Selection of *Trichogramma* for inundative biological control

A study of behavioural variations among strains and species of an egg-parasite genus

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Promotor: dr. J. C. van Lenteren
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in het bijzonder de oecologie der insekten

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Selection of *Trichogramma* for inundative biological control

Proefschrift

ter verkrijging van de graad van

doctor in de landbouwwetenschappen,

op gezag van de rector magnificus,

dr. C. C. Oosterlee,

in het openbaar te verdedigen

op woensdag 11 mei 1988

des namiddags te vier uur in de aula

van de Landbouwuniversiteit te Wageningen

"The behaviour of insect parasites plays a great part in fixing their value as pest controls; and it follows that we must know their behaviour before we can use them efficiently."

G. Salt, 1958

Stellingen

1. Het mislukken van de toepassing van *Trichogramma* voor de bestrijding van insektenplagen is in de meeste gevallen het gevolg van onvoldoende kennis van inter- en intraspecifieke variatie in het zoek- en parasiteringsgedrag van vrouwelijke wespen binnen dit genus.

   F. D. Parker & R. E. Pinnell. Environmental Entomology 3: 935-938 (1974).
   Dit proefschrift.

2. *Trichogramma* vrouwtjes zijn, door beoordeling van de kwaliteit van potentiële gastheren, in staat hun eileggedrag op adaptieve wijze aan te passen aan hun gastheer.

   G. Salt. Proceedings of the Royal Society London (B) 122: 57-75 (1937).
   M. J. van Dijken & J. K. Waage. Entomologia Experimentalis et Applicata 43: 183-192 (1987).
   Dit proefschrift.

3. De hypothese van Klomp *et al.* dat *Trichogramma* vrouwtjes het vermogen tot gastheerdiscriminatie moeten leren door eilegervaring met een ongeparasiteerde gastheer, en dit vervolgens kunnen vergeten, gaat voorbij aan het belang van idee-vorming over wat naïeve wespen feitelijk door een dergelijke ervaring geleerd zouden kunnen hebben.

   H. Klomp, B. J. Teerink & W. C. Ma. Netherlands Journal of Zoology 30: 254-277 (1980).
   J. E. R. Staddon. Adaptive behavior and learning. Cambridge University Press, Cambridge (1983).

4. Het veldonderzoek van Kareiva naar de invloed van habitat opdeling op de stabiliteit van een predator-prooi systeem toont het belang aan van het toetsen van hypothesen over het gedrag van predatoren en parasieten in natuurlijke gemeenschappen van hun prooien of gastheren.

   P. Kareiva. Nature 326: 388-390 (1987).

5. Het primaire doel van insektenbestrijding is niet de bescherming van gewassen, maar het beschermen van landbouwbedrijven tegen een mogelijk faillissement.

   J. H. Perkins. Insects, experts, and the insecticide crisis. Plenum, New York (1982).

6. Bestrijding van de illegale teelt van coca planten in Peru met behulp van de vlinder *Eloria noyesi* zou kunnen leiden tot een entomologische wapenwedloop.

   Butterflies thwart cocaine barons. New Scientist 1598: 27 (4-2-1988).

7. Betiteling van de meest effectieve sluipwesp voor biologische bestrijding van insektenplagen als „Porsche onder de sluipwespen", kan de onterechte indruk wekken dat hier sprake is van een relatief dure methode van bestrijding.

   De Porsche onder de sluipwespen – biologische bestrijding van mineervliegen. Wagenings Universiteitsblad 31: 8 (15-10-1987).

8. De bedrijfsmatige productie van natuurlijke vijanden voor biologische bestrijding kan bevorderd worden door de invoering van „kwekersrecht" voor de geselecteerde en in massakweek gehouden stam van de natuurlijke vijand.

9. Intelligentie blijkt niet alleen uit het verstandelijk vermogen om dingen te doen, maar evenzeer uit het vermogen om dingen te laten.

   R. E. Leaky & R. Lewin. Origins. Dutton, New York (1977).

10. Het feit dat wetenschappelijk onderzoek in toenemende mate gericht is op het oplossen van problemen veroorzaakt door toepassingen van onderzoek, zou tot diepgaande bezinning moeten leiden.

11. Discriminatie van vrouwen op de arbeidsmarkt zal verminderen indien mannen in gelijke mate als vrouwen hun loopbaan aanpassen aan die van hun partner en aan eventuele kinderzorg.

    Vrouwen op de arbeidsmarkt – de aandelen stijgen. Onderzoek NILI-MPW, Wageningen (1987).

12. Ministers zouden net zo gemakkelijk afstand moeten doen van hun functie als van hun dienstauto.

13. Advertenties met de tekst „roken moet mogen" zijn een belediging voor mensen die de rook niet willen zien of kunnen luchten.

14. Bij de pakken neerzitten kan een aangename bezigheid zijn.

Stellingen behorende bij het proefschrift van G. A. Pak: „Selection of *Trichogramma* for inundative biological control".

Wageningen, 11 mei 1988.

Panorama

Wie door dit doek heen stapt verkijkt zich
op de maat van zijn bereik. De wereld is
maar rond en nauw om ons gesteld.
Vanuit een punt gezien een weids gezicht,
met mogelijkheid tot wisseling van zijn;
alleen beweging biedt het juist perspectief:
er komt een eind aan wat geen eind wou zijn.

                    T. van Deel

                    Achter de waterval
                    Gedichten
                    Querido, 1986

CONTENTS

CURRICULUM VITAE

Gerrit Adriaan Pak werd geboren op 23 augustus 1953 in Moerkapelle. Aan het
Christelijk Lyceum te Gouda behaalde hij in 1971 het diploma HBS-B.
Belangstelling voor de levende natuur deed hem kiezen voor een studie
biologie aan de Rijksuniversiteit Leiden. Na het kandidaatsexamen in de
richting Bl-w (1974), studeerde hij verder aan de Landbouwhogeschool te
Wageningen. Hij streefde naar een toegepast wetenschappelijke invulling van
de doctoraal studie door zijn aandacht te richten op de
insektenbestrijding. Het ingenieursdiploma werd behaald in 1978, met als
hoofdvakken entomologie en toxicologie, en een bijvak theoretische
teeltkunde. Aansluitend kon hij zich, met behulp van een beurs van de
"International Telephone and Telegraph Corporation", beschikbaar gesteld door
het "Institute of International Education", specialiseren in de biologische
insektenbestrijding aan de "University of California" te Riverside in de
Verenigde Staten. In 1980 verkreeg hij de titel "Master of Science" in de
entomologie. Het in Riverside verrichte onderzoek aan de minieme
Trichogramma sluipwespjes legde de basis voor het onderzoek waarvan in dit
proefschrift verslag wordt uitgebracht.

    Het onderzoek voor dit proefschrift betreffende de variatie tussen
Trichogramma stammen werd door Gerrit Pak in november 1981 gestart aan de
vakgroep entomologie van de Landbouwuniversiteit te Wageningen.
Aanvankelijk door middel van een tijdelijke arbeidsplaats, en vanaf
december 1982 als wetenschappelijk assistent. Hieraan voorafgaand
orienteerde hij zich gedurende drie maanden in de Sowjet Unie op de
toepassing van Trichogramma in de plaagbestrijding. In november 1984 werd
hij aangesteld als universitair docent bij de vakgroep Entomologie van de
Landbouwuniversiteit. In deze functie verzorgt hij onderwijs in de
toegepaste entomologie en verricht hij onderzoek aan parasiet-
gastheerrelaties ten behoeve van de biologische bestrijding van
plaaginsekten. In 1986 was hij drie maanden werkzaam als gastdocent aan het
"Waite Agricultural Research Institute" van de "University of Adelaide" in
Australië.

PUBLICATIONS

Most chapters of this thesis have been or will be published by different subtitles in a series of papers titled "Behavioural variations among strains of Trichogramma spp.":

Chapter 4:
    Pak G.A. & T.G. van Heiningen, 1985. Behavioural variations among strains of Trichogramma spp.: Adaptability to field-temperature conditions. Entomol. Exp. Appl. 38: 3-13.

Chapter 5:
    Pak G.A., 1986. Behavioural variations among strains of Trichogramma spp.: A review of the literature on host-age selection. J. Appl. Entomol. 101: 55-64.

    Pak G.A., H.C.E.M. Buis, I.C.C. Heck & M.L.G. Hermans, 1986. Behavioural variations among strains of Trichogramma spp.: Host-age selection. Entomol. Exp. Appl. 40: 247-258.

Chapter 6:
    Pak G.A., E.J. de Jong, J.W.M. Kaskens & C.K. Kersten, submitted. Behavioural variations among strains of Trichogramma spp.: Host-species selection.

Chapter 7:
    Pak G.A. & E.J. de Jong, 1987. Behavioural variations among strains of Trichogramma spp.: Host recognition. Neth. J. Zool. (in press).

Chapter 8:
    Pak G.A., A. van Dalen, A. Nieland, N. Kaashoek & N. Hawlitzky, submitted. Behavioural variations among strains of Trichogramma spp.: Host suitability.

Chapter 9:
    Pak G.A., F.A.N. van Alebeek, S.A. Hassan, T.G. van Heiningen & J.C. van Lenteren, submitted. Behavioural variations among strains of Trichogramma spp.: Experimental field releases.

In addition to the preceeding publications, Gerrit Pak has contributed to the research on Trichogramma and biological control by means of several papers related to the present study:

Pak G.A. & E.R. Oatman, 1982. Biology of Trichogramma brevicapillum. Entomol. Exp. Appl. 32: 61-67.

Pak G.A. & E.R. Oatman, 1982. Comparative lifetable, behaviour and competition studies of Trichogramma brevicapillum and T. pretiosum. Entomol. Exp. Appl. 32: 68-79.

Lenteren J.C. van & G.A. Pak, 1984. Can we use Trichogramma spp. to control lepidopteran pests in cabbage? Mitt. Biol. Bundesanst. Land Forstw., Berlin-Dahlem, 218: 119-135.

Schaaf D.A. van der, J.W.M. Kaskens, M. Kole, L.P.J.J. Noldus & G.A. Pak, 1984. Experimental releases of two strains of Trichogramma spp. against lepidopteran pests in a Brussels sprouts field crop in the Netherlands. Med. Fac. Landbouww. Rijksuniv. Gent 49: 803-813.

Jong E.J. de & G.A. Pak, 1984. Factors determining differential host egg recognition of two host species by different Trichogramma spp. Med. Fac. Landbouww. Rijksuniv. Gent 49: 815-825.

Pak G.A. & J.C. van Lenteren, 1984. Selection of a candidate Trichogramma sp. strain for inundative releases against lepidopterous pests of cabbage in the Netherlands. Med. Fac. Landbouww. Rijksuniv. Gent 49: 827-837.

Pak G.A., I. van Halder, R. Lindeboom & J.J.G. Stroet, 1985. Ovarian egg supply, female age and plant spacing as factors influencing searching activity in the egg parasite Trichogramma sp. Med. Fac. Landbouww. Rijksuniv. Gent 50: 369-378.

Heiningen T.G. van, G.A. Pak, S.A. Hassan & J.C. van Lenteren, 1985. Four year's results of experimental releases of Trichogramma egg parasites against lepidopteran pests in cabbage. Med. Fac. Landbouww. Rijksuniv. Gent 50: 379-388.

