underside of the leaf, this category can be regarded as an intermediate zone between upper- and underside.
2. Behaviour:
   a. *Standing still*: no visible body movement, no displacement.
   b. *Spot-movement*: clear body movement but hardly any displacement.
   c. *Walking*: visible displacement.
   d. *Upright posture*: this includes 'pointing' (body raised, antennae pointed forward and slight wing vibration), as well as the typical 'wings erect posture' that usually directly precedes take-off (as 'pointing' but wings completely extended) (cf. Keller, 1985; Figure 8-3).

Data were entered directly into a Tandy 102 microcomputer programmed as event recorder with The Observer. Frequencies and durations of behaviours were also computed with this program. Since frequency distributions of durations were significantly skewed [Kolmogorov test, $P < 0.05$ (SAS, 1985)], differences between means were subjected to non-parametric tests. The Mann-Whitney U test (SAS, 1985) was used to compare pairs of treatment. In case of three treatments, one-way analysis of variance [Kruskal-Wallis test (SAS, 1985)] was applied, after which a distribution-free multiple comparison based on Kruskal-Wallis rank sums (Hollander & Wolfe, 1973) was used to contrast pairs of treatment. The latter test is very conservative, especially when numbers of observations differ between treatments. If means did not differ significantly according to the multiple comparison, while the Kruskal-Wallis test indicated an overall significant treatment effect, the Mann-Whitney U test was used for pairwise comparison of selected treatments.

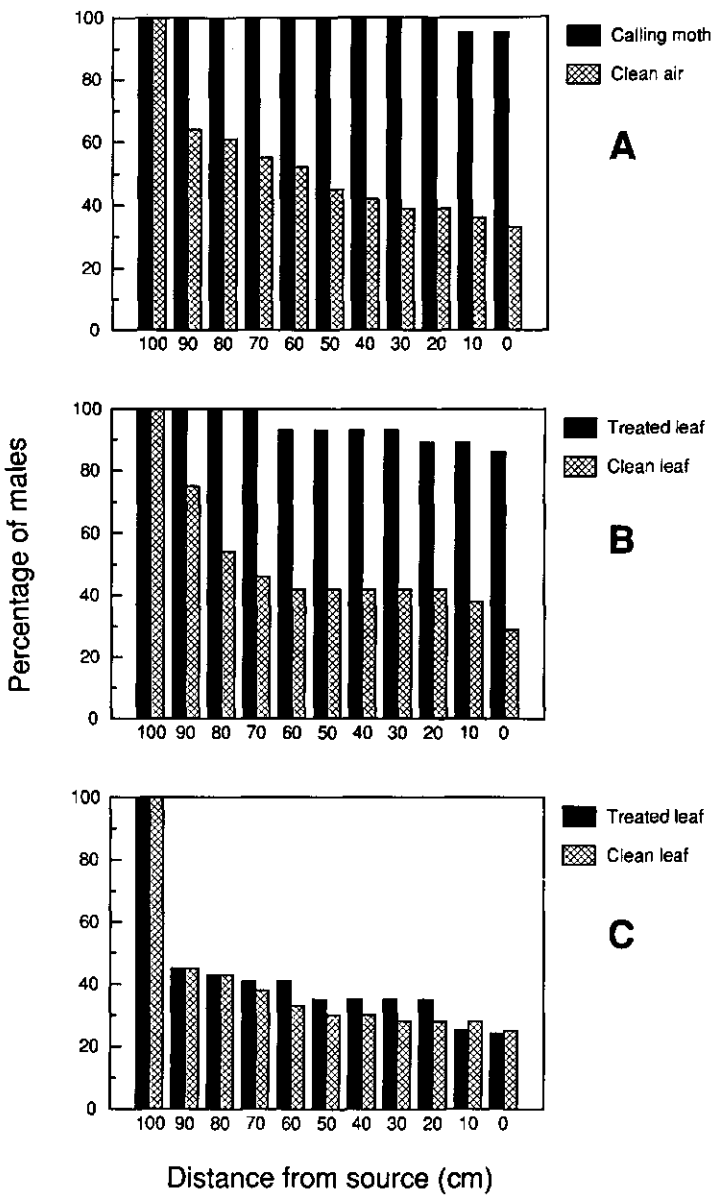

Figure 8-2. Upwind displacement by male *Mamestra brassicae* moths in a wind tunnel, expressed as the percentage of individuals that reached various 10-cm sections. Odour source: **A.** Calling *Mamestra brassicae* moth. **B.** Cabbage leaf previously exposed to the odour of a calling moth, present in the wind tunnel compartment in which it had been treated. **C.** Idem, but leaf present in a clean compartment.

Table 8-1. Behaviour of male *Mamestra brassicae* moths in a wind tunnel when exposed to various olfactory stimuli.

Parameter		Stimulus		P[1]
A.		Clean air	Calling moth	
Number of males tested		33	22	
Locomotion	(occurrence)[2]	69.7	100	***
"	(latency)[3]	97.5 ± 13.2	18.8 ± 5.5	***
Departure	(occurrence)	63.6	100	**
"	(latency)	135.1 ± 15.9	55.1 ± 8.9	***
Contact with screen	(occurrence)	33.3	95.5	**
"	(latency)	119.8 ± 16.6	90.5 ± 12.6	n.s.
Clasper extension	(occurrence)	0	81.8	***
"	(latency)	–	74.2 ± 10.2	–
Walking + fanning	(duration)[3]	39.7 ± 9.0	76.7 ± 11.7	**
B.		Clean leaf	Leaf treated with air passed over calling moth	
Number of males tested		24	28	
Locomotion	(occurrence)	75.0	100	**
"	(latency)	97.3 ± 13.9	42.6 ± 7.6	**
Departure	(occurrence)	75.0	92.9	n.s.
"	(latency)	133.4 ± 14.8	62.8 ± 11.7	***
Contact with screen	(occurrence)	25.0	85.7	**
"	(latency)	145.3 ± 33.4	81.4 ± 12.3	*
Clasper extension	(occurrence)	0	21.4	**
"	(latency)	–	121.3 ± 28.6	–
Walking + fanning	(duration)	74.7 ± 13.4	136.3 ± 14.2	**

Table 8-1, *continued.*

Parameter		Stimulus		P[1]
C.		Clean leaf	Leaf treated with air passed over calling moth (compartment replaced)	
Number of males tested		40	51	
Locomotion	(occurrence)[2]	47.5	54.9	n.s.
"	(latency)[3]	120.0 ± 14.7	109.7 ± 14.6	n.s.
Departure	(occurrence)	45.0	45.1	n.s.
"	(latency)	149.8 ± 15.6	141.7 ± 16.9	n.s.
Contact with screen	(occurrence)	25.0	23.5	n.s.
"	(latency)	188.7 ± 22.1	146.0 ± 17.3	n.s.
Clasper extension	(occurrence)	0	0	–
		–	–	–
Walking + fanning	(duration)[3]	28.2 ± 6.9	39.0 ± 7.9	n.s

1 ns.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.
2 Percentage of males showing behaviour; differences tested with G-test with Williams correction.
3 In seconds (mean ± s.e.); differences tested with Mann-Whitney U test.

Results

Response of male moths to odour-treated leaves

Behaviour of Mamestra brassicae in the wind tunnel

The first experiment was designed to characterize the behaviour of male *M. brassicae* in the wind tunnel, in response to airborne sex pheromone. With a calling female at the upwind end of the tunnel, all tested males initiated locomotion and departed from the release compartment, almost all reached the screen at the upwind end and more than 80 % showed extension of genital claspers (Table 8-1*a*, Figure 8-2*a*). In contrast, in clean air less than 70 % showed locomotion or entered the test section, ca. 30 % reached the upwind end, and no clasper extension was observed. In addition, with a calling moth upwind, locomotion and departure from the release compartment oc-

curred sooner than in clean air. These differences were all highly significant (Table 8-1*a*).

Response of male moths to leaves previously exposed to sex pheromone

Prior to testing males, the wind tunnel system, downwind of the T-shaped piece that held either a odour-treated leaf or a clean leaf, was replaced by clean components. The results indicated that a sufficient amount of sex pheromone had been adsorbed to elicit behavioural responses comparable to those elicited by a calling female. Percentages of males performing relevant behaviours were significantly higher (except percentage of males leaving the release tube), and latencies were significantly shorter in the treated setting compared to the control (Table 8-1*b*, Figure 8-2*b*).

Since we had used the treated leaf together with the T-shaped compartment (including the cork stopper and vial) as odour source in the bioassay, the results obtained could have been due to sex pheromone adsorption to the glass wall of the compartment. In a third experiment we excluded potential pheromone adsorption to glass wall, stopper and vial by replacing all components – except for the leaf itself – by clean material. The results clearly showed that the response found in the previous test was indeed due to pheromone adsorption to and re-release from components other than the leaf: no significant differences were found between treated leaf and control for any parameter (Table 8-1*c*, Figure 8-2*c*). This indicates that either (1) no sex pheromone had been adsorbed to the leaf surface, or (2) adsorption had taken place but the concentration of released pheromone was too low to be perceived by male moths at 1 m distance.

Observations of the males that reached the upwind screen in the last test (clean leaf: N = 10, treated leaf: N = 12) suggested that the latter might be the case. They spent significantly more time in the most upwind compartment with a treated leaf as odour source than with a control leaf (Mann-Whitney U test, one-tailed P = 0.0095). The same was true for the time spent *walking + wing fanning* in that zone (P = 0.035).

Short-distance responses of male moths to odour-treated leaves were further investigated in the next experiment, in which males were exposed to a treated leaf in a closed container. A higher percentage of males became active within 60 s when placed on a treated leaf compared to a clean leaf (Table 8-2). The differences were significant with respect to *walking* and *walking + wing fanning*. The total 2-min observation period was clearly less discriminative: only the difference in occurrence of *walking + wing fanning* was significant.

The results reported so far indicate that the sex pheromone of an individual *M. brassicae* female can be adsorbed to and re-released from the surface of a Brussels sprouts leaf, but not in quantities sufficient to evoke a behavioural response in male

moths at a distance of 1 m. Significant responses were only found at very short distance in the wind tunnel (< 5 cm from the odour source) and in a closed container where the moth was in direct contact with the leaf.

Table 8-2. Behavioural response of male *Mamestra brassicae* moths to contact with a cabbage leaf previously exposed to air passed over a calling female *M. brassicae* vs. a clean cabbage leaf. Expressed as the percentage of males showing key behaviours during a 60- or 120-s interval.

Parameter	Interval (s)	Substrate		P[1]
		Clean Leaf	Treated Leaf	
Number of males tested		12	15	
Walking	60	0	53.3	***
	120	66.7	80	n.s.
Walking + fanning	60	0	33.3	**
	120	0	66.7	***
Flight	60	0	13.3	n.s.
	120	50	40	n.s.

1 G-test with Williams correction. ns.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Behaviour of parasitoids on odour-treated leaves

Behaviour of Trichogramma evanescens wasps on Brussels sprouts leaves

After release on the upperside of a leaf, wasps initiated locomotion immediately or after a short period of preening. Periods of walking were frequently interrupted by periods of standing of variable length, often used for preening activity (as established through a stereo microscope). Sometimes wasps were clearly moving, but there was hardly any displacement. We called this behaviour 'spot-movement'. During walking the wings were mostly held against the abdomen, while the substrate was drummed with the antennae. As soon as an insect reached the leaf margin, it usually walked to the top of the leaf. During walking, the wings were occasionally extended. Upon reaching the highest point, wasps often adopted a typical 'pointing' posture, with the body at an angle of ca. 45° with the substrate, the antennae pointed forward and the wings vibrating (Figure 8-3). In many cases, this posture was often successively assumed in different directions. After this behaviour, wasps either resumed walking, often down the underside of the leaf along the main vein, or flew away. Just prior to flight wasps assumed a typical 'wings erect posture', with wings completely extended (Figure 8-3), for one or a few seconds.

Behaviour of parasitoids on leaves previously exposed to host sex pheromone

In the first experiment, *T. evanescens* females were released individually on leaves that had been treated with either air passed over a calling moth or with clean air. In this test and the following one, behaviours recorded were *walking* vs. *not walking*, and locations recorded were *upperside* vs. *underside*.

Total residence time and percentage of time walking were significantly greater on odour-treated leaves than on control leaves (Table 8-3*a*). The percentage of time spent on upperside vs. underside of the leaf did not differ significantly. However, leaves that had been exposed to air passed over a calling moth also had considerable numbers of moth scales attached to their surface. These scales contain a contact kairomone that elicits klino- and orthokinetic responses and leads to arrestment in *T. evanescens* (Smits, 1982; Noldus & van Lenteren, 1985*b*). Therefore, the experiment was repeated with a nylon gauze (60 mesh, mesh size: 320 µm, 57 % transmittance) inserted in the wind tunnel between the compartments holding the calling moth and the T-piece with the leaf, to prevent contamination of the leaf with scales (Figure 8-1*a*, *ng*). Wind speed was kept at ca. 11 cm/s. After 3 h treatment and subsequent bioassay, gauze and leaf were inspected under a stereo microscope for the presence of scales. The gauze had intercepted ca. 400 scales, while only ca. 10 scales were attached to the leaf. With the modified treatment, a strong behavioural response of the

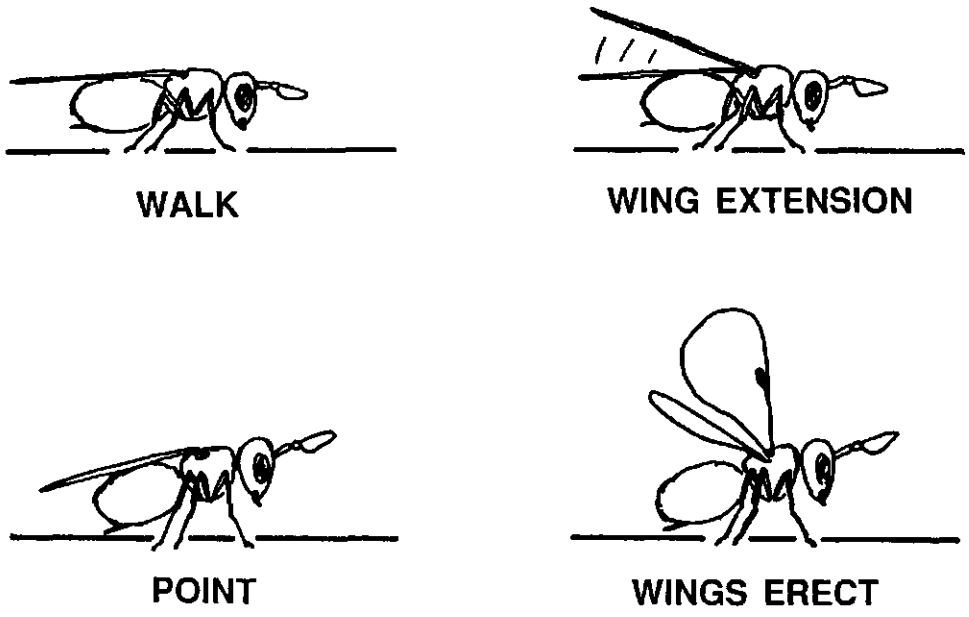

Figure 8-3. Typical postures of *Trichogramma* wasps during locomotory behaviour on a leaf (from Keller, 1985). Reprinted with permission from the author.

parasitoids was still found (Table 8-3*b*). Total residence time and percentage of time spent walking were higher on treated leaves. In addition, wasps spent a significantly larger proportion of their time on the underside of treated leaves compared to control leaves.

Although these results strongly suggested that the behavioural response of wasps was due to adsorption of host sex pheromone to the leaf surface, there were two factors that had yet to be excluded: (1) there was still a possible confounding effect of moth scales, and (2) the observed effect was possibly due not to adsorption of sex pheromone but to adsorption of another odour released by female *M. brassicae* moths ('general body odour'). The experiment was repeated once more with the following modifications. Measurements on a sample of *M. brassicae* scales showed that their length varied between 150 and 950 μm, and their width between 15 and 120 μm (N = 50). To intercept moth scales even more effectively, we used a still finer nylon gauze (400 mesh, mesh size: 32 μm, 25 % transmittance). Inspection of leaves after

Table 8-3. Behaviour of female *Trichogramma evanescens* wasps on a cabbage leaf previously exposed to air passed over a calling or non-calling female *Mamestra brassicae* moth or to clean air.

Gauze	Parameter	Cabbage leaf treatment			P[1]
		Clean air	Air passed over non-calling moth	Air passed over calling moth	
A. Gauze absent	No. wasps tested	43	–[2]	42	
	Total time on leaf (s)	190.0 ± 55.7[3]	–	554.7 ± 120.8	*
	Walking (%)[4]	55.0 ± 2.5	–	60.5 ± 2.3	*
	Underside (%)	13.9 ± 3.9	–	20.3 ± 3.7	n.s.
B. Fine gauze	No. wasps tested	35	–	34	
	Total time on leaf (s)	608.5 ± 191.7	–	1343.0 ± 156.5	***
	Walking (%)	56.1 ± 2.6	–	65.0 ± 1.6	***
C. Very fine gauze	No. wasps tested	89	61	78	
	Total time on leaf (s)	253.1 ± 34.1 a	281.5 ± 43.9 a	569.9 ± 68.7 b	*
	Behaviour				
	Frequency:				
	Walking	15.3 ± 2.0 a	15.3 ± 2.4 a	34.7 ± 4.3 b	***
	Standing still	13.7 ± 1.8 a	14.6 ± 2.2 a	27.6 ± 3.2 b	*
	Spot-movement	2.9 ± 0.4 a	2.4 ± 0.3 a	8.4 ± 1.5 b	**
	Upright posture	2.1 ± 0.4	1.9 ± 0.4	3.0 ± 0.6	n.s.
	Rate (frequency / 100 s):				
	Walking	7.2 ± 0.3	6.4 ± 0.4	6.5 ± 0.3	n.s.
	Standing still	6.3 ± 0.3	6.2 ± 0.3	5.5 ± 0.2	n.s.
	Spot-movement	1.5 ± 0.1	1.2 ± 0.2	1.4 ± 0.1	n.s.
	Upright posture	1.2 ± 0.1	1.1 ± 0.2	1.0 ± 0.1	n.s.
	Duration (s):				
	Walking	124.8 ± 18.4 a	122.8 ± 18.8 a	299.5 ± 40.6 b	*
	Standing still	106.1 ± 17.0 a	137.8 ± 23.9 a	215.0 ± 23.8 b	*
	Spot-movement	17.9 ± 3.4 a	16.3 ± 2.7 a	48.9 ± 8.8 b	**
	Upright posture	4.3 ± 1.3	4.6 ± 1.4	6.6 ± 2.0	n.s.
	Duration (%):[4]				
	Walking	50.8 ± 1.9	48.1 ± 2.4	46.1 ± 1.5	n.s.
	Standing still	39.0 ± 1.6	42.1 ± 2.0	40.7 ± 1.5	n.s.
	Spot-movement	8.2 ± 1.1	7.8 ± 0.6	8.3 ± 0.9	n.s.
	Upright posture	2.4 ± 0.4	2.1 ± 0.5	1.9 ± 0.4	n.s.

Table 8-3, *continued.*

Gauze	Parameter	Cabbage leaf treatment						P[1]
		Clean air		Air passed over non-calling moth		Air passed over calling moth		
	Location (%)							
	Upperside	81.9 ± 2.9	a	84.1 ± 3.6	a	67.6 ± 3.6	b	*
	Underside	7.4 ± 2.2	n.s.	6.9 ± 2.3	**	16.5 ± 2.8		5
	Margin	10.7 ± 1.9	a	8.9 ± 2.1	a	15.5 ± 2.3	b	*
	Percentage of wasps spending more than 1 % time along the margin[6]	98.2 (56)	***	100 (27)	***	95.1 (41)	***	

1 Two treatments: Mann-Whitney U test. Three treatments: Kruskal-Wallis test, followed by distribution-free multiple comparison. Figures followed by different letters are significantly different; n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

2 Not tested.

3 Mean ± s.e.

4 Percentage of the total time.

5 Overall significant treatment effect (Kruskal-Wallis test, $P < 0.001$), but means not significantly different according to multiple comparison. Significance levels between figures obtained by pairwise comparison (Mann-Whitney U test).

6 Margin comprised ca. 1 % of total leaf surface. Percentage based on wasps that reached the margin (in parentheses). Numbers are followed by 2-tailed significance level of difference from 50 % (sign test).

treatment revealed that all scales were successfully intercepted by the gauze. A third leaf treatment, i.e. air passed over a non-calling mated female moth, was added to contrast sex pheromone with 'general body odour'. Behaviours were now classified as *walking, standing still, spot-movement* or *upright posture,* while locations on the leaf were classified as *upperside, underside* and *margin.* The results of the bioassay (Table 8-3*c*) confirmed previous suggestions: we found significant responses of parasitoids only on leaves treated with the odour of a calling *M. brassicae* female, while the two controls did not yield significant differences. On leaves treated with pheromone-loaded air, wasps spent a significantly larger proportion of their time on the underside and along the margin, compared to control leaves. Upon reaching the leaf margin, wasps showed evident thigmotaxis: of the wasps that reached the margin, almost all spent a larger percentage of time in that zone compared to its relative surface area (ca. 1 % of the total leaf). Periods of walking, standing still and spot-movement occurred more frequently on odour-treated leaves than on control leaves.

However, when occurrences of behavioural components were expressed as rates (frequency / unit time), they did not differ significantly. Similarly, the durations of these three behavioural components were significantly larger on pheromone-treated leaves compared to control leaves, but the proportional time allocation to different behaviours were not affected by leaf treatment. Frequency of occurrence or duration of 'upright posture' was not affected by treatment at all.

Mamestra brassicae release most of their sex pheromone during the last 4 h of the scotophase (Subchev, 1983; *Chapter 3*). Since *Trichogramma* does not commence searching activity until the start of the photophase (Ashley *et al.*, 1973), we would not expect a kairomonal use of host sex pheromone to have evolved unless adsorption of the chemicals to leaf surface is capable of bridging the gap in time between release of the cues by the host and perception by the parasitoid. In order to test this hypothesis we examined the behaviour of parasitoids on leaves, treated either directly prior to the test or 4 h earlier. The results (Table 8-4*a*) show that the effect of adsorbed host sex pheromone was still apparent after 4 h: total residence time and percentage of time along the margin and on the underside were larger on pheromone-treated leaves than on control leaves. For several parameters, while Kruskal-Wallis analysis of variance showed a significant treatment effect, distribution-free multiple comparison did not reveal differences between means. However, in all those cases, measurements for leaves treated with the odour of a calling moth were significantly different from those for leaves treated with non-calling moth odour, while the latter did not differ from those for leaves treated with clean air (Table 8-4*a*). As in the previous experiment, there was no effect of treatment on strictly behavioural parameters; therefore, these have not been included in the table.

The results of the previous experiment aroused our interest in the persistence of the observed effect over a longer period. Therefore, the experiment was repeated with a time lag between treatment and bioassay of 24 h. Since the previous experiments had shown that there was no differential effect of treating leaves with odour of a non-calling moth vs. clean air, the latter control was dropped. There was still a significant effect of adsorbed semiochemicals after 24 h: average residence time on treated leaves was still longer than on control leaves (Table 8-4*b*). The higher percentage of time spent on the underside of treated leaves was no longer significant after 24 h.

Table 8-4. Behaviour of female *Trichogramma evanescens* wasps on a cabbage leaf previously exposed to a calling or non-calling female *Mamestra brassicae* moth or to clean air. Leaves were either tested directly after treatment or after a period of 4 or 24 h.

Interval tested	Parameter	Time from treatment to test (h)[2]	Cabbage leaf treatment						P[1]
			Clean air		Air passed over non-calling moth		Air passed over calling moth		
A. 4 hours	No. wasps tested	0	50		61		75		
		4	50		61		75		
	Total time on leaf (s)	0	173.2 ± 31.8[3]	a	174.7 ± 26.1	a	315.2 ± 41.2	b	**
		4	151.5 ± 20.2	n.s.	155.1 ± 20.8	*	263.1 ± 34.1		[4]
	Location (%):[5]								
	Upperside	0	94.1 ± 1.9	n.s.	86.4 ± 3.4	*	74.7 ± 3.8		[4]
		4	92.0 ± 2.3	n.s.	90.4 ± 2.5	*	81.2 ± 3.1		[4]
	Underside	0	2.3 ± 1.4	n.s.	6.3 ± 2.5	*	14.5 ± 3.2		[4]
		4	1.5 ± 1.0	n.s.	1.6 ± 1.0	**	7.8 ± 2.0		[4]
	Margin	0	3.6 ± 1.1	n.s.	7.4 ± 1.9	*	10.9 ± 1.8		[4]
		4	6.5 ± 12.0	n.s.	8.0 ± 2.1	*	11.0 ± 2.0		[4]
B. 24 hours	No. wasps tested	0	–[6]		34		34		
		24	–		34		34		
	Total time on leaf (s)	0	–		164.1 ± 28.9		345.6 ± 45.1		**
		24	–		149.5 ± 21.8		241.9 ± 35.9		*
	Location (%):								
	Upperside	0	–		89.0 ± 3.7		78.6 ± 5.6		n.s.
		24	–		92.5 ± 3.0		86.9 ± 3.7		n.s.
	Underside	0	–		4.1 ± 3.0		12.9 ± 4.9		*
		24	–		0.1 ± 0.1		4.3 ± 2.2		n.s.
	Margin	0	–		6.9 ± 2.5		8.3 ± 2.7		n.s.
		24	–		7.4 ± 2.7		8.8 ± 2.6		n.s.

1 Two treatments: Mann-Whitney U test. Three treatments: Kruskal-Wallis test, followed by distribution-free multiple comparison. Figures followed by different letters are significantly different; n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

2 All means were compared pairwise per column (Mann-Whitney U test). None of the parameters showed an effect of time between treatent and test at the 0.05 significance level.

3 Mean ± s.e.

4 Overall significant treatment effect (Kruskal-Wallis test, $P < 0.01$), but means not significantly different according to distribution-free multiple comparison. Significance levels between figures obtained by pairwise comparison (Mann-Whitney U test).

5 Percentage of the total time.

6 Not tested.

Discussion

Our experiments show that the airborne sex pheromone originating from a single female *Mamestra brassicae* moth can adsorb to a Brussels sprouts leaf and subsequently elicit behavioural responses in conspecific male moths as well as in *Trichogramma* parasitoids. Furthermore, the response of wasps to odour-treated leaves persists for at least 24 h.

Male *Mamestra* moths did not respond to the odour of treated leaves from a distance of 1 m. The only significant male responses were observed close to the upwind screen, i.e. at circa 5 cm from the leaf, and in a container where moths made contact with the leaf. This indicates that re-release of sex pheromone molecules after retention by epicuticular wax, the most likely substrate for adsorption (Baker, 1982; Jeffree, 1986), is very limited. The close-range effect of odour-contaminated leaves would appear of minor significance in the field, since mate location by male *M. brassicae* moths occurs in flight. We must note that in our experiments calling moths were never in contact with leaves. In unconstrained conditions, however, calling *M. brassicae* occasionally press their ovipositor against the substrate (*Chapter 3*). This behaviour could result in a scent mark that attracts males from a larger distance, as shown for *P. gossypiella* (Colwell *et al.*, 1978).

With respect to the third trophic level, our results suggest how host sex pheromones may function as kairomones for egg parasitoids in the field. Since *Trichogramma* wasps do not orient directionally to host odours from a distance (*Chapter 6*), a contact effect on leaves would seem sufficient, since these wasps spend most of their time foraging in the phyllosphere of plants. Persistence of adsorbed chemicals over 4 h may be sufficient to bridge the gap in time between nocturnal sex pheromone release by *M. brassicae* moths and perception by the diurnally foraging wasps. Furthermore, persistence over 24 h or more could even serve as a temporal bridge between moth calling activity and oviposition, since oviposition usually occurs during several nights following calling and mating (Noldus, pers. obs.). However, in a Brussels sprouts field infested by a population of *M. brassicae*, reproductive activity of moths stretches out over several weeks per generation (Pak *et al.*, 1989) [the number of generations varies with the latitude (Bonnemaison, 1965)], so that foraging wasps would presumably use kairomones originating from moths that have been calling while others have been ovipositing.

Adsorption of pheromone to the leaf surface can thus explain how parasitoids orient to semiochemicals released by their host at a different *time*. However, the relation between between pheromone release by moths and its kairomonal role for parasitoids also has important *spatial* aspects. From a spatial perspective, the adaptive value for *Trichogramma* of using host sex pheromone as a kairomone depends on a number of factors: (1) the spatial discontinuity between calling and oviposition

sites of individual female moths, (2) the host density and spatial distribution, which determines to what extent calling and oviposition sites of individual moths overlap, and (3) the active space of calling female moths, i.e. the area downwind of a calling female where sex pheromone is adsorbed to above-threshold level for perception by *Trichogramma*.

Ad 1. Mamestra brassicae moths go through a locomotory phase at the start of the scotophase before they initiate calling or oviposition (*Chapter 3*). Since oviposition usually continues on the nights following mating, a female is not likely to deposit all her eggs near the site where she has called. Some moth species even use different habitats for calling and mating vs. oviposition, e.g. *Ostrinia nubilalis* (Hübner) (Showers *et al.*, 1976). Such migratory behaviour has never been observed in *M. brassicae*. With regard to adsorbed sex pheromone, and with disregard for the presence of conspecific moths, the distance between calling and oviposition sites determines the information content of a moth's chemical traces for a parasitoid. The smaller that distance (and thus the discontinuity), the greater the probability that a responding wasp will encounter other host-related cues within the active space formed by adsorbed volatiles from a moth.