Alebeek F.A.N. van, G.A. Pak, S.A. Hassan & J.C. van Lenteren, 1986. Experimental releases of Trichogramma spp. against lepidopteran pests in a cabbage field crop in the Netherlands in 1985. Med. Fac. Landbouww. Rijksuniv. Gent 51: 1017-1028.

Pak G.A. & J.C. van Lenteren, 1986. Criteria and methods for the pre-release evaluation of different Trichogramma spp. strains. 2nd International Symposium on Trichogramma and other egg parasites, Guangzhou, China, 10-15 november 1986. Publications de l'INRA, in press.

Schmidt J.M. & G.A. Pak, submitted. The temperature dependent time sense of a parasitoid wasp.

SAMENVATTING

Het onderwerp van dit proefschrift is de selectie van natuurlijke vijanden voor de ontwikkeling van biologische plaagbestrijding. Er is onderzoek verricht naar de vraag of de geschiktheid van natuurlijke vijanden voor bestrijding van plagen voorspeld kan worden door middel van laboratorium proeven. Veld proeven voor dit doel zijn doorgaans kostbaar en vergen veel tijd. De beoordeling van natuurlijke vijanden in het laboratorium zou het vinden van een geschikte vijand daarom kunnen versnellen, of de kans dat een geschikte vijand gevonden wordt kunnen vergroten.

Het onderzoek is uitgevoerd met sluipwespen van het geslacht Trichogramma (Hymenoptera, Trichogrammatidae), waarvan de larven als parasiet leven in de eieren van vlinders en motten (Lepidoptera). Trichogramma's worden in diverse landen gebruikt voor biologische bestrijding van rupsenplagen door middel van inundatieve loslatingen. Hierbij wordt een groot aantal parasieten, indien nodig meer dan 100.000 per ha, in het veld losgelaten. De parasieten worden bedrijfsmatig geproduceerd door middel van massakweek op een vervangende gastheer, die relatief gemakkelijk en goedkoop te kweken is, bijvoorbeeld de meelmot (Ephestia kuehniella). Inundatieve loslatingen worden diverse malen binnen een seizoen herhaald, in tegenstelling tot de zogenaamde inoculatieve methoden van biologische bestrijding. Bij inundatieve biologische bestrijding wordt de natuurlijke vijand gebruikt als een biologisch insekticide.

In Nederland zou Trichogramma toegepast kunnen worden als een alternatief voor de intensieve chemische bestrijding van rupsenplagen in kool, onder andere witte kool en spruitkool. Hoofdstuk 1 van dit proefschrift bevat een inleiding van de drie hoofdbestanddelen van deze studie: biologische bestrijding, Trichogramma en het complex van Lepidoptera soorten op kool. De belangrijkste plaagsoorten zijn de kooluil, Mamestra brassicae, het grote koolwitje, Pieris brassicae, en het kleine koolwitje, Pieris rapae.

Het verschil in geschiktheid van diverse soorten natuurlijke vijanden voor bestrijding van een plaagsoort is afhankelijk van de verschillen in biologische eigenschappen tussen deze vijanden (interspecifieke variatie), zoals het vermogen van een parasiet om zich in het plaaginsekt (de gastheer) te kunnen ontwikkelen. Bij Trichogramma is naast interspecifieke

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variatie, tussen één of meer van de ongeveer honderd soorten, ook variatie
in biologische eigenschappen tussen stammen van eenzelfde soort gevonden
(intraspecifieke variatie). Het benutten van deze verschillen voor het
selecteren van geschikte natuurlijke vijanden voor plaagbestrijding, door
middel van vergelijkend onderzoek van soorten, wordt besproken in
hoofdstuk 2. Onderzoekers selecteren geschikte kandidaten voornamelijk op
grond van intuïtie en ervaring (de "trial-and-error" methode) en hebben tot
nu toe nauwelijks gebruik gemaakt van evaluatie onderzoek gebaseerd op
selectie criteria.

Voor het selecteren van een Trichogramma stam die geschikt is voor de
bestrijding van koolrupsen, werd een collectie van zestig geografische
stammen aangelegd. Een stam is een laboratorium kweek van een op een
bepaalde locatie verzameld monster van een soort. De stammen werden
verkregen via buitenlandse instituten of werden locaal verzameld in het
veld. Methoden gebruikt voor het kweken van parasieten en gastheren, en
methoden van observeren van het parasiteringsgedrag van de sluipwespen zijn
beschreven in hoofdstuk 3.

Diverse onderzoekers hebben in de literatuur gemeld dat Trichogramma
ineffectief wordt als de temperatuur lager wordt dan ca. 15°C, wat in het
veld regelmatig gebeurt, vooral in juni. Van alle stammen in de collectie
werd daarom de parasiteringsactiviteit bij 12°C vastgesteld (hoofdstuk 4).
Voor dit selectie criterium bleek een grote variatie tussen de stammen te
bestaan. De locale stammen vielen op door een lage parasiteringsactiviteit.
De stammen met de grootste activiteit in deze toets werden geselecteerd
voor onderzoek van een volgend criterium, het gastheerselectie gedrag.

Gastheerselectie experimenten hebben tot doel vast te stellen of wespen
een voorkeur hebben voor het parasiteren van bepaalde leeftijden of soorten
van gastheereieren. Een voor toepassing geschikte Trichogramma stam zou
niet selectief moeten zijn, maar alle leeftijden van de verschillende
soorten eieren in het veld moeten accepteren en kunnen doden. Uit de
literatuur blijkt dat Trichogramma over het algemeen jonge eieren beter kan
parasiteren dan oude eieren. Bij directe observaties van individuele
vrouwtjes van drie stammen, van verschillende soorten, in keuze proeven met
Mamestra of Pieris eieren van verschillende leeftijden, bleek er geen
voorkeur voor een bepaalde leeftijd te bestaan (hoofdstuk 5). Proeven met
keuze uit twee gastheersoorten toonden echter aan dat vrouwtjes van de

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meeste van tien onderzochte stammen bij voorkeur <u>Mamestra</u> eieren
parasiteren (hoofdstuk 6).

Gastheerherkenning werd onderzocht om vast te stellen hoe <u>Trichogramma</u>
vrouwtjes <u>Mamestra</u> en <u>Pieris</u> eieren kunnen onderscheiden (hoofdstuk 7).
Naast een aantal gastheerspecifieke, fysische eigenschappen van het ei
(grootte, oppervlakte structuur en kleur) bleek ook een specifieke
chemische factor, aanwezig in een substantie op het eioppervlak, een rol te
spelen in het proces van gastheerherkenning. De voorkeur gevonden bij
<u>Trichogramma</u> stammen voor <u>Mamestra</u> eieren komt overeen met de geschiktheid
van eieren voor ontwikkeling van de parasiet (van ei tot wesp). Voor alle
stammen blijkt <u>Mamestra</u> een geschikte gastheer te zijn, terwijl de
geschiktheid van <u>Pieris</u> eieren verschilt tussen stammen (hoofdstuk 8). Dit
wordt waarschijnlijk veroorzaakt door verschillen in chemische en/of
fysische eigenschappen tussen de twee soorten eieren, in combinatie met de
invloed van variabele inwendige factoren (zoals het aantal eieren van de
parasiet dat in het gastheerei gelegd is) en uitwendige factoren
(bijvoorbeeld luchtvochtigheid). <u>Mamestra</u> eieren zijn beter bestand tegen
uitdroging dan <u>Pieris</u> eieren, mogelijk door een meer compacte structuur van
de eischaal.

Van 1982 tot 1985 werd jaarlijks de effectiviteit van twee stammen
beproefd in verschillende proefvakken in een spruitkool veld van ca.
0.5 ha, waarin zich natuurlijke plaagpopulaties bevonden (hoofdstuk 9). De
losgelaten stammen werden geselecteerd op grond van de resultaten van
laboratorium experimenten. Het hoogste percentage parasitisme van <u>Mamestra</u>
eieren werd verkregen met stam 57. Deze stam had van alle stammen de
hoogste activiteit bij lage temperatuur. Voor stam 57 werden echter geen
geparasiteerde eieren van <u>Pieris</u> gevonden. Parasitisme van <u>Pieris</u> eieren
kwam hoofdzakelijk voor in veldjes waarin stam 11 was losgelaten. Vrouwtjes
van deze stam vertoonden in het laboratorium geen gastheervoorkeur. Geen
van de onderzochte stammen was echter zo effectief dat de plaagdichtheid
voldoende gereduceerd werd.

Uit dit onderzoek kan worden geconcludeerd dat het mogelijk is
natuurlijke vijanden van plaaginsekten door middel van eenvoudige
laboratorium experimenten te selecteren. De parasiteringsactiviteit bij een
extreme (beperkende) temperatuur en de gastheerspecificiteit blijken
bruikbare criteria voor de beoordeling van kandidaten op hun geschiktheid

voor plaagbestrijding te zijn. Een effectieve Trichogramma stam voor
toepassing in de koolteelt is echter nog niet beschikbaar gekomen. De
bruikbaarheid van aanvullende selectie criteria voor de selectie van
Trichogramma en gebieden voor verder onderzoek worden besproken in
hoofdstuk 10. Wetenschappelijk onderzoek bij de beoordeling van natuurlijke
vijanden zal het succes van biologische plaagbestrijding kunnen vergroten.

OUTLINE OF THE THESIS

This thesis presents a study of the potential for biological control
of lepidopterous pests on cabbage crops in the Netherlands, by means of
inundative releases of the egg parasite Trichogramma (Hymenoptera,
Trichogrammatidae). The objective of this study is to investigate the
usefulness of a predictive approach to the development of a biological
control program. Chapter 1 provides an introduction to the three basic
elements of this study: the principles of biological control, the use of
Trichogramma as a control agent and the host species occurring on cabbage
crops.

The main theme of this study is the selection, by means of laboratory
investigations and experimental field releases, of candidate strains of the
parasite for practical application. This topic is discussed in chapter 2.
Selection experiments were conducted using a collection of sixty
geographical strains maintained in the laboratory. The methodology of
culturing both parasites and hosts, and of observing female-parasite
behaviour are described in chapter 3. Results of experiments on possible
selection criteria involving various strains are presented in the
subsequent chapters (4-9).

Adaptability of parasitization activity to field-temperature conditions
was investigated for all strains (chapter 4). Strains with a high
performance at low temperature were used for studies on differences in
host-selection behaviour between strains, i.e. host-age selection
(chapter 5) and host-species selection (chapter 6). Studies on host
recognition (chapter 7) and host suitability (chapter 8) were conducted to
determine how and why female wasps distinguish between host species.

Candidate strains selected on the basis of the laboratory research were
released in Brussels sprouts fields with natural pest populations. During
four seasons (1982-85) the performance of two strains in parasitism of the
major host species was compared, in order to test the validity of the pre-
introductory selection criteria (chapter 9). Finally, a comprehensive
discussion of the results of this study is provided in chapter 10.

Chapter 1. GENERAL INTRODUCTION

BIOLOGICAL CONTROL

Biological pest control is, by one of its earliest definitions, the use of natural enemies to control insect pests (Smith 1919). Natural enemies, including predators, parasites and microorganisms, may play a major role in the natural control of insect populations, i.e. the regulation of population density within certain upper and lower limits over time (Huffaker et al. 1971). According to DeBach & Huffaker (1971), the use of biological control is essentially the antithesis of chemical pest control. The use of pesticides may create so-called secondary or upset pests, due to the elimination of natural enemies providing natural control (DeBach 1974).

To date, relatively few pests are controlled by deliberate introductions of natural enemies (e.g. Laing & Hamai 1976, Clausen 1978, Beirne 1985). Contrary to the difference in scale of application between the two methods of pest control, the development and application of biological control appears to be more successful and more profitable than for chemical control (Van Lenteren 1986a). Accounting for environmental side-effects of pesticides might put chemical control at a further disadvantage.

The history of biological control goes back as far as ancient times, when in China nests of the predatory ant Oecophylla smaragdina were placed in citrus trees to control leaf-feeding insects (Doutt 1964). Insect parasitism may have been correctly understood for the first time in 1700 by Van Leeuwenhoek (1702). Successful importation of an exotic natural enemy to control an insect pest first occurred a century ago (1888-89) in California, where the cottony cushion scale (Icerya purchasi) was threatening the new citrus industry (Koebele 1890). The successful introduction of the vedalia beetle (Rodolia cardinalis) from Australia established the biological control method "like a shot heard around the world" (DeBach 1974).

Smith (1919) distinguished between importation of exotic natural enemies (to control exotic pests rather than indigenous pests) and the conservation of indigenous natural enemies (to control both exotic and indigenous pests) as methods of biological control. Importation and

establishment of natural enemies gradually became known as classical biological control, and is commonly defined as the regulation of pest populations at a lower level than would otherwise occur (Caltagirone 1981). Measures to increase the numbers of natural enemies, for example by mass production and periodic introductions or by genetic improvement, have been referred to as biological control by augmentation (DeBach 1974).

DeBach & Hagen (1964) distinguished between small number, inoculative introductions of natural enemies for long-term control, and large scale, inundative introductions for short-term pest management. For biological control in greenhouses, a third method has emerged: seasonal inoculative biological control, in which aspects of both other methods are combined (Van Lenteren 1986a, Van Lenteren & Woets 1988). In contrast with the latter method, inoculative biological control is most feasible in "permanent" crops (orchards, forests), while inundative releases are more applicable to annual crops (Ehler & Miller 1978).

TRICHOGRAMMA

Inundative biological control aims at directly increasing mortality in the pest population, whereby the released natural enemy is used as a biological insecticide (Stinner 1977). The practice of inundative release therefore centers on its cost/benefit ratio as compared to alternatives, especially pesticides. Species of the hymenopterous genus Trichogramma (Westwood 1879) have been used more than any other natural enemy for inundative biological control (DeBach 1974, Stinner 1977). A female wasp is shown in Figure 1.1. Trichogramma has a wide range of hosts, especially among the Lepidoptera (Nagarkatti & Nagaraja 1977). Suitable factitious hosts include the eggs of lepidopterous storage pests of grains, making mass production economically feasible (Ables & Ridgway 1977, Starler & Ridgway 1977).

Experiments with Trichogramma started in the beginning of this century in the USA and the USSR. Development of a method for mass production of Trichogramma on eggs of the Angoumois grain moth, Sitotroga cerealella (Flanders 1929, 1930b), gave rise to a worldwide use of the parasite as a control agent. In a paper with the meaningful title "Is Trichogramma becoming a fad?", Smith & Flanders (1931) subsequently expressed their concern about the usefulness of these applications, on the grounds of a
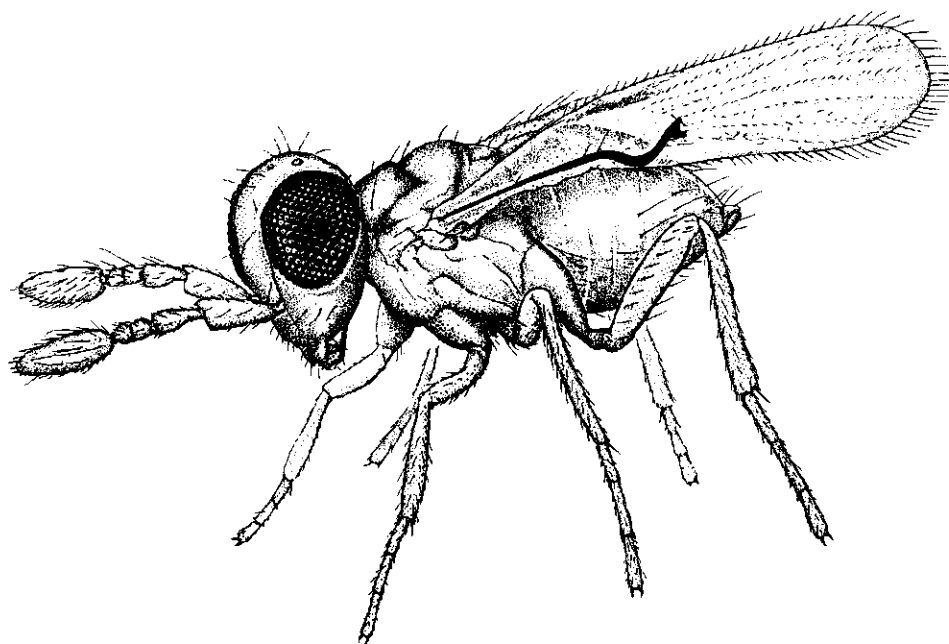
Figure 1.1. A female Trichogramma wasp (drawing by P.J. Kostense).

lack of convincing evidence for practical results. Due to this, and the advent of organic chemical pesticides, interest in Trichogramma faded in western countries (Ridgway et al. 1977). Research on the use of Trichogramma continued in the USSR (Beglyarov & Smetnik 1977) and China (Li 1982), probably due to a lack of pesticides, rather than biological or economical effectiveness of Trichogramma release (Van Lenteren 1987). In these two countries application is presently carried out on several millions of hectares (e.g. Voronin & Grinberg 1981, Li 1986).

Increasing problems with the use of pesticides has stimulated renewed interest in biological control, whether as a method by itself or as part of an integrated pest-control system (e.g. Coppel & Mertins 1977, Franz 1986). For Trichogramma, this renewed interest has led to commercially successful programs in Europe to control the corn borer, Ostrinia nubilalis, by inundative releases of T. maidis (Hassan 1981b, Voegelé 1981, Bigler 1986). Prospective application of Trichogramma against numerous other

lepidopterous pests throughout the world has been reviewed by Stinner (1977). Successful applications are reported for some crops, while results appear to be inconsistent for others. For example, extensive research into the control of Heliothis zea in cotton by releases of T. pretiosum did not provide sufficient results to warrant general adoption of this method in the USA (King et al. 1985, 1986). Effective control of Lepidoptera on cabbage by releases of T. evanescens has been reported for the species Mamestra brassicae (Shchepetilnikova 1974, Hassan & Rost 1985), Pieris rapae (Parker et al. 1971, Parker & Pinnell 1972) and Trichoplusia ni (Zilberg 1972).

LEPIDOPTEROUS PESTS ON CABBAGE

Brassica oleracea (Cruciferae) is cultivated in the Netherlands as a field crop of several varieties, such as Brussels sprouts, white cabbage, red cabbage, and cauliflower. Brussels sprouts make up about 50% of the total area of ca. 15000 ha. Brassica crops are the major outdoor vegetable with an annual wholesale value of Fl. 150 million (De Jonge 1981).

All cabbage varieties are generally infested by a complex of pest species (Figure 1.2) comprising aphids, caterpillars, flea beetles, gall midges and root flies (Theunissen & Freriks 1983). Growers rely on intensive chemical control to protect their crops from insect damage (Minks 1984, Kole & Noldus 1985). Some five to ten broad-spectrum insecticide sprays are usually applied per season. To reduce the use of pesticides, research toward the development of a supervised control system was carried out (Theunissen 1984, Theunissen & Den Ouden 1983, Wit 1982). Action thresholds for the application of insecticides have been determined for aphids and caterpillars on Brussels sprouts, white cabbage and red cabbage (Theunissen & Den Ouden 1985).

Research on non-chemical methods of pest control in cabbage has especially focused on the complex of lepidopterous species, and includes plant resistance (De Ponti 1984), intercropping (Theunissen & Den Ouden 1980), oviposition deterrence (Klijnstra 1985, Klijnstra & Schoonhoven 1987) and inundative biological control (Glas et al. 1981, Van Lenteren et al. 1982). Several lepidopterous species which may occur in cabbage crops are briefly described below. Eggs of these species differ in shape
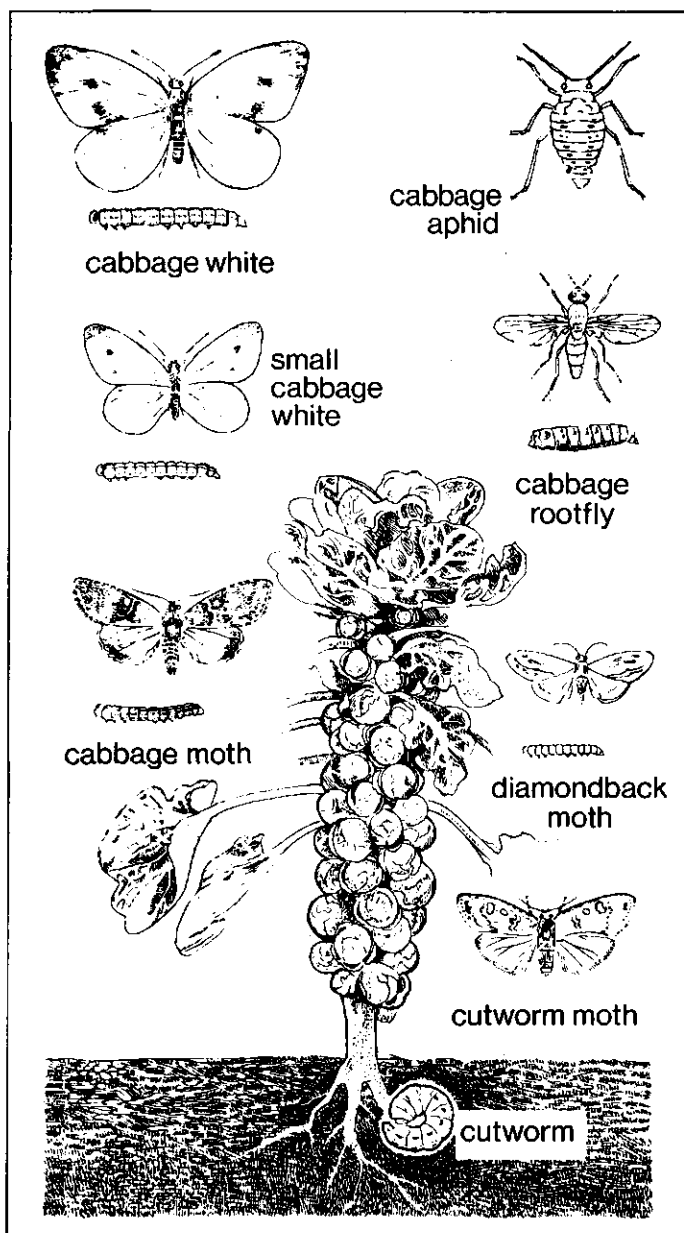
Figure 1.2. Several insect species of the complex of pest species occurring on cabbage plants in the Netherlands. Drawing by W.C.Th. Middelplaats, reproduced with permission from Anonymus (1980).

and/or size, and some are shown in Figure 1.3. Biological information was obtained from various sources, e.g. Bonnemaison 1965, Hoffmann & Schmutterer 1983, Theunissen & Freriks 1983, Hill 1987.