Ad 2. Regardless of the discontinuity between calling and oviposition sites of *individual* moths, and on the assumption that calling and oviposition occur in the same habitat, a larger host density will decrease the spatial discontinuity between active spaces of kairomones and locations of host eggs. The higher the host density, the higher the probability that a moth will oviposit within the active space of a conspecific, and thus bring stimulus and target for foraging parasitoids together.

Ad 3. The first two factors depend on active spaces of a measurable size. But how large is the active space of adsorbed sex pheromone from an individual moth for *Trichogramma*? This depends on numerous factors, most of which are unknown for our Brussels sprouts - *Mamestra* - *Trichogramma* system. First of all, the amount of sex pheromone released by moths. The main component Z11-16:Ac is released at an average rate of ca. 1 ng/FHE (female hour equivalent) (Bestmann *et al.*, 1988; J.H. Tumlinson, pers. comm.). However, it is not certain what the role of this compound is in the kairomonal effect for *Trichogramma* (Noldus & van Lenteren, 1985*a*). A second important factor is the lower concentration threshold for perception of host sex pheromone by *Trichogramma*, which is not known. The active space will further vary with ambient temperature and wind speed (Bossert & Wilson, 1963). The amount and morphology of epicuticular wax of Brussels sprouts vary with light, humidity and temperature (Baker, 1974), which might also affect its properties as an adsorption substrate.

In the three wind tunnel experiments with male *M. brassicae* moths, rather high levels of spontaneous activity were observed, since 25-33 % of the males reached the up-

wind screen in the control situation. There appeared no difference between experiment 1, where the control consisted of clean air, and experiments 2 and 3, where a clean Brussels sprouts leaf was used. However, EAG-responses of *M. brassicae* to cabbage volatiles have been demonstrated (Shimizu & Usui, 1986) and we might thus expect a response in a wind tunnel. The spatial constraints of the experimental set-up may have precluded expression of a behavioural response to plant volatiles, or the amount of plant foliage offered (one leaf) may be too small to elicit a response. The small difference in wind speed between experiment 1 (15 cm/s) and the other tests (11 cm/s) is not likely to explain the lack of response to cabbage odour.

The second wind tunnel experiment showed that the glass compartment holding the leaf had adsorbed a considerable amount of sex pheromone, which was readily re-released, since males were subsequently attracted from 1 m distance. This result is not surprising, since sex pheromones are known to adsorb to glass (Colwell *et al.*, 1978), an attribute which is utilized in volatile collection techniques (Baker *et al.*, 1980, 1981; Witzgall, 1987). Adsorption of lepidopteran sex pheromones has been recorded for various other substrates, including moth scales (Baker *et al.*, 1980; Vogt & Riddiford, 1986), polypropylene (Perry *et al.*, 1988), textile and the human body (Anonymus, 1976; Cameron, 1981).

During the experiments with *Trichogramma* we noticed that leaves became contaminated with moth scales unless a very fine gauze was used as a barrier between calling female and leaf. It has been shown in the lymantriid moth *Orgyia leucostigma* (J.E. Smith) that body scales contain a pheromone that – upon contact – elicits copulation attempts in males (Grant *et al.*, 1987). The response of *M. brassicae* males upon contact with odour-treated leaves, as reported in this study, was perhaps not due to adsorbed sex pheromone but to contamination with scales, since no gauze was used in those experiments. However, this is not likely, since the observed *walking + wing fanning* behaviour does not reflect courtship or mating behaviour – which typically includes display of hair pencils and copulation attempts in *M. brassicae* (Tóth, 1982; Birch *et al.*, 1989) – but rather the initial arousal and orientation behaviour of males upon perception of volatile female sex pheromone.

In the last two experiments wasp residence times were systematically shorter on leaves that had been stored between treatment and bioassay, although differences were not statistically significant (Table 8-4). This might be due to deterioration of leaves. To avoid such problems, leaves should preferably be left attached to plants during treatment and bioassays in future studies.

The various postures observed in *T. evanescens* in our experiments resemble those described for *T. exiguum* (Keller, 1985). The 'pointing' posture, characterized by antennal orientation and wing vibration, has been interpreted as a possible means to determine wind direction and to sample volatile semiochemicals (Keller, 1985). This behaviour could be analogous to the typical wing fanning behaviour of male moths

upon perception of female sex pheromone. A stationary posture with antennae directed up in the air has also been observed as part of the preflight behaviour of larval parasitoids, e.g. *Microplitis croceipes* (Cresson) (Drost *et al.*, 1986; M.A. Keller, pers. comm.), and *Dacnusa sibirica* Telenga (Dicke & Minkenberg, unpubl.).

Wasps showed a significant preference (in terms of time allocation) for the leaf margin, regardless of leaf treatment. This indicates that a similar edge effect found in previous wind tunnel experiments (*Chapter 6*) was not an artefact resulting from the use of a platform with a barrier to the underside, but the expression of a genuine thigmotactic response of wasps to the physical border of the substrate. The function of this behaviour is not known. For the parasitoid it might be a quick way of scanning the leaf for presence of host contact kairomones, since moths and butterflies often cling to the leaf edge while depositing eggs on the underside, and scales may thus be concentrated in the border region of the leaf. Furter, wasps spent relatively more time on the underside of treated leaves compared to control leaves. This seems an adaptive effect since eggs of *M. brassicae* are predominantly laid in batches on the underside of leaves.

Obviously, our conclusions and speculations are based on a limited number of experiments, carried out under standardized conditions. Pheromone adsorption onto leaves under different environmental settings remains to be tested. The interpretation of our results is hampered by our limited knowledge of *M. brassicae*'s behaviour in the field. We need to know the spatio-temporal dynamics of moth calling, mating and oviposition behaviour to determine how host eggs and various types of semiochemicals are distributed in the field. Only then can the adaptive significance of behavioural responses of *Trichogramma* to host odours be fully understood.

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9 Semiochemicals, Foraging Behaviour and Quality of Entomophagous Insects for Biological Control[1]

ABSTRACT – In this chapter the question is addressed whether or not responses to semiochemicals are relevant to the quality of entomophagous insects for inundative biological pest control. Four approaches to answer this question are distinguished: (1) behavioural manipulation with semiochemicals, (2) use of natural intraspecific variation in responses to semiochemicals, (3) artificial selection for intraspecific variation in responses to semiochemicals, and (4) application of simulation models of foraging behaviour. In addition, the possible impact of mass-rearing methods on responses to semiochemicals is discussed. Special attention is paid to *Trichogramma* egg parasitoids: evidence for the use of semiochemicals by *Trichogramma* spp., as well as current mass-rearing and quality control methods are briefly reviewed. From the possible impact of mass rearing on responses to semiochemicals, recommendations for mass rearing, quality control and further research are inferred.

Quality of Entomophagous Insects

The selection of a suitable natural enemy and an appropriate rearing method are essential steps in any biological control program. The *overall quality* of a mass-reared entomophagous insect is determined by the performance in its intended role, i.e. reduction of pest numbers below the economic threshold after release into the field (Huettel, 1976; Boller & Chambers, 1977; Moore *et al.*, 1985; Bigler, 1989). Overall quality encompasses a number of traits related to the species to be controlled, the crop, the climate, and the release strategy to be applied. Many authors have listed desirable traits of natural enemies (e.g., Messenger *et al.*, 1976; Boller & Chambers, 1977; Rosen, 1985; van Lenteren, 1986; Minkenberg & van Lenteren, 1986; Pak, 1988). For inundative and seasonal inoculative biological control these include: adaptability to climatic extremes and various habitats, searching efficiency, host specificity, host discrimination, host utilization (ability to kill a host and/or use it for reproduction), reproductive capacity, and lack of negative side effects. Each of these traits might

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conceivably be affected by mass-rearing procedures, including storage and other aspects of production, which may subsequently lead to altered performance in the field.

Among the traits relevant to quality may be the response of a natural enemy to semiochemicals originating from host habitat (crop) or host. In this chapter I examine to what extent responses to semiochemicals are relevant to quality of entomophagous insects for biological control, and how responses to such substances can be influenced by rearing methods. Examples will mainly be drawn from the literature on parasitic wasps, in particular the genus *Trichogramma*. However, the general considerations apply equally well to predatory insects. Therefore, the words *parasitoid* and *host* can also be read as *predator* and *prey*, respectively, unless explicit reference is made to a guild of parasitoids. The term *natural enemy* refers to entomophagous arthropods throughout this chapter.

Semiochemicals in the Foraging Behaviour of Parasitoids

Terminology and scope

The term semiochemicals refers to the chemicals involved in chemical communication between animals (Law & Regnier, 1971). Pertinent to the present discussion are pheromones (involved in intraspecific interactions), kairomones (involved in interspecific interactions and evoking a response favourable to the receiver) and synomones (interspecific, but with response favourable to both emitter and receiver) (Nordlund & Lewis, 1976; Nordlund, 1981). For a recent discussion of terminology of these information-conveying chemicals, see Dicke & Sabelis (1988).

That semiochemicals play a role in various phases of the foraging behaviour of entomophagous arthropods is a well-documented phenomenon. There is no need to summarize the large amount of evidence published on this topic, as several comprehensive recent reviews are available (Vinson, 1984*a*,*b*, 1985, 1986; Lewis & Nordlund, 1985; Kainoh, 1987). Here we are concerned with those responses to semiochemicals that contribute to the quality of a natural enemy as a biological control agent. The next question is whether or not these responses are affected by mass-rearing procedures.

This review is restricted to stimuli mediating foraging for hosts or prey. Sex pheromones have been identified for parasitoids (Eller *et al.*, 1984; Mohamed & Coppel, 1987) but will not further be discussed in this paper.

Semiochemicals for *Trichogramma* spp.

Trichogramma spp. as biological control agents

Egg parasitoids of the genus *Trichogramma* are the most widely used entomophagous insects in the biological control of insect pests worldwide (King *et al.*, 1985*b*). *Trichogramma* spp. are presently reared for commercial purposes in at least 20 different countries, with the Soviet Union and China leading in acreages of application (Coulson *et al.*, 1982; Li, 1984; Ridgway & Morrison, 1985; Gusev & Lebedev, 1988). Control is mostly attempted through inundative releases, against at least 28 different herbivorous pest species on some 20 different crops (Hassan, 1988; Voegelé *et al.*, 1988). Considerable variability in efficacy of mass releases exists, however, and may be due to the quality of the wasps. In most cases, relative inefficiency of mass-reared *Trichogramma* is compensated for by increasing the frequency of releases or the numbers of released wasps. However, such compensatory measures do not solve the underlying problem, and therefore a critical discussion of mass rearing and quality control in *Trichogramma* seems appropriate.

Foraging behaviour of Trichogramma

For foraging *Trichogramma* spp., three levels of resource distribution are relevant: the host habitat, host aggregations within the habitat, and individual hosts (i.e. host eggs). Due to the minute size of adult *Trichogramma* wasps (0.5 - 1 mm) and their inability to fly upwind in any but the slowest winds, movement between potential host habitats and location of host aggregations may be passive rather than active in most cases (Keller & Lewis, 1985; Keller *et al.*, 1985). Their small size also hampers direct observation of foraging behaviour in the field, and therefore most of the evidence reviewed below originates from laboratory experiments. So far, plant odours, host sex pheromones, host scales, egg odours, and contact chemicals associated with host eggs, have been identified or suggested as semiochemicals for *Trichogramma* spp. These groups of cues will briefly be reviewed below.

Plant odours

Several authors have documented differential rates of parasitism of one host species by *Trichogramma* on different plant species. For example, Rabb & Bradley (1968) compared parasitism of *Manduca sexta* eggs by *T. minutum* on three solanaceous plants and found parasitism on tomato and jimsonweed but not on tobacco. A similar difference between parasitization rates by *Trichogramma* spp. on tomato and tobacco has been observed by Martin *et al.* (1981) for *H. virescens*. Bar *et al.* (1979) found

parasitism of *Heliothis armigera* by *T. semifumatum* on tomato, but not on cotton. In a study of *Choristoneura fumiferana* on balsam fir, higher percentages of egg parasitism by *T. minutum* were observed with increasing proportions of non-budworm-host trees in a stand (Kemp & Simmons, 1978). Effects of polycultures vs. monocultures on parasitism by *Trichogramma* spp. appear ambiguous: Altieri *et al.* (1981) reported higher rates of parasitism in soybean/corn intercropping plots vs. soybean monocultures, and Nordlund *et al.* (1984) found that interplanting corn with tomatoes and beans increased parasitism over corn grown in monoculture. However, Andow & Risch (1987) observed higher parasitism rates in corn monocultures vs. polycultures of corn/bean/squash and corn/clover.

Some of these authors suggested that olfactory cues are used in host-habitat selection by *Trichogramma*. However, this can not be concluded from such indirect evidence, because the behavioural mechanism is unknown and many other factors may have caused the observed differences. For example, structures on the leaf surface may trap *Trichogramma* (Rabb & Bradley, 1968) or reduce walking velocity and thus lead to lower searching success (Treacy *et al.*, 1985, 1986; Keller, 1987).

There is also direct evidence on the role of plant odours in *Trichogramma* foraging (Table 9-1). Altieri *et al.* (1981, 1982) were able to increase rates of parasitism by *Trichogramma* spp. by spraying plants with an extract of *Amaranthus* sp. The

Table 9-1. Evidence for plant odours that function as semiochemicals for *Trichogramma* spp.

Parasitoid	Source	Response	Reference
T. semifumatum	cotton	repellence in olfactometer	Bar *et al.* (1979)
Trichogramma sp. *T. pretiosum*	*Amaranthus* sp. corn	increased parasitism	Altieri *et al.* (1981)
T. pretiosum	*Amaranthus* sp.	increased parasitism	Altieri *et al.* (1982)
T. pretiosum	tomato	preference in Y-tube olfactometer[1], increased parasitism	Nordlund *et al.* (1985*a*,*b*)
T. sp.p. *buesi*	cotton	repellence in T-tube olfactometer[1]	Cabello & Vargas (1985*a*)
T. maidis	corn	preference in 4-arm olfactometer[1]	Kaiser (1988)

1 Tested against clean air

mechanism causing enhanced parasitism after the addition of such plant chemicals was not examined. Nordlund *et al.* (1985*a,b*, 1987) showed that the higher rates of parasitism found in tomato (Nordlund *et al.*, 1984) might be explained by tomato volatiles: in the laboratory, parasitism rates of *H. zea* eggs by *T. pretiosum* could be increased by application of tomato extract and parasitoids showed a positive response to tomato odour in a Y-tube olfactometer. Recently, Kaiser (1988) documented arrestment of *T. maidis* by the odour of corn leaves in a four-armed airflow olfactometer. An opposite effect of plant odours has also been recorded. Bar *et al.* (1979) found that *T. semifumatum* was repelled by the odour of cotton in an olfactometer. Repellency by cotton odour has also been recorded for *T.* sp.p. *buesi* (Cabello & Vargas, 1985*a*).