Agrotis spp., cutworms (Noctuidae)

Cutworms, for example those of the turnip moth, A. segetum, are polyphagous caterpillars which feed at night and spend the day hiding in the leaf litter or in the soil. They may be serious pests throughout the world, because the larvae cut through the stems of seedling plants. In larger plants the stems are hollowed out, which may cause wilting. One generation usually occurs in temperate regions. The white, globular eggs are laid in irregular clusters near the base of plants or on the soil. The first larval instars feed on the plant foliage and later descend to the soil, becoming cutworms. The cutworms have a grey-brown, greasy appearance, are stout-bodied and measure 40 mm when full-grown. They overwinter in the soil and pupate in the spring.

Autographa gamma, the silver-y-moth (Noctuidae)

A. gamma is a widespread pest in Europe, Asia and North America. Serious damage occurs only occasionally. A. gamma eggs are globular, ribbed and white and usually laid singly. The larvae are semi-loopers and quite polyphagous leaf eaters. They are green and measure 25 mm when full-grown. Pupation takes place on the plant, the pupa is enclosed inside a silken cocoon. In Europe this species usually is bivoltine.

Mamestra brassicae, the cabbage moth (Noctuidae)

M. brassicae is a serious pest on many crops in Europe and temperate Asia. Its eggs are similar to those of A. gamma, but are laid in clusters on the underside of leaves. Young larvae darken to brown or black and measure 45 mm. On cabbage they tunnel to the heart of the plant or into sprouts. Pupation takes place in the soil. Pupae overwinter or give rise to a second generation at the end of the summer.

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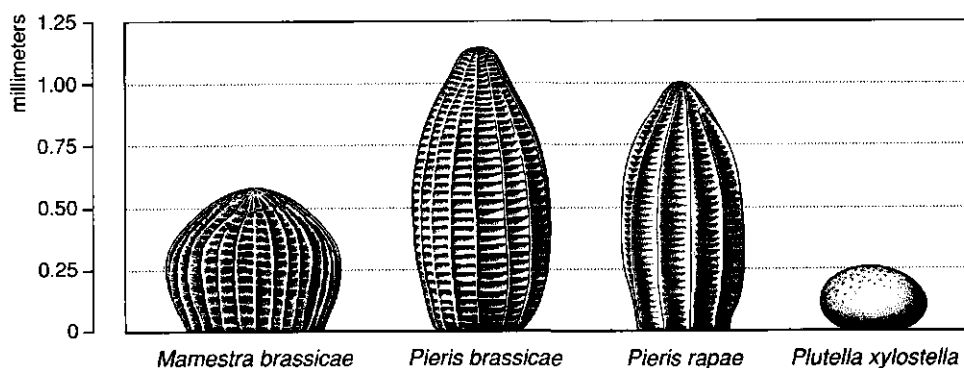
Figure 1.3. Eggs of four lepidopterous species occurring on cabbage, presenting different shapes and sizes of host for the egg parasite Trichogramma (drawing by P.J. Kostense).

Pieris brassicae, the large white butterfly (Pieridae)

P. brassicae is a serious pest of cruciferous crops in Europe, North Africa and South-west Asia. It is a bivoltine migrant species which varies in abundance between years. The eggs are bright yellow, spindle-shaped and ribbed and laid in clusters on the underside of leaves. The larvae feed gregariously, keeping very close together when young. Body coloration is yellowish-green with large black spots and stout bristles and a dorsal yellow line. An infested plant usually becomes defoliated. Full-grown larvae (40 mm) leave plants to pupate on an elevated structure.

Pieris rapae, the small white butterfly (Pieridae)

P. rapae is a widespread and very serious pest on cruciferous crops in the northern hemisphere and Australia. Its common name in North America is the imported cabbageworm. Its eggs are similar to those of P. brassicae, but smaller and pale yellow instead of bright yellow. They are laid singly on the underside of leaves, and are rather evenly spread over the crop area. Larvae are green with a yellow dorsal line and have many small setae. Full-grown they are 25 mm long. Damage due to feeding in the heart of the plant and consequent faecal contamination is more important than leaf-feeding

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damage. Larvae pupate on the plant. Two generations are usual in temperate regions; under warm conditions breeding may be continuous.

Evergestis forficalis, the garden pebble moth (Pyralidae)

E. forficalis may cause damage to cruciferous crops in Europe and Japan. Closely related species of the same genus are pestiferous in other parts of the world. Clusters of overlapping, flat eggs are laid on the underside of leaves. Larvae are pale green with a dark dorsal stripe and yellowish lateral stripes. Their full-grown size is 20 mm. Pupation takes place inside a silken cocoon, just under the surface of the soil. Evergestis is mono- or bivoltine.

Plutella xylostella, the diamondback moth (Yponomeutidae)

P. xylostella is a widespread cosmopolitan pest of cruciferous crops, especially serious in the warmer parts of the world. Tiny yellowish eggs are usually laid singly on the upper surface of the leaves. Larvae are pale green and measure about 10 mm when full-grown. They pupate in a gauzy cocoon attached to the plant. Damage consists of small pits in the foliage and may become economic if several larvae occur per leaf. In the tropics P. xylostella has overlapping generations year-round.

Chapter 2. SELECTION OF CANDIDATE NATURAL ENEMIES

PRE-INTRODUCTORY RESEARCH

The aim of the present study is to test whether the development of an
inundative biological control program can be directed by predictions on the
performance of candidate natural enemies deriving from pre-introductory
experiments in the laboratory. The general course in the development of a
biological control program has been described by several authors (e.g.
Zwölfer et al. 1976, Pschorn-Walcher 1977). The major phases are
(1) collection of information on pest and natural enemies, (2) exploration
for natural enemies, (3) selection of candidates, (4) mass production and
release and (5) evaluation of results.

     The selection of candidate natural enemies, which is generally
considered the most critical phase for the success of a biological control
program, is an issue surrounded by conflicting ideas about the usefulness
of pre-introductory research (Van Lenteren 1980, Ehler 1982). This "art
versus science" conflict (Harris 1973) seems to be one between theoretical
ecologists and practitioners of biological control (Way 1973, Van Lenteren
1980). In extremo, the theoreticians believe that it is possible to
determine the "best" natural enemy by pre-introductory studies (e.g.
Beddington et al. 1978, Waage & Hassell 1982, Miller 1983), whereas the
practitioners advocate a minimum of such studies and prefer to test each
prospective candidate directly in the field by a trial-and-error method
(Legner 1969, DeBach & Huffaker 1971). Nevertheless, most practitioners
express ideas about attributes that an effective natural enemy should
possess, although they rarely seem to use these as a check list of
selection criteria (Van Lenteren 1980, 1983).

CRITERIA FOR SELECTION

Several researchers have provided lists of criteria for the evaluation of
natural enemies (e.g. Flanders 1947, Doutt & DeBach 1964, Coppel & Mertins
1977, Huffaker et al. 1977, Pschorn-Walcher 1977, Waage & Hassell 1982,
Rosen & Huffaker 1983). In general, criteria are deduced from empirical
records of successful natural enemies by means of a combination of

intuition and theoretical considerations. Main categories of criteria are searching capacity, specificity, power of increase, adaptability, synchronization and feasibility of culture.

Most criteria have emerged from experience with inoculative biological control programs, where the aim is long-term control. In contrast, seasonal inoculative and inundative biological control aim at short-term or immediate control. As a consequence, some criteria may be relaxed or become unimportant (e.g. synchronization), whereas others become more important (e.g. culture method), because the applier can manipulate time and frequency of the releases and the number of released enemies (Hoy 1976, Rabb et al. 1976, Van Lenteren et al. 1982). Characteristics related to the ability of rapid host-finding and -attack by the enemy may therefore be of major importance (Stinner 1977, Waage & Hassell 1982, Barclay et al. 1985).

A comprehensive list of nine selection criteria, compiled from the literature (after Van Lenteren 1986a,b), is presented in Table 2.1. The relative importance of each criterion for the three methods of biological control is indicated. Major criteria for the inundative method are climatic adaptation, the absence of negative traits (e.g. hyperparasitism or competition) and a good culture method. The importance of density responsiveness is a matter of controversy between theoretical ecologists (Van Lenteren 1986a). The debate centers around explanations of how an effective natural enemy maintains a low pest density, for example by regulation of a pest-equilibrium (e.g. Waage & Hassell 1982, Murdoch et al. 1985). Inundative control requires the killing of hosts soon after release of the natural enemy, but not necessarily reproduction of the latter. From this point of view density responsiveness is unimportant for inundative control. Alternatively, density responsiveness can be important if it is related to, for example, the host-finding ability of the natural enemy.

The order of criteria in Table 2.1 does not suggest a ranking of steps to conduct an evaluation program. A procedure for selecting natural enemies for the three methods of biological control was proposed by Van Lenteren (1986a). Unacceptable negative aspects render a natural enemy unsuitable for any method. If negative aspects do not play a role, a natural enemy may be considered for inundative releases if it attacks the pest insect and is able to kill it under the applicable field conditions. The next important criterion then becomes the ability to culture the natural enemy.

Table 2.1. Criteria for pre-introductory evaluation of natural enemies for different methods of biological control.

Selection criterion	Biological control method		
	Inoculative	Seasonal inoculative	Inundative
1. Seasonal synchronization with host	+	−	−
2. Internal synchronization with host	+	+	−
3. Climatic adaptation	+	+	+
4. Negative traits	+	+	+
5. Attack on various host plants	+	−	−
6. Culture method	−	+	+
7. Host specifity	+	−	−
8. Reproductive potential	+	+	−
9. Density responsiveness	+	+	±

+ = important; − = not important

SELECTION OF TRICHOGRAMMA

The feasibility of mass production on factitious hosts has promoted extensive use of the egg parasite Trichogramma for inundative biological control programs. In production systems the hosts used are generally grain-feeding Lepidoptera such as the rice meal moth, Corcyra cephalonica (Brenière 1965b), the Mediterranean flour moth, Ephestia kuehniella (Daumal et al. 1975), and the Angoumois grain moth, Sitotroga cerealella (Morrison & King 1977, Hassan 1981a). In addition to efficient mass production, application systems have been developed for manual delivery in small fields of 1-2 ha (Bigler 1986, Bigler & Brunetti 1986) and aerial delivery in fields larger than 10 ha (Morrison et al. 1978, Bouse & Morrison 1985).