One could envisage parasitoids being repelled by the odour of toxic plants, and thus being protected from oviposition in unsuitable host insects. Such odours would then serve as kairomones for the parasitoids. However, I have not found any evidence for this.

Host sex pheromones

Various pieces of direct and indirect evidence indicate that within a habitat *Trichogramma* spp. are able to distinguish between host-infested and uninfested areas. In this phase, foraging behaviour seems to be mediated by odours originating from adult female host insects (Table 9-2). The first observation of this phenomenon was made by Lewis *et al.* (1982), who established increased rates of parasitism by *Trichogramma* spp. in cotton plots treated with a synthetic sex pheromone blend of *Heliothis zea*. In an airflow olfactometer *T. pretiosum* responded to the odour released by calling virgin moths (Noldus, 1988*b*; *Chapter 5*). The same has been found for the noctuid *Mamestra brassicae* and *T. evanescens* (Noldus & van Lenteren, 1985*a*; *Chapter 4*). Wind tunnel experiments showed that both *Trichogramma* spp. are not attracted to the pheromone source; the odour suppresses phototactic upward flight and leads to arrestment of the parasitoid (Noldus *et al.*, 1988*a,b*; *Chapter 6*). A recent study by Kaiser (1988) indicates that *T. maidis* is arrested by the sex pheromone of its host *Ostrinia nubilalis*. For *Pieris brassicae*, a host of *T. evanescens*, no female sex pheromone is known, but virgin female butterflies release volatiles that attract wasps in an olfactometer, while mated butterflies do not elicit a response in the parasitoids (Noldus & van Lenteren 1985*a*; *Chapter 4*).

Other authors used a 'diffusion olfactometer' to study olfactory orientation of *Trichogramma* and recorded responses to adult host odours (Ferreira *et al.*, 1979; Bourarach & Hawlitzky, 1984; Zaki, 1985). These results remain inconclusive, however, because large groups of insects were tested simultaneously, behavioural observations were not made, and only small proportions of wasps 'responded'.

Table 9-2. Evidence for adult host odours that function as semiochemicals for *Trichogramma* spp.

Parasitoid	Host	Source	Response	Reference
T. pretiosum	*Heliothis zea*	host sex pheromone gland, synthetic sex pheromone	increased parasitism, preference in 4-arm olfactometer[1], arrestment, suppressed flight	Lewis *et al.* (1982), Noldus (1988*b*), Noldus *et al.* (1988*a*), *Chapter 5, 6*
T. evanescens	*Mamestra brassicae*	host sex pheromone gland	preference in 4-arm olfactometer[1], arrestment, suppressed flight	Noldus & van Lenteren (1985*a*), Noldus *et al.* (1988*a,b*), *Chapter 4, 6*
T. cordubensis	*Heliothis armigera, Earias insulana*	adult female moth	preference in T-tube olfactometer	Cabello & Vargas (1985*a*)
T. sp.p. *buesi*	*Ephestia kuehniella*	adult female moth	preference in T-tube olfactometer	Cabello & Vargas (1985*a*)

1 Tested against clean air.

Host scales

The response to contact kairomones present in scales originating from the body of adult host insects is a well-documented aspect of *Trichogramma* foraging behaviour. Laing (1937) first demonstrated that contact of *T. evanescens* with 'traces' of *S. cerealella* or *M. brassicae* led to increased residence times and higher host-finding rates in contaminated areas. Decades later, Lewis *et al.* (1971) found that traces left by ovipositing *H. zea* or *Plodia interpunctella* moths increased parasitism by *T. evanescens*. Body scales of the moths contained the kairomone (Lewis *et al.*, 1972), and a hexane extract of these scales sprayed over plants could increase rates of parasitism by *T. evanescens* and *T. achaeae* in laboratory, greenhouse and field (Lewis *et al.*, 1972, 1975*a*). Inhibition of flight and klinokinesis are the major components leading to intensified searching in contaminated areas and enhanced host finding (Beevers *et al.*, 1981; Morrison & Lewis, 1981; Gardner & van Lenteren, 1986). The response of *Trichogramma* to host scales appears to be a general phenomenon, as it has been described for several *Trichogramma*-host combinations (Table 9-3).

Chemical analysis of *H. zea* scale extracts yielded tricosane as the most active component for *T. evanescens* (Jones *et al.*, 1973). Gueldner *et al.* (1984) identified a

Table 9-3. Evidence for host scales that contain semiochemicals for *Trichogramma* spp.

Parasitoid	Host	Response	Reference
T. evanescens	*Sitotroga cerealella* *Mamestra brassicae*	arrestment	Laing (1937)
T. evanescens	*Heliothis zea*, *Plodia interpunctella*	increased parasitism	Lewis *et al.* (1971, 1972), Jones *et al.* (1973)
T. achaeae	*Heliothis zea*	increased parasitism	Lewis *et al.* (1975*a*)
T. pretiosum	*Heliothis zea*	increased parasitism	Lewis *et al.* (1975*b*), Gueldner *et al.* (1984)
T. evanescens[1]	*Mamestra brassicae*	arrestment	Smits (1982), Noldus & van Lenteren (1985*b*)
T. evanescens[1]	*Pieris brassicae*	arrestment	Noldus & van Lenteren (1985*b*), Gardner & van Lenteren (1986)
T. evanescens[1]	*Pieris rapae*	arrestment	Noldus & van Lenteren (1985*b*)
T. exiguum, *T. maltbyi*, *T. minutum*, *T.* sp.nr. *pretiosum*	*Heliothis zea*, *Manduca sexta*, *Ostrinia nubilalis*	arrestment	Thomson & Stinner (1989*b*)
T. minutum	*Choristoneura fumiferana*	arrestment	Zaborski *et al.* (1987)
T. nubilale	*Ostrinia nubilalis*	arrestment	Shu & Jones (1988)

1 Presently named *T. maidis* (strain no. 11 in Pak & van Heiningen, 1985).

number of organic acids in the scales of *H. zea* which were attributed a minor kairomonal role for *T. pretiosum*. Recently, Shu & Jones (1988) isolated three dimethyl-nonatriacontanes from the hexane extract of scales of *O. nubilalis*. The relative contribution of each of these components to the kairomonal effect of the scales for *T. nubilale* remains to be elucidated.

Host egg odours

No conclusive evidence exists about the possible involvement of odours originating from eggs in host finding by *Trichogramma* spp. Laing (1937) mentioned that *T. evanescens* did not react to the presence of eggs of *Sitotroga cerealella* or *Mamestra brassicae* from a distance of less than 5 mm if those eggs were invisible for the parasitoid. Ferreira *et al.* (1979) reported attraction of eight *Trichogramma* spp. to the eggs of *Ephestia kuehniella* in a diffusion olfactometer. However, due to the lack of air flow, their method does not allow conclusions with regard to orientation behaviour. The same applies to the results of Ferreira-Anunciada & Pintureau (1981) and Bourarach & Hawlitzky (1984).

Contact chemicals associated with host eggs

Upon contact with a potential host, *Trichogramma* wasps engage in a specific examination behaviour, i.e. an evaluation of various host characteristics (host recognition), after which the object is accepted (host acceptance) or rejected for oviposition. Besides physical properties, such as perceived host size (Salt, 1935; Schmidt & Smith, 1985; Pak & de Jong, 1987), semiochemicals play a role in host recognition (Table 9-4). The secretion from the accessory gland of *H. zea*, present as a coating on the egg, contains a kairomone that mediates host recognition by *T. pretiosum* (Nordlund *et al.*, 1987). A similar effect was obtained with an egg wash of *Pieris brassicae*, inducing host acceptance in *T. maidis* (Pak & de Jong, 1987). Applied on a leaf surface, this material led to intensified search behaviour and arrestment of *T. evanescens* (= *T. maidis*) (Noldus & van Lenteren, 1985*b*). In some cases, the chemicals present on the host egg elicit rejection rather than acceptance, as shown for *T. buesi* when offered eggs of *P. brassicae* (Pak & de Jong, 1987). The results of Taylor (1969) for *T. semifumatum* and *Estigmene acroea* point at a similar effect. In these cases the term synomone is more appropriate than kairomone (Pak & de Jong, 1987). Host-recognition mediators are probably proteinaceous and of very low volatility (Strand & Vinson, 1983; Nordlund *et al.*, 1987; Vinson *et al.*, 1988*a*). *T. evanescens* did not respond to an egg wash of *P. brassicae* in an olfactometer (Noldus & van Lenteren, 1983).

During host examination *Trichogramma* wasps also discriminate between parasitised and unparasitised hosts. This ability was first demonstrated by Salt (1934), who later proved that external as well as internal chemical markers mediate host discrimination (Salt, 1937*b*). Host discrimination is now considered to be a general phenomenon among parasitic insects (van Lenteren, 1981). Like in most parasitoids, host discrimination appears to function only intraspecifically in *Trichogramma* (Ables *et al.*, 1981). It aids *Trichogramma* in the avoidance of superparasitism, although superparasitism can be an adaptive strategy under certain cir-

Table 9-4. Evidence for contact chemicals associated with host eggs that function as semiochemicals for *Trichogramma* spp.

Parasitoid	Host	Source	Active component(s)	Response	Reference
T. evanescens[1]	*Pieris brassicae*	host accessory gland	not known	host recognition, arrestment	Noldus & van Lenteren (1985*b*), Pak & de Jong (1987)
T. buesi	*Pieris brassicae*	host accessory gland	not known	host rejection	Pak & de Jong (1987)
T. buesi, *T. maidis*[2]	*Mamestra brassicae*	host accessory gland	not known	host acceptance	Pak & de Jong (1987)[3]
T. pretiosum	*Heliothis zea*	conspecific female wasp	not known	host discrimination	Ables *et al.* (1981)
T. dendrolimi	not relevant	–	leucine, phenyl-alanine isoleucine, histidine	oviposition into artificial medium	Wu & Qin (1982)
T. pretiosum, *T. minutum*	*Heliothis virescens*	egg contents	KCl, $MgSO_4$	oviposition into artificial medium	Nettles *et al.* (1982)

1 Presently named *T. maidis* (strain no. 11 in Pak & van Heiningen, 1985).
2 Previously referred to as *T. evanescens* (Smits, 1982; Noldus & van Lenteren, 1985*a*,*b*; Gardner & van Lenteren, 1986; *Chapter 4*).
3 Indirect evidence.

cumstances (van Dijken & Waage, 1987). The external marking pheromones employed by *Trichogramma* spp. appear to be of low, though distinct volatility (Salt, 1937*b*). To date, no information exists on their chemical nature.

Semiochemicals and Quality of Entomophagous Insects for Biological Control

Introduction

The ability of an insect to respond to a certain semiochemical has presumably evolved under and is maintained by natural selection. Does this mean that this ability is relevant for the animal's quality in the context of biological control? Present-day agro-ecosystems are often very different from the natural context in which these responses evolved, especially with regard to seasonal inoculative or inundative biological control. Therefore, many natural responses may be superfluous, and others that are uncommon in natural populations may be advantageous. I distinguish four approaches to investigate whether responses to semiochemicals are relevant for quality, and if so, which responses are relevant. These are: (1) manipulation of behaviour with semiochemicals, (2) use of natural intraspecific variation in responses to semiochemicals, (3) artificial selection for intraspecific variation in responses to semiochemicals, and (4) application of simulation models of foraging behaviour, with varying responses to semiochemicals. Each approach has its own merits and limitations, and they should be regarded as complementary rather than as alternatives.

Manipulation of behaviour with semiochemicals

Behavioural manipulation has been suggested by several authors as a means to improve the quality of inundatively released natural enemies (e.g., Vinson, 1977; Haskell *et al.*, 1981; Greany *et al.*, 1984; Wall, 1984; Lewis & Nordlund, 1985; Coppel, 1986; Powell, 1986; van Lenteren, 1987), but has rarely reached beyond the realms of speculation. Modification of behaviour can be brought about prior to or after release in the field. If manipulation leads to a change in performance then responses to semiochemicals may be relevant to quality. However, such evidence only proves that the insects can use the chemical cues, and that they did use them in a particular experimental setting with a resulting increase in foraging success. It does not provide direct proof that insects will also use these cues in a setting where the cues are not artificially added to the environment, and that responses to such cues can be a limiting factor for searching efficiency. A recent study on the winter moth and its tachinid fly parasitoid *Cyzenis albicans* showed that attack rates on apple trees by feral flies could be increased by an application of oak foliage extract (Roland *et al.*, 1989). This corroborated the results of wind tunnel experiments, which had indicated the presence of an attractive odour in oak leaves.

Most experimental work on the possibilities of behavioural manipulation of parasitoids outside the laboratory has been done with *Trichogramma* spp. by W.J. Lewis and cooperators in Tifton (Georgia, U.S.A.). They have been able to show that chemical stimuli which evoke behavioural responses in the laboratory can be employed to increase rates of parasitism by *Trichogramma* spp. in greenhouse and field settings. Confinement of released parasitoids to the target area, i.e. prevention of unwanted dispersal, has been the key phrase throughout these series of experiments (Lewis & Nordlund 1985; Lewis *et al.*, 1985).

As mentioned above, plant extracts can be used to increase parasitism (Altieri *et al.*, 1981, 1982), although the mechanism is not clear. Results on employment of kairomones show a more direct link between behaviour in the laboratory and field performance. Initially, a 'blanket treatment' of moth scale extract resulted in increased egg parasitism and seemed a very promising employment strategy for enhancing *Trichogramma*'s field performance (Lewis *et al.*, 1972, 1975*a,b*). However, with increasing plot size, this turned out to be true only at high host densities; at low or medium densities a homogeneous treatment led to arrestment of wasps in host-free areas and a resulting decrease of parasitoid effectiveness. The application of diatomaceous earth particles impregnated with kairomone extract around host oviposition sites gave the desired effect of enhanced local search without reduced movement at larger scale (Lewis *et al.*, 1979). This, however, was a very unpractical and labour-intensive method, and certainly not feasible for large-scale application. By augmenting the host density with sterilized moth eggs at the start of the season, the problem of low density could be circumvented and an easier kairomone application pattern could be employed (Nordlund *et al.*, 1981; Gross *et al.*, 1981*a*, 1984). However, this still did not yield a commercially feasible situation. From the correlation between high activity of moths and *Trichogramma* performance (Lewis *et al.*, 1979), Lewis *et al.* (1985) inferred that volatile cues might be critical for consistent benefit of released wasps independent of host density. This was supported by the finding that a synthetic sex pheromone blend of *H. zea* increased rates of parasitism by *Trichogramma* spp. in cotton plots (Lewis *et al.*, 1982). The behavioural mechanism underlying this effect is much like the response to contact kairomones: *Trichogramma* is not *attracted* by host sex pheromone but the odour *arrests* the parasitoids and suppresses upward (phototactic) flight (Noldus, 1988*b*; Noldus *et al.*, 1988*a*; *Chapters 5, 6*).

Besides treating parasitoids behaviour *in situ*, a pre-release experience of parasitoids with contact kairomones from host scales can suppress the tendency of naive wasps to disperse, and lead to increased confinement to the target area and higher parasitism rates (Gross *et al.*, 1975).

Natural intraspecific variation

Variation between populations, biotypes or strains of a species is a well-established phenomenon (Mackauer, 1976). Races or strains of a species may differ greatly with respect to any of their quality components and related behavioural traits. The response to certain semiochemicals can be among those properties, but field testing of strains differing in that respect is necessary to determine the relevance for quality. Obviously, this applies to all potential quality traits. But it is also virtually impossible to find strains that differ in such a small number of traits that variation in performance can reliably be attributed to one of those traits. The possibility of covariance between the trait under study and another trait, that has not been measured, but which is actually responsible for an observed variation in performance, can hardly ever be ruled out. So a correlation between variation in responses to semiochemicals in the laboratory and performance in the field does not prove that this particular response is a quality trait. This also applies to variation obtained through selective breeding and certainly for the study of interspecific variation. Obviously, with each extra source of variation, the number of field releases necessary before a conclusive judgement can be made increases exponentially.

As far as *Trichogramma* is concerned, intraspecific variation was first described more than fifty years ago. However, much of the early work is hard to interpret due to the dubious taxonomic status of many of the races, ecotypes and strains mentioned in papers (Quednau, 1960; Nagarkatti & Nagaraja, 1977). A good example are reports on differences in fecundity, temperature sensitivity and host preference between a 'yellow race' and a 'grey race' of *T. minutum* (Flanders, 1930*b,c*; Harland & Atteck, 1933; Lund, 1934). Later these races received the status of separate species. During the last decades the taxonomy of the genus *Trichogramma* has made considerable progress (Voegelé, 1988), but still many problems exist. Also, various studies on variation between strains of *Trichogramma* spp. have recently been published (Smith & Hubbes, 1986*a*; Huo *et al.*, 1988; Ouyang *et al.*, 1988; Pak, 1988), including a comparative study between laboratory behaviour and field performance (Pak & van Heiningen, 1985). To date, no evidence on intraspecific variation in responses to semiochemicals has been published.

Artificial selection on intraspecific variation

Selective breeding of natural enemies has been used mostly for genetic improvement, e.g. the enhancement of temperature tolerance, pesticide tolerance, etc. (Messenger *et al.*, 1976; Hoy, 1985; Vinson, 1986). However, it can also be applied for the evaluation of potential quality traits. If a genetic basis for the variability in responses to certain

semiochemicals within a population is proven, lines with diverging responses to certain cues can be selected. Next, results of tests of such lines in the field should indicate whether such a selection should be incorporated into mass-rearing procedures, and whether the particular response should be monitored during quality control. To date, examples of such an approach are very rare. A recent study by Prévost & Lewis (1989) indicates a genetic basis for responses to host odours in the larval parasitoid *Microplitis croceipes*, but selection has not yet been attempted.

Selective breeding in *Trichogramma* spp. has been the subject of several studies. Urquijo (1946, 1950) reported improved fecundity and parasitisation activity of *T. minutum* after selection during many generations. However, as far as the second trait is concerned, due to the lack of direct observations it is not clear what was exactly selected for. The absence of a control (non-selected line) further decreases the value of these accounts. Brenière (1965) obtained positive results with selection for fecundity in *T. australicum*, and Ram & Sharma (1977) also succeeded to increase fecundity, but not sex ratio, in *T. fasciatum*. Fecundity, longevity and sex ratio are not affected by the age of the mother wasps at oviposition (Davis & Burbutis, 1974). Ashley *et al.* (1974*a*) selected *T. pretiosum* for improved heat tolerance and locomotory activity. Further reports on selective breeding involve flight propensity (Steel, 1981), heat tolerance (Lopez & Morrison, 1980) and insecticide resistance (Hsiu *et al.*, 1988). Recently, Chassain & Boulétreau (1987) showed that variation in the manner in which *T. maidis* distributes its eggs among host eggs within a cluster is genetically based. The relationship between this trait and quality was not examined.

These accounts show that variation in components of foraging behaviour of *Trichogramma* spp. has a genetic basis. The basis for behavioural variation in responses to semiochemicals has yet to be explored.

Application of simulation models

Simulation models of natural enemy foraging behaviour and population dynamics may aid in identifying the importance of responses to semiochemicals. An example is a study of the predatory mite *Phytoseiulus persimilis* and the two-spotted spider mite *Tetranychus urticae*, where a model for local predator-prey dynamics could only be validated after incorporation of arrestment of *P. persimilis* by kairomones left by *T. urticae* (Sabelis & van der Meer, 1986). System analysis can provide an estimate of the relevance of various responses to the animal's performance as a biological control agent.

For *Trichogramma* spp., simulation models have been used to investigate various aspects related to biological control. Models have been developed for the maximization of the output of fit mated females in a mass rearing, with special

reference to aspects as development rate, sex ratio and clutch sizes (Goodenough *et al.*, 1983; Waage & Lane, 1984; Waage & Ng, 1984; Terytze & Mentscher, 1987), and for the efficacy of inundative releases, with emphasis on the role of the total plant leaf surface (Knipling & McGuire, 1968; Need & Burbutis, 1979; Kanour & Burbutis, 1984; Chiang *et al.*, 1986), host spatial distribution (Hassell, 1982), or initial host density (van Hamburg & Hassell, 1984). Although non-random distribution of parasitism in the field after release has been described (Allen & Gonzalez, 1974), all these models, in so far as they deal with foraging behaviour, assume that parasitoids search at random, independent of the spatial scale. However, the probability of encounters with host egg clusters is likely to be a function of cluster size (van der Schaaf *et al.*, 1984). Also in Yano's (1978) specific model for individual searching behaviour of *T. dendrolimi*, only success-motivated area-restricted search after oviposition is included and semiochemicals are not mentioned. This in spite of ample laboratory evidence of responses of *Trichogramma* to host scales and other cues (see above).

According to Goodenough & Witz (1985), the main reason for the exclusion of small-scale foraging components from models for host-parasitoid dynamics in the field is the fact that *Trichogramma*'s searching behaviour can hardly be studied under field conditions. But one can still simulate different foraging strategies and test model predictions against observed patterns of parasitism in the field. Very recently, a model has been presented for the distribution of parasitism by *Trichogramma* in the field as a function of the distance from the release point (Chernyshev *et al.*, 1988). Input consists of search parameters such as linear speed and turning angle, based on laboratory measurements. The model succeeds in predicting parasitism on egg cards placed in the field, but underestimates the parasitism of naturally laid eggs. Based on this discrepancy the authors suggest that the response to host kairomones must be an important component in *Trichogramma*'s foraging behaviour in the field.

Are responses to semiochemicals relevant for quality of *Trichogramma*?

So far I have made an inventory of all responses to semiochemicals reported for *Trichogramma* spp. and I have reviewed existing evidence on the relevance of such responses for the quality of biological control agents. There is no doubt that *Trichogramma* parasitoids use semiochemical cues in their foraging behaviour in the field. Under certain circumstances it has been possible to enhance the performance of mass-released insects by artificial employment of such semiochemicals. Apparently, searching efficiency at various spatial scales, including retention in target areas, is a key to success and is mediated in part by various chemical cues. This makes adjustment to semiochemicals related to the agro-ecosystem in which *Trichogramma* has to

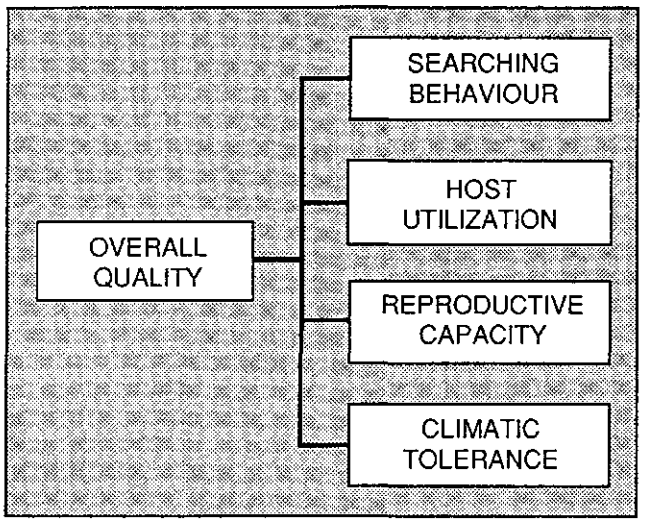

Figure 9-1. Diagram of major quality components for *Trichogramma* spp.

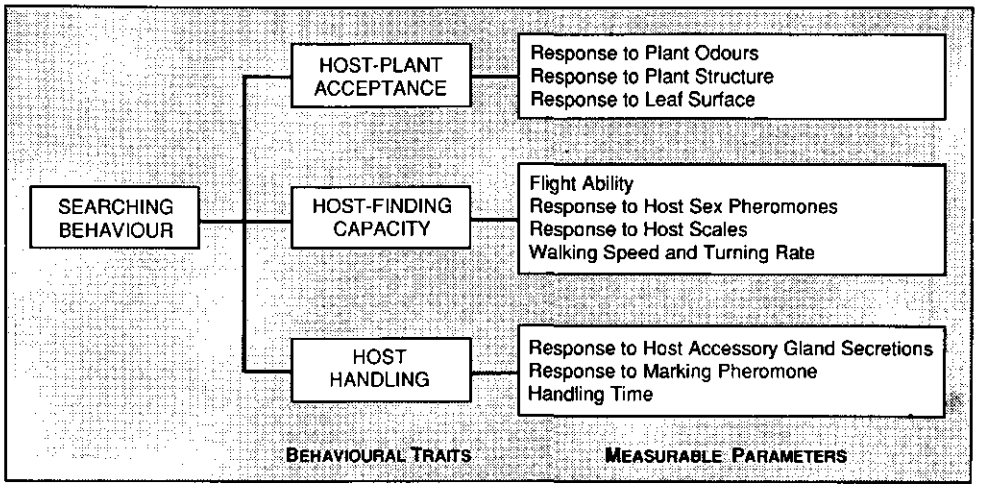

Figure 9-2. Diagram of behavioural traits and measurable parameters related to searching behaviour of *Trichogramma* spp.

function an important attribute of effective parasitoids. Wasps should preferably respond to kairomones originating from the pest species to be controlled, and not be repelled by odours from the crop on which it occurs. However, apart from information derived from manipulative experiments, the contribution to quality of responses to semiochemicals is still ill-defined. Studies on naturally occurring or artificially selected intraspecific variation, as well as simulation models of *Trichogramma*'s foraging behaviour have hardly addressed responses to semiochemicals so far.

With the information presently available we can identify searching behaviour, host utilization, reproductive capacity and climatic tolerance as major quality components for *Trichogramma* wasps (Figure 9-1). Searching behaviour, where semiochemicals are involved, has been worked out in more detail in Figure 9-2. It is divided here into three behavioural traits: host-plant acceptance, host-finding capacity and host handling. Measurable parameters include the response to plant odours, host sex pheromones, host scales, host accessory gland secretions and marking pheromones. The diagram further contains parameters related to responses to the physical environment (plant structure, leaf surface) as well as to the vigour of parasitoids (flight ability, walking speed and turning rate, handling time). Handling time consists of drumming, drilling and oviposition time (Pak *et al.*, 1986). If considerable variation in such responses is observed, they should be screened during the selection of potential control agents (Gross, 1981; Pak, 1988), as well as during quality control programs.

Obviously, if manipulative employment of semiochemicals becomes part of inundative biological control programs, behavioural traits related to semiochemicals become even more important for quality of *Trichogramma*. However, so far, success has depended on very elaborate application patterns (Gross, 1981), which are neither practical nor feasible (Gardner & van Lenteren, 1986).

Mass Rearing of *Trichogramma*

Rearing *Trichogramma* on lepidopteran eggs depends on a continuous, dependable supply of acceptable host eggs. Natural host species can be used for mass rearing, such as *Heliothis* spp. (Lewis *et al.*, 1976; Morrison, 1985), *Ostrinia nubilalis* (Burbutis & Goldstein, 1983) or *Trichoplusia* spp. (Morrison, 1985). However, most commercial mass rearings of *Trichogramma* spp. are based on an easily, cheaply and more dependably reared factitious host. Most commonly used are the Angoumois grain moth, *Sitotroga cerealella*, and the Mediterranean flour moth, *Ephestia* (= *Anagasta*) *kuehniella* (Flanders, 1930*a*; Morrison *et al.*, 1976; Bigler, 1986; Hassan *et al.*, 1984). In Asia, most mass rearings are based on eggs of the oak silkworm, *Antheraea pernyi*, the eri silkworm, *Philosamia cynthia ricini*, or the rice meal moth, *Corcyra cephalonica*

(Coulson *et al.*, 1982; Li, 1982; Cadapan, 1988).

The desire to increase the efficiency of mass rearing has encouraged research into *in vitro* rearing of *Trichogramma* spp. (Thompson, 1986; Vinson *et al.*, 1988*b*). A suitable artificial medium for continuous rearing should possess a nutritional value allowing immature development of the parasitoids leading to emergence of fit adults, and it should be acceptable for oviposition by adult wasps. These two obstacles have been overcome during the past 15 years by research groups in the United States, China and France (Table 9-5). In China, the method now approaches practical utilization.

Low-temperature storage techniques in mass rearing of *Trichogramma* offer ways to gradually build up a stock of host and/or parasitoid material, in order to have a sufficient number of parasitoids available at the start of the release season. Further, year-round rearing implies more efficient use of rearing equipment. Different kinds of low-temperature storage should be distinguished: storage of unparasitised host eggs and long- or short-term storage of parasitised eggs. Low-temperature storage of unparasitised eggs was already mentioned by Mokrzecki & Bragina (1916), but has only recently been investigated in detail. Various storage programs have been proposed, with storage in liquid nitrogen or at temperatures between -10 and +5 °C, for periods up to 2-8 months, and have been described for most of the common factitious hosts, i.e. *E. kuehniella* (Voegelé *et al.*, 1974), *S. cerealella* (Gennadiev *et al.*, 1985; Morrison, 1988) and silkworms (Coulson *et al.*, 1982; Li, 1982; Hu & Xu, 1988; Wang *et al.*, 1988). Low-temperature storage of parasitised host eggs, via induced diapause of *Trichogramma*, was already suggested by Salt (1940) as a means to solve long-distance transportation problems. Peterson (1931) already men-

Table 9-5. Published reports on advances of *in vitro* rearing of *Trichogramma*.