Negative effects of Trichogramma inundations have not been considered important in the past, but will be discussed in chapter 10. Few criteria presently seem to be relevant to the selection of a suitable Trichogramma

for field release. This may be the reason that Trichogramma workers
commonly use trial-and-error methods to select a candidate strain. The
prospective Trichogramma strain either is collected locally, on the grounds
of expecting an optimal adaptation to pest and environment in local
populations (e.g. Oatman & Platner 1971, 1978, Oatman et al. 1983, Ridgway
et al. 1981, Sh.M. Grinberg pers. comm.), or is imported from a colleague
who uses it successfully against the same pest species (e.g. Parker 1970,
Hassan 1981b, Voegelé 1981, Bigler 1983).

Selecting Trichogramma by means of trial-and-error releases in the
field has produced good results, but not consistently (Stinner 1977).
Ridgway et al. (1977) and Ridgway & Morrison (1985) identified selection of
effective parasite species or strains as one of the necessities to improve
the success record for Trichogramma application.

INTER- AND INTRASPECIFIC VARIATION

In pre-introductory selection programs of candidate natural enemies
researchers make use, among others, of the fact that pest species usually
have a different complex of natural enemies for different parts of their
natural range (Messenger et al. 1976a). Biological differences among species
or populations of natural enemies, whether morphological, physiological,
ecological or behavioural, are the result of geographical variations in
environmental and biological interactions and reflect adaptations in
populations to the local environment (Messenger & Van den Bosch 1971).
Little is known, however, about qualitative and quantitative genetic
differences between conspecific populations of natural enemies which differ
in biological traits (Caltagirone 1985, Unruh et al. 1986).

In the past, reseachers have shown a certain awareness of the
importance of genetic variation for the success of (inoculative)
introductions of natural enemies (e.g. Wilson 1965, Remington 1968,
MacKauer 1976, Hoy 1985). A prolific use of different terms in the
literature to characterize different populations of conspecific natural
enemies, including form, race, ecotype, biotype, strain and polymorphs,
bears witness of a similar awareness among practitioners of biological
control (Messenger & Van den Bosch 1971, Diehl & Bush 1984).

Introduction of differently adapted strains or species may improve the

success of a biological control program, as shown in California for the aphelinid genus _Aphytis_ controlling California red scale, _Aonidiella aurantii_, and olive scale, _Parlatoria oleae_ (DeBach et al. 1971, Rosen & DeBach 1979). Messenger & Van den Bosch (1971) reviewed several examples of limited adaptiveness in geographical strains of natural enemy species, such as climatic tolerance, host specificity and evasion of host-immunity response, which reduced their effectiveness as control agents. Nevertheless, they concluded that this vast potential resource of conspecific strains of natural enemies with different ecological and behavioural adaptabilities has hardly been tapped to improve biological control.

In the present study use is made of inter- and intraspecific differences among strains of _Trichogramma_. In fact, one of the earliest examples of differences between strains of a natural enemy concerns the nearctic species _T. minutum_ (Messenger & Van den Bosch 1971). In the past, only a few species of _Trichogramma_ were recognized, due to taxonomic difficulties (Nagarkatti & Nagaraja 1977). Differences in structure of male genitalia were used to describe new species by Nagaraja & Nagarkatti (1969) and gave rise to extensive revision of the genus (Voegelé & Pintureau 1982). Therefore, it is difficult to relate previous accounts of biological differences between ecotypes of _Trichogramma_ (Kot 1979), or strains (Diehl & Bush 1984), with their present taxonomic status. Inter- and/or intra-specific differences have been reported among _Trichogramma_ for various biological characteristics:
(1) Adaptation to plant structure or habitat (Flanders 1937, Flanders & Quednau 1960, Walter 1983a, Thorpe 1985);
(2) Adaptation to climatic conditions (Quednau 1957, Pintureau et al. 1980, Pak & Oatman 1982b, Lopez et al. 1982);
(3) Fecundity (Flanders 1935, Russo & Voegelé 1982, Walter 1983b);
(4) Longevity (Brenière 1965c, Russo & Voegelé 1982, Harrison et al. 1985);
(5) Intrinsic rate of increase (Orphanides & Gonzales 1971, Pak & Oatman 1982b, Smith & Hubbes 1986a);
(6) Host suitability (Salt 1938, Parker & Pinell 1974);
(7) Walking speed (Biever 1972, Boldt 1974);
(8) Parasitization rate (Boldt et al. 1973, Shcheptilnikova 1974, Bournier 1982);

(9) Attraction to hosts (Schieferdecker 1965, 1968, Ferreira et al. 1979);

(10)Response to searching stimulants (Jones et al. 1973, Gueldner et al. 1984).

RESEARCH PROGRAM

In the present study the usefulness of pre-introductory selection of candidate strains of Trichogramma is evaluated for an inundative release program against lepidopterous pests on cabbage (chapter 1). Research on the feasibility of this program in the Netherlands began with a trial-and-error type of approach to the selection of suitable candidate parasites (Glas et al. 1981). Two Trichogramma species, T. evanescens and T. cacoeciae, were obtained from S.A. Hassan (Darmstadt, FRG) and released in field plots of Brussels sprouts. At the same time, host-selection experiments were conducted in the laboratory.

In order to conduct a more extensive pre-introductory selection program, a collection of sixty Trichogramma strains was maintained in the laboratory (chapter 3). These strains were obtained in 1982 from laboratory cultures at various foreign institutes, or collected from local fields. A strain refers to the cultured offspring of a sample taken from a field population at a certain time and locality (Diehl & Bush 1984). Each strain was given a number (1-60). Table 2.2 lists the species name for each strain, the host species or family it was collected from and the institute or country of origin. Most strains are European and collected from Ostrinia nubilalis or Mamestra brassicae.

Strains were identified on morphological characters, especially male genital structure (Pointel 1977), according to the classification system of Voegelé & Pintureau (1982). Consequently, T. evanescens and T. maidis are considered distinct species (Pintureau & Voegelé 1980). Apparently there is no general agreement about the separate status of these two species (Pintureau & Babault 1980, 1981, Pintureau et al. 1982), so that some Trichogramma workers only use the name T. evanescens (S.A. Hassan, A.P. Sorokina pers. comm.). During the course of the present research, T. brassicae (Voegelé 1982) was renamed T. buesi (Voegelé 1985).

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Table 2.2. Species, host and country of origin for the collection of geographical laboratory strains of Trichogramma maintained since 1982 at the Department of Entomology, Agricultural University, Wageningen, the Netherlands.

Strain no.		Species	Host	Origin
1	(A 138)	T. evanescens	Pieris rapae	France
2	(A 256)	T.*lutea	Heliothis armigera	South Africa
3	(A 16)	T. maidis	Ostrinia nubilalis	Moldavia, USSR
4	(A 81)	T. brassicae	Mamestra brassicae	France
5	(A 108)	T. embryophagum	Laspeyresia pomonella	Ukraine, USSR
6	(A 45)	T. embryophagum	Pandemis sp.	France
7	(A 163)	T. maidis	Mamestra brassicae	Moldavia, USSR
8	(D -)	T. maidis	Ostrinia nubilalis	Moldavia, USSR
9	(D -)	T. embryophagum		Germany, GDR
10	(D H80)	T. maidis	Mamestra brassicae	Holland
11	(D H81)	T. maidis	Mamestra brassicae	Holland
12	(Z OL30)	T. maidis	Ostrinia nubilalis	Moldavia, USSR
13	(Z HMO)	T. maidis	Mamestra brassicae	Hungary
14	(Z RUE)	T. dendrolimi		Romania
15	(A 254)	T. schuberti	Ostrinia nubilalis	France
16	(A 21)	T. pintoi		USSR
17	(A 258)	T. rhenana	Ostrinia nubilalis	France
18	(A 261)	T. ostriniae	Ostrinia nubilalis	China
19	(A 2)	T. oleae	Glyphodes unionalis	Yougoslavia
20		T. exiguum	Heliothis zea	Peru
21	(A 68)	T. nagarkatti	Heliothis zea	Mexico
22		T. dendrolimi		Romania
23	(UCR)	T. pretiosum	Heliothis zea	California, USA
24	(UCR)	T. exiguum	Heliothis zea	Alabama, USA
25	(UCR)	T. platneri	Amorbia essigana	California, USA
26	(UCR)	T.sp.(group chilonus)	Noctuidae	USA
27	(UCR)	T. minutum	Manduca sp.	California, USA
28	(IpD)	T. embryophagum	Zeiraphera diniana	Poland
30	(IpD)	T. dendrolimi	Pieris brassicae	Czechoslovakia
31	(IpD)	T. embryophagum	Zeiraphera diniana	Czechoslovakia
32		T. embryophagum	Leucoma salicis	Holland
33		T. maidis	Mamestra brassicae	Ukraine, USSR
34	(K 82)	T. maidis	Pieris brassicae	Moldavia, USSR
35	(K 163)	T. maidis	Mamestra brassicae	Moldavia, USSR
36		T. evanescens	Mamestra brassicae	Portugal
37	(K 168)	T. maidis	Mamestra brassicae	Moldavia, USSR
38	(K 177,2)	T. maidis	Mamestra brassicae	Ukraine, USSR
39	(K 178,3)	T. maidis	Mamestra brassicae	Ukraine, USSR
40	(K 179,1)	T. maidis	Mamestra brassicae	Ukraine, USSR
41	(K 140)	T. maidis	Ostrinia nubilalis	Moldavia, USSR
42	(K 142)	T. maidis	Ostrinia nubilalis	Moldavia, USSR
43	(K p.ach)	T. brassicae		Poland
44	(K 191,1)	T. embryophagum	Mamestra brassicae	Ukraine, USSR
45	(K 3b)	T. semblidis		Ukraine, USSR

Table 2.2. (continued)

Strain no.		Species	Host	Origin
46	(K 188b1)	T. pintoi	Cassida nebulosa	Moldavia, USSR
47	(K 212a,28)	T.sp.(near sibiricum)		Moldavia, USSR
48	(K 180)	T. semblidis	Phytometra gamma	Germany, GDR
49	(K 194,2)	T. evanescens	Pieris brassicae	Ukraine, USSR
50	(K prin)	T. principium		USSR
51	(USDA)	T. exiguum		Missouri, USA
52		T. maidis	Mamestra brassicae	Holland
53		T. schuberti	Mamestra brassicae	Holland
54		T. maidis	Pieris rapae	Holland
55		T. maidis	Mamestra brassicae	Holland
56	(USDA)	T. evanescens	Pieris rapae	France
57		T. evanescens	Chilo sp.	Egypt
58	(UCR)	T. brevicapillum	Vanessa sp.	California, USA
59		T. dendrolimi	Archips rosana	Holland
60	(A -)	T. maidis (vestigial)	Ostrinia nubilalis	France

* = Trichogrammatoidea
Strain number at laboratory of origin in parentheses; A = Antibes, France, INRA
Station de Zoologie et de Lutte Biologique; D = Darmstadt, Fed. Rep. Germany,
Table 2.2 (continued)
Institut für Biologische Schädlingsbekämpfung; Z = Zürich, Switzerland, Station
Fédérale de Recherches Agronomique; UCR = Riverside, USA, University of
California, Division of Biological Control; IpD = Ivanka pri Dunaji,
Czechosloviakia, Institute of Experimental Phytopathology and Entomology; K =
Kishiniev, Moldavian Republic, USSR, Allunion Institute of Biological Methods of
Plant Protection; USDA = United States Dept. of Agriculture, Beneficial Insect
Introduction Laboratory, Beltsville, USA.