Country	References
U.S.A.	Rajendram & Hagen (1974), Hoffman *et al.* (1975), Rajendram (1978*a*,*b*), Nettles *et al.* (1982, 1983, 1985), Morrison *et al.* (1983), Strand & Vinson (1985), Xie *et al.* (1986*a*,*b*, 1988), Irie *et al.* (1987), Vinson *et al.* (1988*b*)
China	Guan *et al.* (1978), Hubei Research Group (1979), Liu *et al.* (1979), Wu *et al.* (1980, 1982), Gao *et al.* (1982), Liu & Wu (1982), Wu & Qin (1982), Dai *et al.* (1988), Li *et al.* (1988), Qin & Wu (1988)
France	Grenier & Bonnot (1988)

tioned the successful storage of *T. minutum* at 4.4 °C for 6 months. Recently, this technique has received renewed interest, and a storage method for *T. maidis* has been reported yielding 89 % emergence after 9 months storage at 3 °C (Voegelé *et al.*, 1986). Short-term storage of parasitised host eggs at a lower temperature reduces the development rate and can be used to program the emergence of adult wasps. Stinner *et al.* (1974*a*) were the first to develop such a system for *Trichogramma*. This technique has subsequently been incorporated into schemes for mass-rearing and release of *Trichogramma* (Morrison *et al.*, 1978; Bouse & Morrison, 1985).

Impact of Mass Rearing on Responses to Semiochemicals

Introduction

Information is accumulating on how different rearing methods can affect responses to semiochemicals. A *response* is a change in behaviour as the result of a stimulus. A *change in response* can indicate that: (1) the insect can no longer perceive the stimulus, (2) the response threshold has changed, (3) dose-response relations have changed, (4) the behaviour upon perception changes in a different manner (change of motor pattern), (5) the response relative to another stimulus has changed (change of preference). Unless explicit reference to one of these aspects is made, no distinction between them is made in the following sections. Aspects of mass rearing with a potential impact on quality include the rearing substrate (natural or factitious host, artificial medium), host diet, presence or absence of host kairomones and food for adults, and abiotic conditions such as temperature, humidity and light. In theory, all the quality components can be affected. This includes searching behaviour, where responses to semiochemicals are involved.

Continuous rearing of natural enemies outside the environment in which they have to perform can lead to adverse genotypic and/or phenotypic changes. Components of the insect's quality that have a genetic basis can undergo selection in an unwanted direction, or may change because of lack of directed selection (Mackauer, 1976; Bartlett, 1984, 1985). Further, rearing conditions can exert non-genetic influences, either through the physiology of the developing larvae or by directly affecting the adults. Many behavioural traits depend on or are modified by the experience of the adult insect, which is also common in parasitoids (Vet, 1983, 1988). Behavioural plasticity (of which learning is but one example) with respect to responses to semiochemicals may be regarded as a quality trait in itself, which may be affected by mass rearing.

Genetic effects

A rearing environment where substantial searching activity of natural enemies is not required for host finding and where semiochemicals originating from the plant-host complex in which they are to be released are absent, may lead to changes or disappearance of responses to such cues. Such changes may not occur immediately; for example, Weseloh (1987) did not find such an effect after rearing *Cotesia melanoscela* in the laboratory for 25 generations.

Genetic changes can be detected by comparison of the quality of the colony in the course of time. Thus, Ashley *et al.* (1973) found a decrease in field performance of a strain of *T. pretiosum* in the course of four years of laboratory rearing. Alternatively, a simultaneous comparison between laboratory-reared and wild insects can be made (Nagarkatti & Nagaraja, 1978; Nagarkatti, 1979; Southard *et al.*, 1982; Hawlitzky & Boulay, 1988). Thomson & Stinner (1988) found no difference in response to *H. zea* scales between laboratory-reared and field-collected *T. exiguum*. I have made such a comparison with regard to the olfactory responses of *T. pretiosum* to the sex pheromone of its host *H. zea*. Exposing adult female wasps to a synthetic sex pheromone blend in a wind tunnel leads to prolonged searching times on a platform and a higher incidence of landing vs. upward phototactic flight (Noldus *et al.*, 1988*a*; *Chapter 6*). Furthermore, the proportion of insects landing on the platform increases with increasing dosage of the olfactory stimulus (*Chapter 7*; Figure 9-3). These experiments were carried out with a strain of *T. pretiosum* that had been reared in the laboratory on eggs of *H. zea* for 15 years without infusion of field individuals. These parasitoids had probably not been exposed to host sex pheromone for more than 450 generations, since the eggs were always thoroughly rinsed with sodium hypochlorite before exposure to parasitoids. Moreover, there had certainly been no selection for responses to host-searching cues, since rearing occurred on egg cards in test tubes and hardly any searching activity of the wasps was required. How would this laboratory strain compare with *T. pretiosum* parasitising *H. zea* in the field, with respect to the response to host sex pheromone in the wind tunnel? To investigate this, parasitoids were collected from cotton fields near Tifton (Georgia, U.S.A.) by applying a *H. zea* egg to ca. 100 plants for 24 h. After that, eggs were recollected and incubated. The emerging progeny crossed successfully with the laboratory strain of *T. pretiosum*. After laboratory rearing for 10 generations, both strains, the 'old' laboratory strain and the 'new' strain were tested in the wind tunnel according to the procedure described by Noldus *et al.* (1988*a*; *Chapter 6*). The old strain showed the same response as in previous experiments, with respect to the intercept as well as the slope of the dose-response curve (Figure 9-3). The new strain showed an almost identical dose response, although the intercept was higher, indicating an overall higher propensity to land on the platform and a weaker phototactic response. However, the

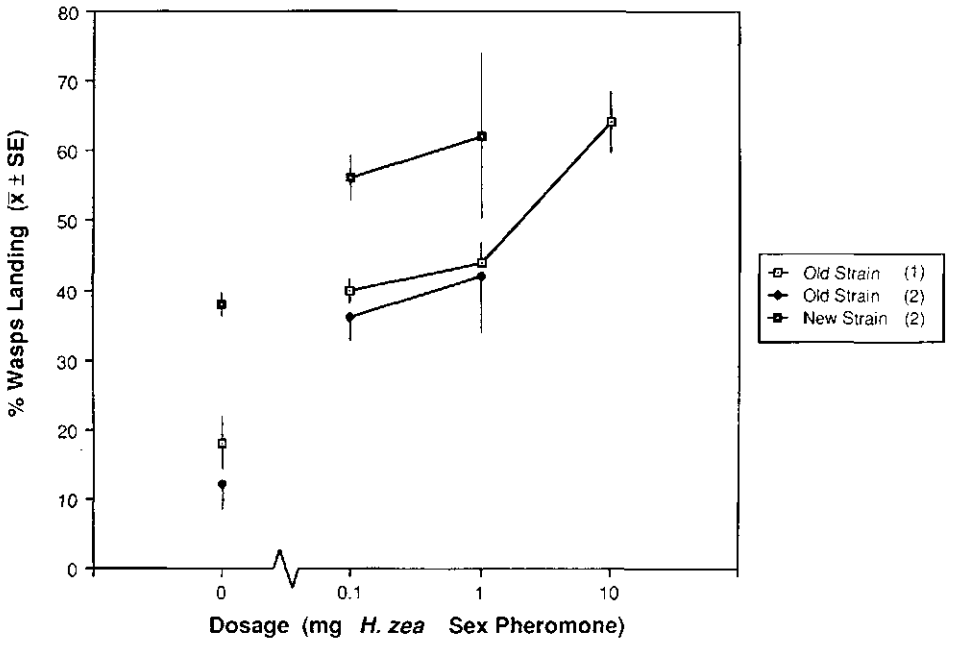

Figure 9-3. Response of *Trichogramma pretiosum* females to the sex pheromone of *Heliothis zea*: proportion of wasps landing on a platform in a wind tunnel as a function of pheromone dosage. (1) Data from Chapter 7; (2) Comparison of a 15-year old and a recently initiated laboratory strain. See Chapters 6 and 7 for details on experimental procedures.

strength of the response to the odour is reflected by the slope, not the intercept of the line. Rearing the new strain in the laboratory for 10 generations may already have been long enough to cause genetic changes, but yet these results indicate that in spite of rearing *T. pretiosum* for a large number of generations in the absence of host sex pheromone, the dose response to this sex pheromone is still similar to the one of a recently initiated lab strain.

Detecting a change in quality in a laboratory culture by itself says nothing about what caused the change. Furthermore, a deterioration in field performance does not directly indicate which quality trait has been affected. Explicit cause-effect studies are required to answer those questions. For example, a study by Stein (1960) suggested that the rearing host may influence the field performance of mass-released *Trichogramma* spp. Later work supported this notion (Lewis *et al.*, 1976; Bigler *et al.*,

1982). However, effects of rearing host species have been found for development rate, morphology, size, fecundity, longevity, sex ratio, locomotory capacity, searching capacity and host preference (see Appendix).

Rearing parasitoids on host species other than the target pest species may influence the host preference of the emerging wasps (King *et al.*, 1985). As host recognition and acceptance are mediated by a complex of physical as well as chemical factors, changes in host preference may be caused by selection of parasitoids with altered responses to host recognition chemicals. Zaborski *et al.* (1987) found that the rearing host had no influence on the response of *T. minutum* to the scales of *C. fumiferana*. Other effects of the rearing host may involve semiochemicals, such as 'searching capacity' as measured (but not clearly defined) by Stinner *et al.* (1974*b*) and host preference. Interpreting changes in host preference is complicated by the fact that differences in egg surface chemicals are associated with differences in size and shape, and other physical characterstics. Increased acceptance by *Trichogramma* spp. of a host species after rearing on it have been found by Flanders (1935), Salt (1940), Taylor & Stern (1971), Kaiser & Pham-Delègue (1988) and Kaiser *et al.* (1989), but none of these authors mentioned a chemical basis. In three species of phytoseiid mites, the rearing prey altered the response to prey odours but the preference was left intact (Dicke, 1988*b*).

In vitro rearing of parasitoids requires chemical stimulants to induce oviposition into the medium by the adult wasps. These have recently been identified for *Trichogramma* spp. (Nettles *et al.*, 1982; Wu & Qin, 1982; Table 9-4) as well as for a larval parasitoid (Tilden & Ferkovich, 1988). Rearing insects on such media may lead to selection for parasitoids which no longer recognize and accept the target host in the field. Rearing predators on a (semi)artificial diet can also have severe effects on their responses to semiochemicals. This phenomenon has been studied in most detail in predatory mites (Dicke, 1988*a*; Dicke *et al.*, 1989).

Non-genetic effects

Preimaginal conditioning is a rare phenomenon in parasitic insects (Vet, 1988) and has never been recorded for *Trichogramma*. However, several accounts on adult learning in relation to responses to semiochemicals exist. In *T. pretiosum* and *T. achaeae* a pre-release exposure to host scales has shown to suppress dispersal and lead to higher parasitisation success (Gross *et al.*, 1975). Similar effects were found of a pre-release oviposition experience (Gross *et al.*, 1981*b*; Nordlund *et al.*, 1981). In the case of oviposition experience, semiochemicals may be involved in the learning process, but this has never explicitly been investigated. Thomson & Stinner (1989*b*) found that the response of *T. pretiosum* to host scales decreased after contact with scales but in-

creased after an oviposition experience. This effect was not further tested in relation to parasitisation success. They also found that preferences for the scales of a certain host species were not modified by experience with either scales or host eggs. The same has been found for *T. evanescens* and *P. brassicae* (Gardner & van Lenteren, 1986). Effects of experience on responses to semiochemicals by *Trichogramma* spp. cannot be generalized. For example, oviposition by *T. maidis* in an egg of *O. nubilalis* on corn increased the response to odour of corn as well as to odour of corn in combination with eggs and sex pheromone of *O. nubilalis* in an airflow olfactometer (Kaiser, 1988). In contrast, in experiments with *M. brassicae* and cabbage, an oviposition experience in a complete plant-host complex had a very strong effect on behaviour of *T. evanescens* in a wind tunnel (increased walking speed, longer search paths and increased retention on a platform) regardless of the odour offered, so that the response to host sex pheromone was no longer expressed (Noldus *et al.*, 1988*b*). An oviposition experience can also cause an immediate increase of host acceptance, as has been shown for *T. maidis* (Kaiser *et al.*, 1987, 1989; Kaiser & Pham-Delègue, 1988). But again, it is not known to what extent semiochemicals are involved here.

The diet on which the rearing host is fed may have a profound effect on the behaviour of emerging parasitoids. For example, rearing the larval parasitoids *Microplitis croceipes* or *M. demolitor* on *H. zea* larvae fed on an artificial diet rather than on the host plant cowpea, leads to a significant reduction of oriented flights towards *H. zea* larvae on cowpea by the emerged wasps (Drost *et al.*, 1988, Hérard *et al.*, 1988*b,c*).

Chilling parasitised hosts in order to program the emergence of the parasitoids can influence the subsequent responses of these adult wasps to host odours. This effect has recently been shown for the larval parasitoid *Microplitis demolitor* by Hérard *et al.* (1988*a*), who found that chilling pupae not only negatively affected the reproductive performance of emerging adults, but also rendered most adult females unresponsive to volatile semiochemicals. A similar effect might occur in *Trichogramma* as a result of low-temperature storage of parasitised host eggs. However, this has not yet been investigated.

Finally, low humidity during rearing might lead to irreversible damage to a wasp's receptor system, as has been found in tephritid fruit flies (Städler *et al.*, 1987).

Quality Control of *Trichogramma*

Current quality control methods

Crucial in biological control programs using *Trichogramma* is the selection of a suitable species/strain for release (Pak, 1988). Next, an appropriate rearing method has to be chosen. Obviously, arguments at this point stem from economic as well as quality considerations (Marston & Ertle, 1973; Chambers, 1977). Based on knowledge of the many aspects of mass rearing that can affect the quality of released *Trichogramma* spp., a decision with regard to rearing host (or medium) and other rearing conditions can be made. Several authors have stressed the importance of a broad genetic base of a mass rearing, to be ensured by a large founder population and regular replacement with fresh field material (Mackauer, 1976; Morrison & King, 1977; King & Morrison, 1984; van Lenteren, 1986). Once a particular rearing method has been selected, the quality of the produced wasps has to be monitored.

In most current mass rearings of *Trichogramma*, main emphasis has been put on production control, rather than on product control (*sensu* Chambers & Ashley, 1984), i.e. quality control measures mainly deal with maximizing output of mated female wasps (e.g. Terytze & Mentscher, 1987). Often, the only parameters regularly monitored are percentage parasitism, percentage emergence, sex ratio and longevity (King & Morrison, 1984; Gennadiev, 1985; Morrison, 1985; see also Appendix). However, these production-oriented parameters may not relate at all to field performance. For example, while fecundity may be important in the context of inoculative control (Coulson *et al.*, 1982), searching capacity rather than fecundity may be a limiting factor for field performance of inundatively released *Trichogramma* spp. in many cases, depending on the distribution of host eggs in the field (Hirose *et al.*, 1976; van der Schaaf *et al.*, 1984).