Chapter 3. GENERAL MATERIALS AND METHODS

PARASITE CULTURES

Each Trichogramma strain (Table 2.2) was maintained on eggs of the
Mediterranean flour moth, Ephestia kuehniella (Pyralidae). Strains were
reared in culture tubes (16x1.8 cm) plugged with cotton. The tubes were
kept in a climatic cabinet at ca. 15°C, 50% relative humidity (rh) and
18L:6D h photoperiod during pupal development of the parasites. To feed the
wasps, a droplet of honey was placed in the tube prior to, or upon their
emergence. Emerged parasites were transferred to a higher temperature, ca.
25°C, 50% rh and 18L:6D h photoperiod in another climate cabinet. After 1-2
days (d) about 250 wasps were provided with ca. 1000 fresh host eggs on an
"egg card". Egg cards were prepared by sprinkling host eggs on a wet piece
of paper index-card (ca. 13x1 cm). After the card had dried (to the air or
with the aid of a blow drier) the eggs would adhere to the paper.

Wasps were allowed to parasitize the eggs for 2-4 h, after which they
were removed from the eggs. The egg card was then transferred to a clean
tube and incubated at ca. 25°C, 50% rh and 18L:6D h photoperiod. After 4 d
of incubation parasitized eggs turned black. A three quarter section of the
card was then removed for storage in a refrigerator (ca. 8°C, 35% rh and
continuous scotophase). The remaining quarter section of the card was
transferred to the 15°C cabinet to slow down the rate of development. Under
these conditions, development from egg to adult was about 20 d.

Parasite strains used for experiments were cultured as above, but in
several replicate tubes and kept constantly in a climatic room at 25±1°C,
65±5% rh and 16L:8D h photoperiod. Under these conditions, the
developmental time of Trichogramma was 10 d. Parasites were isolated, if
required for a test, by placing a single black host (containing one
parasite pupa) into a gelatin capsule.

Trichogramma are facultatively gregarious parasites (see below). The
size of E. kuehniella eggs (0.5x0.3 mm) is such, that female wasps usually
lay a single egg per host for this species. Female Trichogramma emerging
from E. kuehniella eggs are suboptimal (average headwith 0.22 mm, compared
to 0.26 mm for females developing gregariously in larger hosts), which
results in a reduced fecundity (Klomp & Teerink 1967, 1978).

HOST CULTURES

E. kuehniella larvae were reared on an oatmeal diet in plastic food
containers (17x12x4.5 cm) closed with a lid. About 100 larvae were kept per
container on 60 g oatmeal supplemented with 10 g yeast and 15 g wheatgerm,
in a room kept at ca. 25°C, uncontrolled humidity and 10L:14D h
photoperiod. Moths were transferred to an oviposition cage with a fine
gauze bottom. Eggs were laid singly through the gauze and were collected by
means of a funnel emptying into a jar. Eggs were removed daily and those
used for preparation of egg cards were first rendered inviable by freezing
(3 h at -20°C) or U.V. irradiation (4 h at 163.8 J/m^2.h) (Singh 1969,
Voegelé et al. 1974, Maninder & Varma 1980). This sterilization of host
eggs was neccessary to prevent caterpillars, emerging from unparasitized
eggs, feeding on the parasitized eggs. Surplus, sterile eggs were stored in
a refrigerator at ca. 8°C for up to 14 d and, if necessary, used for
rearing without apparent reduction of their quality as a host (Voegelé et
al. 1974).

Cultures of the experimental host species Mamestra brassicae, Pieris
brassicae and P. rapae were maintained in the laboratory. Larvae of each
species were reared in cages on Brussels sprouts plants in a room at ca.
25°C, uncontrolled humidity and 18L:6D h photoperiod. The plants were grown
in pots in a greenhouse. P. brassicae and P. rapae butterflies were kept in
large oviposition cages (ca. 1x1x1 m) at 25°C and exposed to daylight. The
butterflies could feed on a 10% sugar-water solution. Each day a host plant
was placed in the cages to stimulate oviposition. P. brassicae and P. rapae
eggs were laid, respectively, in large clusters and singly on the underside
of the leaves. Eggs were removed from the leaves by probing gently with a
fine, wet brush under each egg. At 25°C, development of P. brassicae lasted
4 d and that of P. rapae 3-4 d.

M. brassicae moths were kept at ca. 25°C, uncontrolled humidity and
6L:18D h photoperiod in oviposition cages as described by Knott et al.
(1966). Moths could feed on a 10% water-sugar solution. Eggs were laid in
clusters on the inside of the tissue-paper sides of the cage and were
collected daily. Eggs used for culturing were surface sterilized by
exposure to formic-acid vapour (10% solution) during 4 h. Eggs needed for

experiments were harvested shortly after egg laying and transferred to controlled conditions. They could be readily removed from the tissue paper with a fine brush after the paper had been made wet. The development time at 25°C was 4 d.

OBSERVATIONS

In several experiments the behaviour of female Trichogramma toward one or more host eggs was recorded. Observations were carried out with the aid of a dissecting microscope and fiber optical illumination to maintain a constant temperature. Trichogramma are pro-ovigenic and readily parasitize eggs soon after emergence (Pak & Oatman 1982a). Upon contact with a host egg, females exhibit a characteristic parasitization behaviour, which has been described in detail by e.g. Klomp et al. (1980). The major phases of the parasitization process are shown in Figure 3.1, the function and the duration of each phase are presented in Table 3.1. After contact, the host is examined by antennal drumming, which is followed by the drilling through the egg shell with the ovipositor. Oviposition begins with insertion of the ovipositor and, depending on the size of the host, one or more eggs are laid (Salt 1935, Klomp & Teerink 1962, Schmidt & Smith 1985a,b).

After withdrawal of the ovipositor, the wasps may host feed on the exudate oozing from the egg-shell puncture. A host may be parasitized in a few minutes (Table 3.1), depending on its size, the hardness and thickness of the chorion (Salt 1940), and ambient temperature. A host may be rejected during any phase of the process of examination and parasitization, and prior to this also after a brief initial contact without drumming or visually from a short distance (Van Dijken et al. 1986).

An ethogram of the parasitization behaviour has been presented by Van Dijken et al. (1986). Most Trichogramma spp. reproduce by arrhenotokous parthenogenesis, while thelytoky and deuterotoky have been reported for some species (Birova 1970). Suzuki et al. (1984) provide a detailed account of the egg-laying behaviour. They describe a method to determine the number of eggs laid and how to discern whether an egg becomes fertilized when it is laid, based on movements in the female's abdomen. Since in arrhenotokous reproduction fertilized eggs produce females, it is possible to evaluate the sex ratio of the progeny allocated per egg (Van Dijken & Waage 1987).
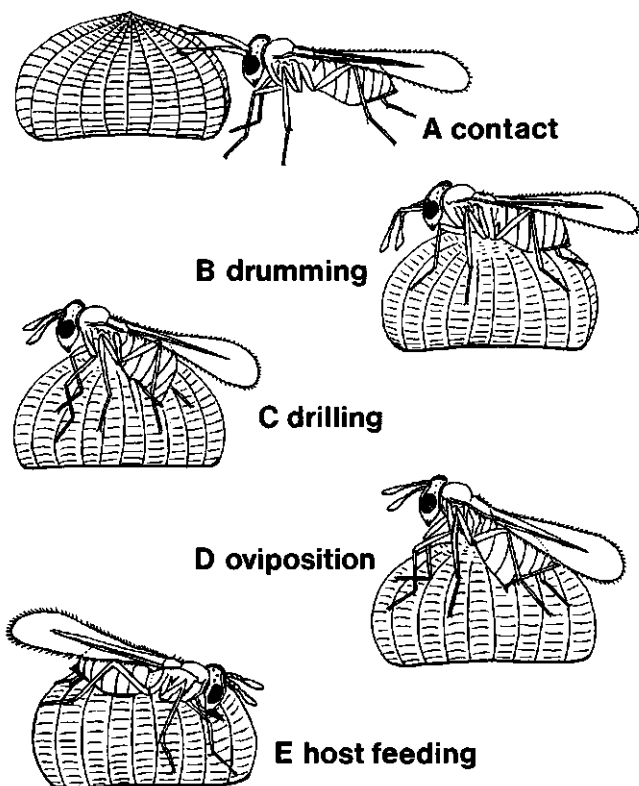
Figure 3.1. Different phases of the oviposition behaviour of the egg
parasite Trichogramma (drawings by Ha Quang Hung).

The strategy of progeny and sex allocation of Trichogramma in relation to
variable host and/or parasite densities has been discussed by Waage (1986).

At the beginning of the present research Suzuki's method was not known.
In most experiments the number of eggs laid per host was determined by
dissection. Parasitized eggs were placed in a droplet of water on a
microscope slide and then gently squeezed under a coverslip. The parasite
eggs could then be identified and counted under a microscope. Female
Trichogramma were prepared in the same way to determine the number of
mature eggs in the ovaria or oviducts.

In several experiments of the present research observations were made
on females encountering previously self-parasitized hosts. Trichogramma are
known to discriminate between parasitized and unparasitized hosts and lay

40

Table 3.1. Approximate durations of the major phases of the parasitization behaviour of a _Trichogramma_ female on a medium-sized host egg (ca. 1 mm diameter) at 20°C.

Behaviour	Supposed function	Duration (s)
contact	examination	5
drumming	examination, host-volume measurement	30
drilling	penetration	60
ovipositing	progeny production	200
host feeding	nutrition	300

eggs in those hosts containing the least number of eggs (Van Lenteren et al. 1978, Van Lenteren 1981). Host discrimination in parasitic wasps was first discovered by Salt (1937a), who found that females of _T. evanescens_ marked their hosts both internally and externally and that discrimination may occur by external examination with the antennae and/or by internal examination with the ovipositor. Klomp et al. (1980) showed that the ability to discriminate is not acquired until a female has had oviposition experience with an unparasitized host (Van Dijken & Waage 1987).

Chapter 4. ADAPTABILITY TO FIELD-TEMPERATURE CONDITIONS

ABSTRACT

The effect of temperature on parasitization activity was studied for
various laboratory strains of Trichogramma spp., in order to assess the
adaptability to climatic conditions in candidate strains for inundative
biological control in the Netherlands. In three strains tested at
temperatures ranging from 12-30°C, the number of females becoming active
increased linearly with temperature, until nearly all females parasitized
at 20 or 25°C. The number of hosts parasitized increased with increasing
temperature to a maximum at 20-25°C and declined at 30°C. Handling time
decreased asymptotically with increasing temperature.

The results for sixty strains exposed to 12°C suggest that
Trichogramma strains vary in adaptability to low temperature. The number of
females parasitizing and the number of host eggs parasitized differed
significantly between strains. These two traits were not correlated, which
suggests that they are controlled by different temperature dependent
mechanisms.

The results of this study make it possible to select candidate
strains for field testing and for further pre-introductory laboratory
studies on the basis of a high performance at 12°C. Indigenous strains were
characterized by a low activity at 12°C, which makes their usefulness for
inundative releases doubtful.