Although quality control for *Trichogramma* is still in its infancy in many respects, progress is being made. The importance of locomotory capacity in *Trichogramma* has been recognized and selection for this trait forms a standard component of several *Trichogramma* mass rearings (Morrison & King, 1977; Bigler *et al.*, 1988). In China, mass rearing also includes selection for flight capacity (Coulson *et al.*, 1982), a procedure which has recently also been adopted in some western systems (Bigler, 1986; Bigler *et al.*, 1987). Some rearing systems include alternation of rearing host (Bigler, 1986; Bigler *et al.*, 1987; S.A. Hassan, pers. comm.), selection for temperature tolerance (Hassan, pers. comm.), or provision of additional food for adult wasps (Coulson *et al.*, 1982).

Recommendations for quality control of *Trichogramma*

Due to the diversity of agro-ecosystems in which *Trichogramma* spp. are used for biological control, generalizations with regard to quality are hard to make. Quality should be viewed in the context of a particular crop-pest system, with factors such as plant architecture, leaf surface characteristics, host-egg distribution, plant- and host-related semiochemicals, taken into consideration.

Quality of *Trichogramma* obviously includes more than fecundity, longevity and sex ratio. A general awareness of the importance of behavioural characteristics for quality is growing, and should now be reflected in the design of mass-rearing methods as well as in the selection of quality control procedures. However, *maximization* of a quality trait is not synonymous with *optimization*. For example, *Trichogramma* wasps should have sufficient locomotory and flight capacities, but a too strong innate propensity of flight may lead to rapid dispersal from the target area. The desired level of a particular trait should be verified by means of comparative field experiments to determine the contribution of the trait to overall performance, as has recently been done for locomotory capacity (Bigler *et al.*, 1988).

Behavioural traits that are not under continuous selective pressure in the rearing have to be monitored to assess possible changes in the course of time. This includes especially the various components of host finding and selection under field conditions (Neuffer, 1982; Voegelé *et al.*, 1986). However, product control requires a specified quality standard (Chambers & Ashley, 1984), and what is the standard for *Trichogramma*? The development of theoretical models for rearing as well as the implementation of behavioural traits in quality control is still hampered by our limited knowledge of *Trichogramma*'s foraging behaviour under natural circumstances (Keller *et al.*, 1985) and of the relative importance of genetic vs. learned aspects of its behaviour (Waage *et al.*, 1985). As far as semiochemicals are concerned, the review presented above shows that a fair amount of information exists on the responses of *Trichogramma* spp. to plant or host cues under experimental conditions, but that these responses have rarely been related to quality. This neglected area certainly deserves closer attention. Simple bioassays for behavioural studies in the laboratory – which exist for the measurement of host preference (van Dijken *et al.*, 1986; Wäckers *et al.*, 1987) – should be developed for responses to semiochemicals, e.g. the response to host scales. However, eventually, the step to the field has to be made to assess the relevance of responses to semiochemicals for overall quality of *Trichogramma*. Behavioural manipulation of *Trichogramma* with semiochemicals will have drastic implications for quality control but, for the time being, Vinson's (1986) statement that this is "... an exciting potential rather than a reality" is still valid. Basic research on the role of these cues in the behavioural ecology of *Trichogramma* is still a pressing necessity.

In vitro rearing of *Trichogramma* has not yet developed to such a level that quality control comes in the picture (Thompson, 1986; Qin & Wu, 1988). So far, researchers have mainly dealt with morphological aspects of the emerging wasps or whether or not they are fecund (Strand & Vinson, 1985). Future mass-rearing systems based on artificial rearing may involve even less inherent selection for behavioural quality than the present ones. In that case, quality control based on behavioural traits becomes even more important.

In conclusion, to be truly successful with semiochemicals and natural enemies we must (1) know which traits of the natural enemy are desirable, (2) know the mechanism(s) by which semiochemicals influence those traits, (3) have the ability to measure and manipulate those traits, and (4) have the technology to manipulate those traits prior to and/or after release. Such information and technology may require considerable research investment, but without it we will be operating in the dark with regard to application of semiochemicals as well as to what problems are caused by rearing under unnatural conditions.

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Appendix: Mass Rearing and Quality of *Trichogramma*

The following tables list published evidence for effects of mass rearing on various aspects of the quality of *Trichogramma* spp. Each table deals with one of the following aspects of mass rearing: (1) rearing host species, (2) temperature, (3) relative humidity, (4) photoperiod and light intensity, (5) density of wasps relative to host eggs, (6) presence of food for adult wasps, (7) diet of rearing host, (8) rearing on artificial medium, (9) exposure of wasps to host kairomones, (10) oviposition by wasps prior to release, (11) low-temperature storage of host eggs prior to parasitisation, (12) short-term low-temperature storage of parasitised host eggs, and (13) long-term low-temperature storage of parasitised host eggs. The information is provided to illustrate the variety of possible effects on parasitoid quality attached to mass rearing. It may serve as a starting point for further reading, for scientists as well as biological control practitioners.

1. Rearing host species

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Field performance	*Heliothis zea* > *Sitotroga cerealella*	Lewis *et al.* (1976)
	Ostrinia nubilalis > *Ephestia kuehniella*	Bigler *et al.* (1982)
	E. kuehniella = *S. cerealella*	Hassan *et al.* (1978), Bigler *et al.* (1987)
Longevity	*S. cerealella* > *E. cautella*	Flanders (1930*a*)
	S. cerealella > *T. ni*	Marston & Ertle (1973)
	H. zea > *T. ni*	Ashley *et al.* (1974*b*)
	Galleria mellonella > *S. cerealella*	Boldt & Martson (1974)
	E. kuehniella > *S. cerealella*	Lewis *et al.* (1976)
	H. armigera = *Corcyra cephalonica* > *Cadra cautella* > *S. cerealella*	Navarajan Paul *et al.* (1981)
	O. nubilalis = *E. kuehniella*	Bigler *et al.* (1982)
	Choristoneura fumiferana > *E. kuehniella*	Smith & Hubbes (1986*a*,*b*), Smith *et al.* (1986)
	E. kuehniella = *S. cerealella*	Bigler *et al.* (1987)
	T. ni > *S. cerealella*	Hohmann *et al.* (1988*a*)
Fecundity	*S. cerealella* < arctiid sp.[1]	Flanders (1930*a*)
	Trichoplusia ni > *S. cerealella*	Marston & Ertle (1973) Hohmann *et al.* (1988*a*)
	H. zea > *T. ni*	Ashley *et al.* (1974*b*)
	H. virescens > *S. cerealella*	Stinner *et al.* (1974*b*)
	E. kuehniella > *S. cerealella*	Lewis *et al.* (1976)

1. Rearing host species, *continued*

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Fecundity	*H. armigera* = *C. cephalonica* > *C. cautella* > *S. cerealella*	Navarajan Paul *et al.* (1981)
	O. nubilalis > *E. kuehniella*	Bigler *et al.* (1982)
	Choristoneura fumiferana > *S. cerealella*	Houseweart *et al.* (1982)
	C. fumiferana > *E. kuehniella*	Smith & Hubbes (1986*a*,*b*), Smith *et al.* (1986)
	E. kuehniella = *S. cerealella*	Bigler *et al.* (1987)
	O. nubilalis > *S. cerealella*	Neuffer (1987)
	M. brassicae > *S. cerealella*	Neuffer (1987)
Sex ratio	*C. fumiferana* = *E. kuehniella*	Smith & Hubbes (1986*a*), Smith *et al.* (1986)
	E. kuehniella = *S. cerealella*	Bigler *et al.* (1987)
	T. ni = *S. cerealella*	Hohmann *et al.* (1988*a*)
Development rate	*E. kuehniella* > *Agrotis c-nigrum* > *S. cerealella*	Salt (1940)
	E. kuehniella = *Bupalus* sp. > *Cimex lectularius* > *G. mellonella*	Quednau (1957)
	T. ni > *S. cerealella*	Butler & Lopez (1980)
Size	*Agrotis c-nigrum* > *E. kuehniella* > *S. cerealella*	Salt (1940)
	Mamestra brassicae > *E. kuehniella* > *S. cerealella*	Salt (1940, 1941)
	T. ni > *S. cerealella*	Boldt (1974), Hohmann *et al.* (1988*a*)
	H. armigera = *C. cephalonica* > *Cadra cautella* = *S. cerealella*	Navarajan Paul *et al.* (1981)
	C. fumiferana > *S. cerealella*	Southard *et al.* (1982)
	E. kuehniella > *S. cerealella*	Bigler *et al.* (1987)
Morphology	*T. semblidis* on *Sialis lutaria*: apterous males	Salt (1941)
Locomotory activity	*T. ni* vs. *S. cerealella*: higher % walking, higher speed	Biever (1972)
	T. ni vs. *S. cerealella*: larger distance traveled, higher % walking, higher speed	Marston & Ertle (1973), Boldt (1974)
	G. mellonella vs. *S. cerealella*: larger distance, higher speed	Boldt & Martson (1974)
	O. nubilalis vs. *E. kuehniella*: larger distance, higher speed	Bigler *et al.* (1982)

1. Rearing host species, *continued*

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Locomotory activity	*E. kuehniella* = *S. cerealella*	Bigler *et al.* (1987)
Searching capacity	*H. virescens* > *S. cerealella*	Stinner *et al.* (1974*b*)
Response to host scales	*C. fumiferana* = *S. cerealella*	Zaborski *et al.* (1987)
Host preference	*Estigmene acroea* vs. *S. cerealella*: lower acceptance of *S.c.*	Flanders (1935)
	E. kuehniella vs. *S. cerealella*: no effect on preference for *E.k.*	Salt (1935, 1940)
	A. c-nigrum vs. *S. cerealella*: lower acceptance of *S. cerealella*	Salt (1940)
	S. cerealella vs. *T. ni*: preference for *S.c.*[2]	Taylor & Stern (1971)
	C. cephalonica vs. *H. armigera*, *C. cautella*, *S. cerealella*: no effect on preference for *C.cephalonica*.[3]	Navarajan Paul *et al.* (1981)
	S. cerealella vs. *O. nubilalis*: higher acceptance of *S.c.*	Neuffer (1987)
	E. kuehniella vs. *O. nubilalis*: higher acceptance of *E.k.*	Kaiser *et al.* (1988), Kaiser & Pham-Delègue (1988)

1 Species not mentioned.
2 After 100 generations.
3 Wasps had been reared on *C. cephalonica* for many generations.

2. Temperature

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Longevity	25-32 °C (4)[1]: decrease	Schulze (1926)
	13-38 °C (10): decrease	van Steenburgh (1934)
	10-30 °C (5): decrease	Lund (1938)
	12-33 °C (7): maximum at 15 °C	Quednau (1957)
	12-33 °C (10): decrease, variable trends	Russo & Voegelé (1982*b*)
	17-35 °C (6): 20-30 °C no effect;	
	17, 35 °C shorter	Calvin *et al.* (1984)
	15-35 °C (5): decrease	Harrison *et al.* (1985)

2. Temperature, *continued*

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Longevity	13-27 °C (6): variable trends	Smith & Hubbes (1986*a*)
	20-35 °C[2]: decrease	Cabello & Vargas (1988)
	20-35 °C (4): decrease	Gou (1988)
Fecundity	15-40 °C (12): maximum at 26-29 °C	Schulze (1926)
	10-30 °C (5): maximum at 25 °C	Lund (1938)
	10-32 °C (10): maximum at 20 °C	Stschepetilnikova (1939)
	12-33 °C (7): maximum at 25-27 °C	Quednau (1957)
	12-34 °C (7): maximum at 26 °C	Savescu (1970)
	16-33.5 °C (11): maximum at 27 °C	Savescu (1973)
	15-35 °C (5): maximum at 25 °C	Frantsevich (1978)
	12-33 °C (10): maxima at 25, 25-30, 25 and 20-25 °C	Russo & Voegelé (1982*b*)[3]
	17-35 °C (6): no consistent trend, > 25 °C decrease	Calvin *et al.* (1984)
	20-30 °C (5): decrease	Cabello & Vargas (1985*c*)
	13-27 °C (6): maximum at 23 °C	Smith & Hubbes (1986*a*)[4]
	20-35 °C[2]: maximum at 25 °C	Cabello & Vargas (1988)
	20-35 °C (4): maximum at 25 °C	Gou (1988)
Sex ratio	17-32 °C (4): no consistent effect	Lund (1934)
	13-38 °C (10): no effect	van Steenburgh (1934)
	10-30 °C (5): maximum % females at 25 °C	Lund (1938)
	12-33 °C (10): no effect, except at 12-13 and 30-32 °C for 2 species	Russo & Voegelé (1982*b*)
	17-35 °C (6): no effect	Calvin *et al.* (1984)
	16-33 °C (6): maximum % females at 22-25 °C	Zhang (1984)
	15-35 °C (5): slight differences, maximum % females at 20 °C	Harrison *et al.* (1985)
	13-27 °C (6): no effect	Smith & Hubbes (1986*a*)
	20-30 °C (5): switch from thelytoky to arrhenotoky at 28 °C	Cabello & Vargas (1985*c*)
	20-35 °C[2]: decrease of % females	Cabello & Vargas (1988)
Development rate	25-32 °C (7): increase	Schulze (1926)
	17-32 °C (4): increase	Lund (1934)
	10-38 °C (11): < 13, > 35 no development, 13-35 increase	van Steenburgh (1934)
	13.5-32 °C (9): increase	Stschepetilnikova (1939)
	11-35 °C (15): increase	Quednau (1957)

2. Temperature, *continued*

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Development rate	10-43 °C (6): 16-32 °C increase, 10, 43: no emergence	Stern & Bowen (1963)
	12-34 °C (7): increase	Savescu (1970)
	16-33 °C (10): increase	Savescu (1973)
	15-34 °C[5]: increase[6]	Butler & Lopez (1980)
	11-34 °C (13): increase	Russo & Voegelé (1982*a*)
	17-35 °C (6): maximum at 30 °C	Calvin *et al.* (1984)
	16-33 °C (6): increase	Zhang (1984)
	15-35 °C (5): increase	Harrison *et al.* (1985)
	13-27 °C (6): increase	Smith & Hubbes (1986*a*)
	20-35 °C[5]: increase	Cabello & Vargas (1988)
Size	12-26 °C (4): no effect, 26-34 °C (4): decrease	Savescu (1970)
	18-27 °C (4): no effect, 27-33 °C (6): decrease	Savescu (1973)
Morphology	27-43 °C (5): minimum % brachyptery at 32 °C	Gross (1988)

1 In parentheses: number of constant temperatures compared.
2 Comparison of 3 constant and 3 fluctuating temperatures.
3 Fecundity measured as number of eggs parasitised during the first week.
4 Fecundity measured as first-day fecundity.
5 Comparison of 10 constant and 6 fluctuating temperatures.
6 Slightly lower at temperatures > 32 °C.