INTRODUCTION

Climatic tolerance of introduced natural enemies is generally considered by
biological control workers as a dominant factor determining a species'
establishment and effectiveness in inoculative biological control programs
(e.g. DeBach 1965a,b, Messenger et al. 1976a,b, Hokkanen 1985). This is
exemplified by the importation of two geographical strains of the aphidiid
parasite Trioxys pallidus, from France and Iran, into California for
control of the walnut aphid, Chromaphis juglandicola (Messenger & Van den
Bosch 1971). The Iranian strain readily colonized all climatic zones,
whereas the French strain did not get established in the harsher climatic

zones, such as the Central Valley.

Climatic adaptation may also be an important criterion in the evaluation of candidate natural enemies for inundative biological control programs (chapter 2). This chapter deals with the effect of temperature on the parasitization activity of various strains of Trichogramma, in order to utilize temperature dependent activity as a criterion to select candidate strains for field application (chapter 1). Attempts from 1960 to 1962 in the Netherlands to control leafrollers (Tortricidae) in apple orchards with releases of an imported Trichogramma sp., demonstrated adverse effects of cool weather on the performance of the parasites (De Jong 1963). Parker et al. (1971) observed the same phenomenon after releasing a Polish T. evanescens strain in cabbage fields against Pieris rapae in Missouri, USA. Parasitism declined to insufficient levels when the daily temperature averaged below 15.5°C. Kot (1979) reported a reduced effectiveness of Trichogramma during periods of cool or hot weather.

Several studies have dealt with tolerance of immature Trichogramma to high or low temperature extremes (Kot 1964, Lopez & Morrison 1980a,b, Nagarkatti 1979, Venkatraman & Govil 1952). Biever (1972) and Boldt (1974) studied the locomotory behaviour of females of T. evanescens and T. minutum at various temperatures and found that their walking speed increased between 20°C and 35°C, but decreased at 40°C. Eidmann (1934) determined the activity thresholds for the same two species and observed the beginning of coordinated movements at a temperature of 5°C and 8°C, respectively. The upper limit for normal walking behaviour was 38°C. Russo & Voegelé (1982) studied the fecundity of four Trichogramma species at various temperatures and found a variation of 11–15°C for the lower parasitization threshold and of 32–34°C for the upper threshold.

In order to determine the influence of temperature on the parasitization activity of Trichogramma, three strains of different species from the collection of laboratory strains (chapter 2) were studied at temperatures ranging between 12 and 30°C. During the field-release season, June through August, temperatures below 15°C occur frequently in the Netherlands, especially in June (Figure 4.1). High temperature extremes probably are not a matter of concern in the Netherlands, since the maximum seldom is above 30°C. The parasitization activity of all strains in the collection was therefore studied at a low temperature extreme only. A

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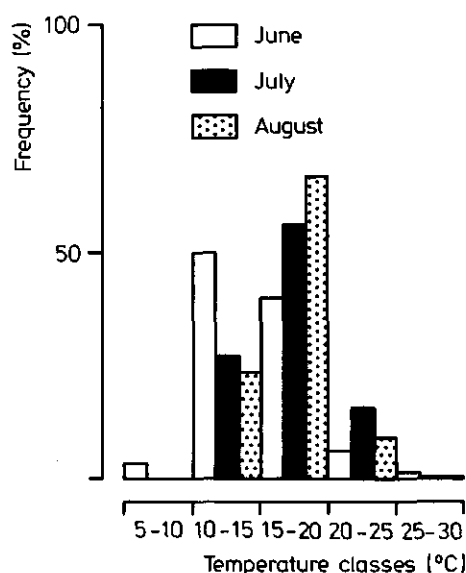
Figure 4.1. Frequency distribution of average daily temperatures (1.5 m above ground, 1974-1983) in June, July and August for Wageningen, the Netherlands.

temperature of 12°C was chosen, because this is within the range of reported lower temperature limits for activity in Trichogramma. To facilitate this multi-strain experiment, female wasps were only observed during a limited host-exposure period: directly for 2 h and, subsequently, indirectly for another 22 h.

MATERIALS AND METHODS

Standard procedures were used to culture parasites and hosts (chapter 3). During the first part of development (egg through prepupal stage) all strains were reared at $25 \pm 1°C$ and $60 \pm 5\%$ rh to prevent induction of diapause. Parasites were transferred to a climate room, at $17 \pm 1°C$, $60 \pm 5\%$ rh and 16L:8D h photoperiod, after host eggs had turned black. For each test of a strain, wasps were isolated in gelatin capsules and 40 females, 12-24 h old, were collected for testing the parasitization activity at 12°C. Females were put individually in glass vials (70x22 mm) plugged with

cotton. An egg card (40x15 mm, chapter 3), carrying ca. 50 eggs of Ephestia kuehniella (1-2 d old), was introduced into each vial. Preliminary studies showed that this was an abundant host supply for the duration of the exposure period.

Immediately after introduction of the egg cards, the vials were exposed to the test conditions of $12\pm1°C$, $50\pm5\%$ rh and 1000 lux illumination in a container hanging in a water bath and covered with a glass plate. After being thus exposed for 2 h, the egg cards were exchanged for fresh ones, which were then exposed for 22 h. The 2 h period was chosen to avoid total depletion of the egg complement of females, which might cause them to become inactive. For each strain, 15 unexposed females were dissected to determine the number of mature eggs in the ovaria (chapter 3).

During the first exposure, some females were observed with a dissecting microscope and the duration of the different phases of the oviposition behaviour was measured with a stopwatch (chapter 3). Each female in the test was observed every 30 minutes and recorded as to whether she was walking, standing still or parasitizing. No observations were made during the second exposure. Females were removed after the second exposure. Exposed egg cards were left in the vials and incubated at room temperature. Parasitization rates were determined by recording, for each card, the number of host eggs which turned black.

Females of strains 11 (T. maidis), 23 (T. pretiosum) and 48 (T. semblidis) were tested the same as above and at a range of higher temperatures: $17\pm1°C$, ($60\pm5\%$ rh), $20\pm1°C$ ($60\pm5\%$ rh), $25\pm1°C$ ($60\pm5\%$ rh) and $30\pm1°C$ ($80\pm5\%$ rh). In contrast with the 12°C test, the parasites were continuously reared and kept in the climate room at the respective conditions of each test. At each condition, 15 unexposed females were dissected to determine the number of mature eggs in the oviducts or ovaria (chapter 3).

Individual females in a test responded to exposure by either parasitizing one or more host eggs, or by remaining inactive. The activity of a strain (i) is here defined as the proportion $\underline{P}_i$ of parasitizing females in a test. An estimate of $\underline{P}_i$ is $\hat{\underline{P}}_i = \underline{k}_i/n_i$, where $\underline{k}_i$ = number of parasitizing females and n_i = number of tested females. The sampling distribution of a proportion $\underline{P}_i$ is binomial with expectation:
$\mu_{\underline{P}_i} = \underline{P}_i$ and $\sigma_{\underline{P}_i} = \sqrt{[\frac{1}{n_i} \cdot \underline{P}_i(1-\underline{P}_i)]}$, but may for large values of n_i ($n_i > 30$)
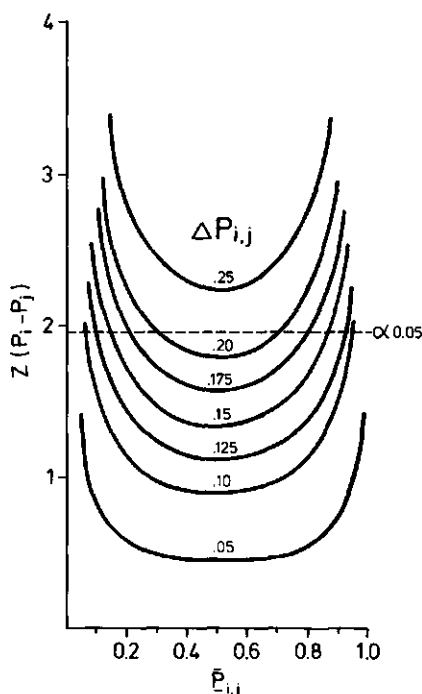
Figure 4.2. Relationship between the standardized variable $Z(P_i-P_j)$ and the mean proportional activity $\bar{P}_{i,j}$ at various levels of the difference $\Delta P_{i,j}$ in activity between females of two **Trichogramma** strains i and j for $n_i=n_j=40$.

be approximated by a normal distribution (Spiegel 1975). For evaluation of the difference $\Delta P_{i,j} = |\underline{P}_i - \underline{P}_j|$ between strains i and j, with respective activities $\underline{P}_i$ and $\underline{P}_j$, the standardized variable is:

$$Z(P_i-P_j) = [(\underline{P}_i-\underline{P}_j)-\mu_{P_i-P_j}]/\ \sigma_{P_i-P_j} \quad \text{(Spiegel 1975)}$$

Consider the null hypothesis that there is no difference between the activities of strains i and j, that is Ho:$\underline{P}_i=\underline{P}_j$. Assuming $\underline{P}_i=\underline{P}_j=\underline{P}_{i,j}$, then $\underline{P}_{i,j} = (n_i\underline{P}_i + n_j\underline{P}_j)/(n_i + n_j)$. Under the null hypothesis $\mu_{P_i-P_j} = 0$ and $\sigma_{P_i-P_j}=\sqrt{[\sigma^2 P_i+\sigma^2 P_j]}= \sqrt{[\underline{P}_i(1-\underline{P}_i)/n_i]+[\underline{P}_j(1-\underline{P}_j)/n_j]} = \sqrt{[\bar{\underline{P}}_{i,j}(1-\bar{\underline{P}}_{i,j})/(n_i+n_j)]}$

Evaluation of the standardized variable under the null hypothesis gives:

$$Z(P_i-P_j) = (\underline{P}_i-\underline{P}_j-0)/\ \sigma_{P_i-P_j} = (\underline{P}_i-\underline{P}_j)/\ \sigma_{P_i-P_j} = \Delta P_{i,j}/\ \sigma_{P_i-P_j}$$

At a significance level of $\alpha = 0.05$ the null hypothesis is accepted for $Z(P_i-P_j) < 1.96$. Figure 4.2 shows the relationship between $Z(P_i-P_j)$ and $\underline{P}_{i,j}$ for different values of $\Delta P_{i,j}$ and $n_i=n_j=40$. For example, a value of $\Delta P_{i,j} \approx 0.25$ will be significant at the 5% level for any $\underline{P}_{i,j}$; a value of

0.2 will be significant for $\underline{P}_{i,j} < 0.3$ only.

Differences in the mean number of hosts eggs parasitized and the mean duration of the oviposition behaviour between strains were tested for significance by Student t-test.

RESULTS

In each of the three strains studied at temperatures from 12 to 30°C, the duration of the oviposition behaviour was related to temperature by an inverse curvilinear relationship (Figure 4.3). Significant negative correlations ($P < 0.05$) were determined between the logarithms of the mean handling time and temperature.

The proportion parasitizing females of strains 11, 23 and 48 increased linearly ($P < 0.05$) with temperature between 12 and 25°C at 24 h exposure (Figure 4.4). At 25°C nearly all females were parasitizing and the proportional activity at this temperature was not different ($P < 0.05$) from that at 30°C (in strain 48 also not different from the activity at 20°C). For 2 h exposure the trend is similar, but less consistent, which suggests a variable delay in the activity response between females, especially at the lowest test temperatures. At 30°C, there was no difference ($P < 0.05$) in activity during 2 h and 24 h exposure.