3. Relative humidity

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Longevity	0-15 mm s.d.[1]: decrease	Lund (1938)
	0-100 % (8): 0-82 no effect, > 82 decrease	Quednau (1957)
	20, 60, 80 %: decrease	Calvin *et al.* (1984)
	29-92 % (5)[2]: no effect	Gou (1988)

3. Relative humidity, *continued*

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Fecundity	0-15 mm s.d.: decrease	Lund (1938)
	40-95 % (4): maximum at 75-80 %	Stschepetilnikova (1939)
	0-100 % (8): 0-82 slight decrease, > 82 sharp decrease	Quednau (1957)
	20, 60, 80 %: increase	Calvin *et al.* (1984)
	29-92 % (5): no effect	Gou (1988)
Sex ratio	30-100 % (4): no effect	Lund (1934)
Development rate	30-100 % (4): increase, except at 17 °C	Lund (1934)
	40-95 % (4): no effect	Stschepetilnikova (1939)
	0 vs. 80, 95 %: increase	Quednau (1957)
	20, 60, 80 %: increase	Calvin *et al.* (1984)
Morphology	10-100 % (7): minimum % brachyptery at 60-80 % r.h.	Gross (1988)
Locomotory activity	50 vs. 80 %: no effect	Boldt (1974)

1 mm s.d. = mm Hg saturation deficiency (0 mm s.d. = 100 % r.h.).
2 In parentheses: number of relative humidities compared.

4. Photoperiod and light intensity

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Longevity	0 vs. 24 h: increase	Lund (1938)
	0, 12, 24 h: decrease	Klink (1964)
	0.5 vs. 24 h: decrease	Orphanides & Gonzalez (1970)
	10, 14, 24 h: increase	Rounbehler & Ellington (1973)
	12, 14, 16 h: increase	Calvin *et al.* (1984)
Fecundity	0 vs. 24 h: no effect	Lund (1938)
	12 vs. 0, 24 h: higher	Klink (1964)
	0.5 vs. 24 h: at 30 °C decrease, at 20 °C no effect	Orphanides & Gonzalez (1970)
	10, 14, 24 h: increase	Rounbehler & Ellington (1973)
	12, 14, 16 h: no effect	Calvin *et al.* (1984)
Sex ratio	12, 14, 16 h: no effect	Calvin *et al.* (1984)

4. Photoperiod and light intensity, *continued*

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Development rate	distant light vs. darkness: increase at 21 °C, no effect at 16, 27, 32 °C	Stern & Bowen (1963)
	12, 14, 16 h: increase	Calvin *et al.* (1984)

5. Density of wasps relative to host eggs

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Sex ratio	Higher density: increase of % males	Salt (1937*a*), Kochetova (1972), Waage & Lane (1984), Hohmann *et al.* (1988*b*)
Size	Higher density: decrease	Salt (1937*a*)
Morphology	Higher density: increase of % imperfectly developed wasps	Salt (1937*a*)

6. Presence of food for adult wasps

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Longevity	Increase	Lund (1938), Narayanan & Mookherjee (1956), Ashley & Gonzalez (1974), Stinner et al. (1974*b*), Cabello & Vargas (1985*b*), Hohmann *et al.* (1988)
Fecundity	Increase	Lund (1938), Narayanan & Mookherjee (1956), Ashley & Gonzalez (1974), Cabello & Vargas (1985*b*)
	No effect	Neuffer (1987)
Sex ratio	No effect	Cabello & Vargas (1985*b*)

7. Diet of rearing host

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Longevity	Greengram > sorghum = groundnut	Navarajan Paul *et al.* (1975)
	Jowar + yeast > jowar + vitamin B > jowar > jowar + groundnut > jowar + casein	Katiyar (1962)
Fecundity	Greengram > groundnut <>[1] sorghum	Navarajan Paul *et al.* (1975)
	Jowar + yeast > jowar + vitamin B > jowar > jowar + groundnut > jowar + casein	Katiyar (1962)
Sex ratio	Groundnut vs. greengram, sorghum: no effect	Navarajan Paul *et al.* (1975)
	Jowar + yeast > jowar + vitamin B > jowar > jowar + groundnut > jowar + casein	Katiyar (1962)
Development rate	Sorghum > groundnut > greengram	Navarajan Paul *et al.* (1975)
Size	Groundnut > greengram > sorghum	Navarajan Paul *et al.* (1975)

1 Different effects with *T. australicum* (= *T. chilonis*) and *T. japonicum*.

8. Rearing on artificial medium

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Size	No effect	Strand & Vinson (1985)

9. Exposure of wasps to host kairomones

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Field performance	Increase	Gross *et al.* (1975)
Longevity	Increase	Nordlund *et al.* (1976)
Fecundity	Increase	Nordlund *et al.* (1976)

10. Oviposition by wasps prior to release

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Searching efficiency	Increase	Gross *et al.* (1981*b*)

11. Low-temperature storage of host eggs prior to parasitisation

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Fecundity	60 days at 4 °C: no effect	Voegelé *et al.* (1974)
	8 months in liquid nitrogen: no effect	Hu & Xu (1988)
Sex ratio	6-8 months in liquid nitrogen: no effect	Gennadiev (1985)
	8 months in liquid nitrogen: no effect	Hu & Xu (1988)
	21 days in liquid nitrogen: no effect	Morrison (1988)
Duration of oviposition	60 days at 4 °C: no effect	Voegelé *et al.* (1974)

12. Short-term low-temperature storage of parasitised host eggs

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Longevity	Decrease with duration of storage	Venkatraman & Govil (1952), Stinner *et al.* (1974*b*)
Fecundity	Decrease with duration of storage	Venkatraman & Govil (1952)
Sex ratio	Temperatures < 8 °C: increase % males Slight increase % females with duration of storage	Schread & Garman (1934) Venkatraman & Govil (1952)
Morphology	Increasing % wing deformity with increasing duration of storage	Schread & Garman (1934)

13. Long-term low-temperature storage of parasitised host eggs

Aspect of quality of *Trichogramma*	Effect	Reference(s)
Field performance	90-180 days at 3 °C: no effect	Voegelé *et al.* (1986)
Fecundity	75 days at 1.7-4.4 °C: decrease	van Steenburgh (1934)
Sex ratio	40-180 days at 4.4 °C: no effect	Peterson (1931)
Parasitisation capacity	90-180 days at 3 °C: no effect	Voegelé *et al.* (1986)

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Glossary

The following list gives definitions of technical terms as used in this dissertation. These are not necessarily the oldest definitions. A literature reference indicates the source of the given definition or a further discussion of its meaning.

Allelochemical
An infochemical that mediates an interaction between two individuals that belong to different species (Dicke & Sabelis, 1988).

Allomone
An allelochemical that is pertinent to the biology of an organism (organism 1) that, when it contacts an individual of another species (organism 2), evokes in the receiver a behavioural or physiological response that is adaptively favourable to organism 1 but not to organism 2 (Dicke & Sabelis, 1988).

Anemotaxis
A directed reaction of a motile organism towards (positive) or away from (negative) wind or air currents (Lincoln *et al.*, 1982).

Arrestant
A chemical that causes an organism to aggregate in contact with it, the mechanism of aggregation being kinetic or having a kinetic component (Dethier *et al.*, 1960).

Arrestment
Increased residence time of an organism in an area after perception of a stimulus. This term is used with recognition of the fact that it merely describes the end result of a response and does not imply a specific underlying orientation mechanism (Kennedy, 1978). See also *Arrestant.*

Attractant
A chemical that causes an organism to make oriented movements towards its source (Dethier *et al.*, 1960).

Attraction
Oriented movement towards the source of a stimulus. This term is used with recognition of the fact that it merely describes the end result of a response and does not imply a specific underlying orientation mechanism (Kennedy, 1978). See also *Attractant*.

Calling
Generic term to indicate the behaviour of insects whereby a signal is emitted to attract one or more conspecifics. In this context referring to the behaviour of female noctuid moths during release of volatile sex pheromone, characterized by a typical body posture with extruded abdominal tip.

Communication disruption
A pest control technique aiming at disruption of the pest's communication; usually referring to sexual communication (mating disruption). It involves widespread application of one or more components of the pest's sex pheromone, a pheromone mimic, or some known behaviour modifier. The insects are then unable to locate their mates when using their own pheromone system and mating is therefore reduced or eliminated.

Event recording
Technique in behavioural research whereby sequences of observed events and the time at which they occur are recorded. Accomplished by means of an event recorder, which can be a dedicated device or a microcomputer programmed as such.

Geotaxis
A directed reaction of a motile organism towards (positive) or away from (negative) the direction of gravity (Lincoln *et al.*, 1982).

Infochemical
A chemical that, in the natural context, conveys information in an interaction between two individuals, evoking in the receiver a behavioural or physiological response that is adaptive to either one of the interactants or to both (Dicke & Sabelis, 1988).

Kairomone
An allelochemical that is pertinent to the biology of an organism (organism 1) that, when it contacts an individual of another species (organism 2), evokes in the receiver a behavioural or physiological response that is adaptively favourable to organism 2 but not to organism 1 (Dicke & Sabelis, 1988).

Klinokinesis
A change in the rate of random movement of an organism (kinesis) in which the rate of change of direction (frequency of turning movements) varies with the intensity of the stimulus (Lincoln *et al.*, 1982).

Mating disruption
See *Communication disruption.*

Olfactometer
An apparatus used to measure olfactory responses of animals. Usually based on air flow (airflow olfactometer, e.g. T-tube, Y-tube, four-armed olfactometer), sometimes on molecular diffusion (diffusion olfactometer).

Orthokinesis
A change in the rate of random movement of an organism (kinesis) in which the rate of locomotion (linear velocity) varies with the intensity of the stimulus (Lincoln *et al.*, 1982).

Parasitoid
An organism of which the larva feeds within (endoparasitoid) or upon (ectoparasitoid) the living body of another organism eventually causing the death of the host. Examples: egg parasitoid, larval parasitoid, pupal parasitoid.

Pheromone
An infochemical that mediates an interaction between organisms of the same species whereby the benefit is to the origin-related organism ([+,-] pheromone), to the receiver ([-,+] pheromone) or to both ([+,+] pheromone) (Dicke & Sabelis, 1988).

Phototaxis
A directed reaction of a motile organism towards (positive) or away from (negative) a light stimulus (Lincoln *et al.*, 1982).

Primer pheromone
A pheromone that triggers a chain of physiological events in the recipient (Wilson, 1963).

Releaser pheromone
A pheromone that produces a more or less immediate and reversible change in the behaviour of the recipient (Wilson, 1963).

Semiochemical
A chemical involved in the interactions between organisms (Nordlund, 1981).

Sex pheromone
A pheromone involved in mate-location or courtship behaviour. In this context almost exclusively referring to volatile chemicals released by female moths to attract males.

Strain
The cultured offspring of a sample taken from a field population at a certain time and locality (Diehl & Bush, 1984).

Synomone
An allelochemical that is pertinent to the biology of an organism (organism 1) that, when it contacts an individual of another species (organism 2), evokes in the receiver a behavioural or physiological response that is adaptively favourable to both organism 1 and 2 (Dicke & Sabelis, 1988).

Thigmotaxis
A directed response of a motile organism to a continuous contact with a solid surface (Lincoln *et al.*, 1982).

Author Index

Literature references with more than one author have only been indexed on the first author.

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Curriculum Vitae

Lucas Paul Johan Joseph Noldus werd op 24 November 1959 geboren te Roosendaal. Hij behaalde in 1977 het eindexamen V.W.O. B (cum laude) aan het St. Oelbertgymnasium te Oosterhout. In datzelfde jaar begon hij zijn studie Biologie aan de Rijksuniversiteit Leiden. In 1980 werd het kandidaatsexamen afgelegd (cum laude), waarna hij de volgende doctoraalonderzoeken verrichtte:

— Morfologische en biometrische aspecten van de groei van de schedel van de bruinvis, *Phocoena phocoena* (onder begeleiding van Dr. C. Smeenk);

— Chemische stimuli die een rol spelen in het zoekgedrag van de eiparasiet *Trichogramma evanescens* (onder begeleiding van Prof. Dr. J.C. van Lenteren).

Gedurende zijn doctoraaltijd was hij aangesteld als studentassistent bij de vakgroep Diermorfologie (1981-1983). Het doctoraalexamen werd afgelegd in december 1983. Na een drie-maands aanstelling als gastmedewerker bij de vakgroep Populatiebiologie van de Rijksuniversiteit Leiden was hij in 1984 gedurende zes maanden werkzaam op het Department of Biology, Beijing Normal University (Beijing, China), onder supervisie van Prof. Dr. Xu Rumei. Hier verrichtte hij onderzoek naar ruimtelijke verdeling, voortbeweging en voedingsgedrag van de kaswittevlieg, *Trialeurodes vaporariorum*.

Het in dit proefschrift beschreven onderzoek, waarmee in Leiden onder begeleiding van Prof. Dr. J.C. van Lenteren een eerste begin was gemaakt, werd voortgezet terwijl hij gedurende een jaar verbonden was aan het Insect Biology & Population Management Research Laboratory in Tifton (Georgia, Verenigde Staten) in 1985, onder supervisie van Dr. W.J. Lewis. Het grootste deel van het onderzoek werd uitgevoerd op de vakgroep Entomologie van de Landbouwuniversiteit Wageningen, waar hij was aangesteld als wetenschappelijk assistent van februari 1986 tot en met mei 1989.

Curriculum Vitae

Lucas Paul Johan Joseph Noldus was born on 24 November 1959 in Roosendaal. In 1977 he graduated (with honours) from the St. Oelbertgymnasium in Oosterhout. In the same year he started his study Biology at the University of Leiden. The 'kandidaatsexamen' was passed in 1980 (with honours), after which he conducted the following two graduate research projects:

— Morphological and biometrical aspects of the growth of the skull of the harbour porpoise, *Phocoena phocoena* (under supervision of Dr. C. Smeenk);
— Chemical stimuli that play a role in the searching behaviour of the egg parasitoid *Trichogramma evanescens* (under supervision of Prof. Dr. J.C. van Lenteren).

During his graduate studies he was employed as teaching assistant at the Department of Animal Morphology (1981-1983). The 'doctoraalexamen' was passed in December 1983. After a three-month assignment as voluntary researcher at the Department of Population Biology, University of Leiden, he worked in 1984 during six months as a visiting scientist at the Department of Biology, Beijing Normal University (Beijing, China), under supervision of Prof. Dr. Xu Rumei. During this period he conducted research on spatial distribution, movement, and feeding behaviour of the greenhouse whitefly, *Trialeurodes vaporariorum*.

The research described here was initiated in Leiden under supervision of Prof. Dr. J.C. van Lenteren, and continued during a one-year assignment at the Insect Biology & Population Management Research Laboratory in Tifton (Georgia, U.S.A.) in 1985, under supervision of Dr. W.J. Lewis. The bulk of the research was carried out at the Department of Entomology, Wageningen Agricultural University, from February 1986 through May 1989.