Oviposition rates increased with temperature, reaching a maximum at 25°C (Figure 4.4). The mean number of eggs laid was lower at 30° than at 25°C (not significant in strain 23). At 25° and 30°C most females were found motionless, often on top of a host egg, after a certain part of the 2 h exposure had lapsed. Dissections showed that quiescence was induced if the egg complement was nearly depleted. Thus, the eggs laid during the second exposure, by females that had parasitized in the preceeding 2 h exposure, probably had matured during this second exposure. Under this assumption, egg maturation (in 22 h) averaged 11.5, 13.6, and 13.6 in strains 11, 23 and 48, respectively, at 25°C and 8.4, 8.0 and 10.9, respectively, at 30°C. The number of mature ovarial eggs in females 12–24 h old did not appear to be related to the rearing temperature, the means varying between 20 and 30 eggs per female (Table 4.1).

Figure 4.5 shows the proportion of parasitizing females (activity) and the mean number of hosts parasitized per (active) female for each strain at

48
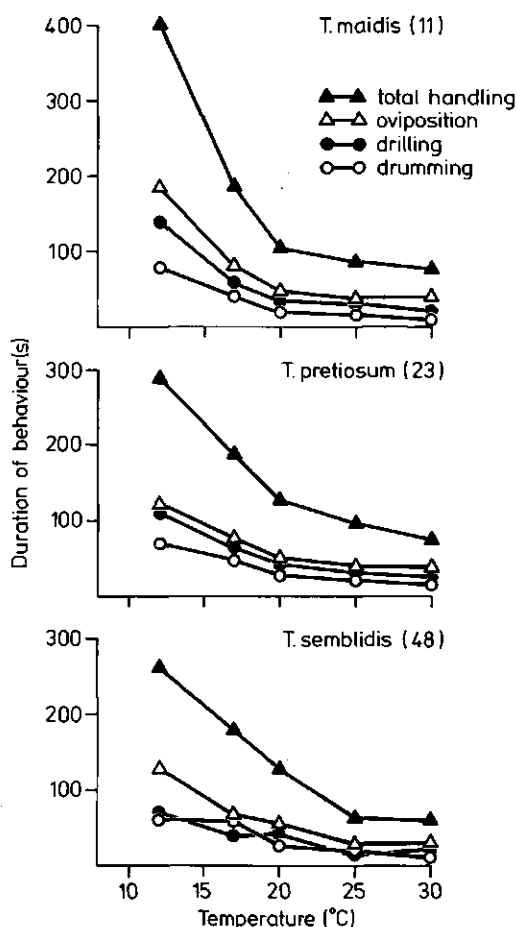
Figure 4.3. Relationship between temperature and the duration of the
drumming, drilling and oviposition phases of the parasitization behaviour,
and the total handling time, for three _Trichogramma_ spp. strains.

2 h and 24 h exposure (first and first plus second exposure period) at
12°C. The difference between 24 h and 2 h exposure represents activity in
the 22 h exposure of those females that were not active in the preceeding
2 h exposure. The activity in the first 2 h was not a consistent proportion
of the activity in 24 h, but varied among strains from 0.0 to 0.96. Thus,
in some strains activity was restricted to the second exposure period,
whereas in other strains the proportion of parasitizing females in the
first exposure period hardly increased during the second exposure. Females
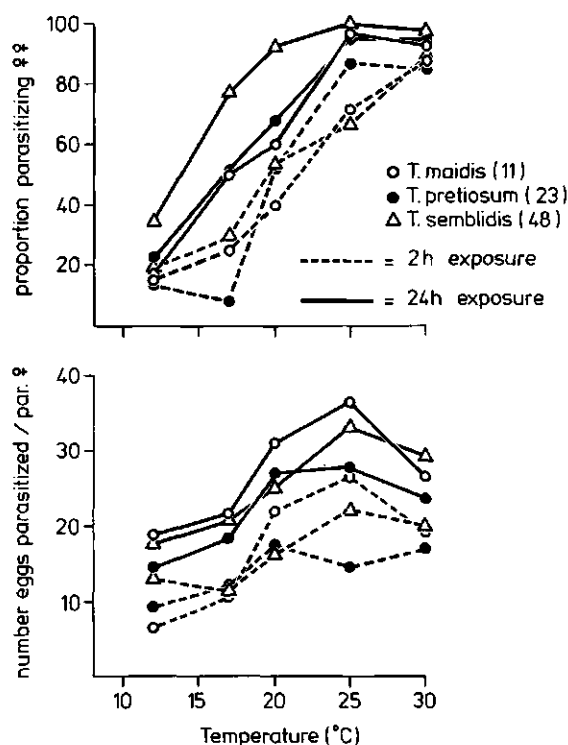
Figure 4.4. Relationship between temperature and proportional activity and between temperature and oviposition rate for three _Trichogramma_ spp. strains during 2 h and 24 h exposure.

within strains apparently differed in searching motivation and/or in their reaction to the low temperature condition. The 24 h exposure data therefore appear to be more reliable for the evaluation of activity differences among strains than the data for 2 h exposure. Direct observations during the 2 h exposure showed that most of the parasitizing females became active during the first half hour of the test. Non-parasitizing females usually were immobile; walking was seldom observed.

The data on activity at 12°C demonstrate a large variation among strains for this trait. The frequency distribution for activity of strains, for data grouped into ten classes of activity (from 0 to 1), shows several strains in each class, except in the three highest classes (Figure 4.6). Strains 1, 7, 14, 16, 22, 24, 26, 35, 38, 43 and 57 had an activity > 0.6
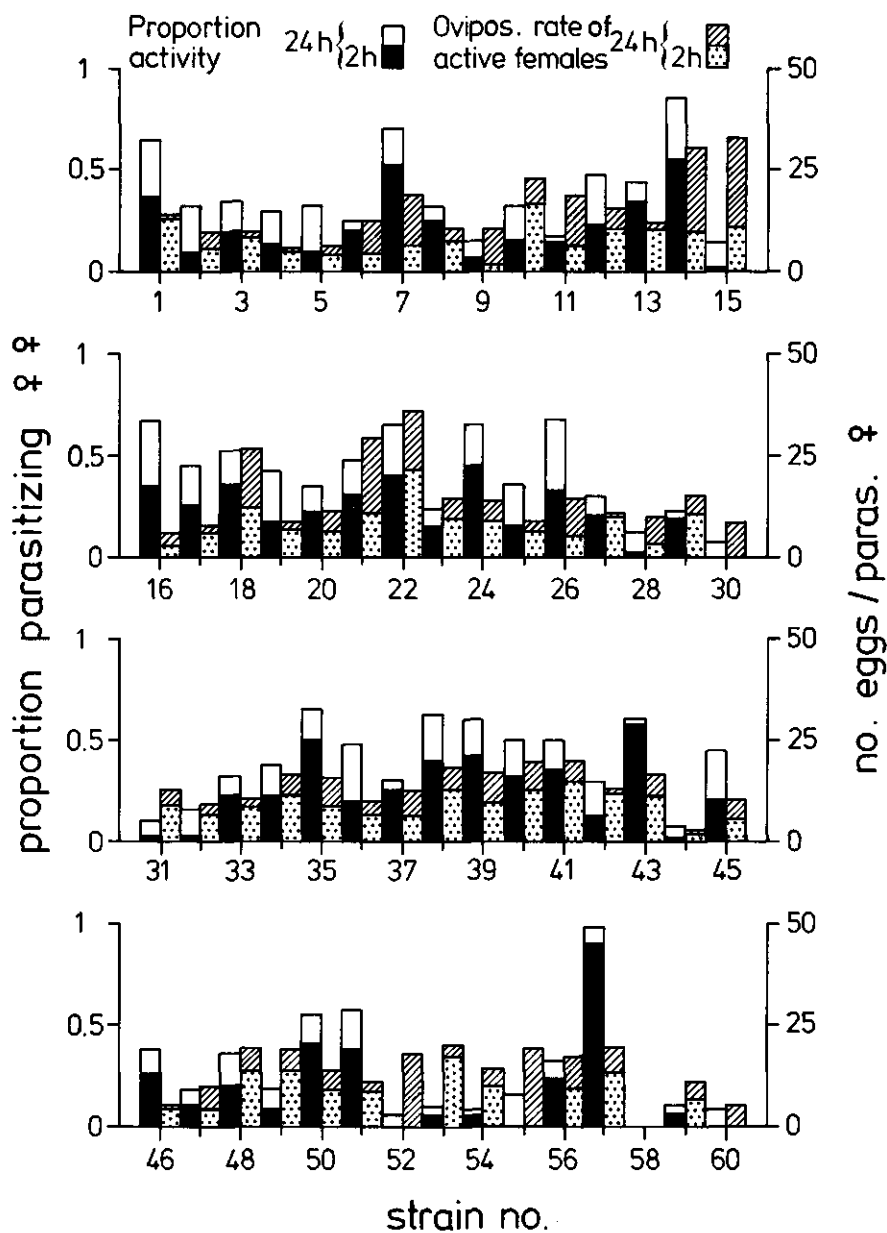
Figure 4.5. Proportional activity and parasitization rate of females of 60 *Trichogramma* spp. strains during 2 h and 24 h exposure at 12°C.

Table 4.1. Number of mature eggs (means $\pm$ 95% confidence limits) in ovaria
and oviducts of 12-24 h old females (n=15) of three <u>Trichogramma</u> spp.
strains reared at different temperatures.

Temperature (°C)	Number of mature eggs[1]		
	<u>T. maidis</u> (11)	<u>T. pretiosum</u> (23)	<u>T. semblidis</u> (48)
17	35.0+2.2 a	19.6+2.2 b	28.0+2.0 a
20	25.5+2.2 b	28.7+2.0 a	25.0+2.3 ab
25	29.8+2.7 ab	27.3+2.3 a	20.4+1.8 b
30	26.3+1.6 b	29.4+1.5 a	23.7+1.9 ab

[1] Means within strains followed by the same letter are not significantly
different, P < 0.05.

(24 h exposure). The activity of these strains is significantly higher
(P < 0.05) than that of 36 strains with an activity < 0.4 (Figures 4.2
and 4.3). The variation in activity among strains within the <u>T. evanescens/
T. maidis</u> complex is also significant, the means ranging from 0.05 to 0.7.
Native strains (10, 11, 32, 52, 53, 54, 55, 59) are characterized by a low
activity response (< 0.3) at 12°C.

Mean oviposition rates per active female varied widely among strains,
from 2.7 (strain 44) to 35.7 (strain 22) at 24 h exposure. Means calculated
from data for a few active females show a large variance, impeding
statistical comparison of mean differences between strains. Where
comparisons could be made, significant differences (P< 0.05) were found
between strains, even between strains within the same species. Mean
oviposition rates at 24 h exposure generally were higher than those at 2 h
exposure. Thus, depletion of the egg complement did not occur during the
2 h exposure. A correlation between oviposition rates at 2 h and 24 h was not
apparent.

The counts of ovarial eggs indicated that females with a high
oviposition rate utilized most of their egg complement during the 24 h
exposure period. Additional egg maturation appeared to occur during the
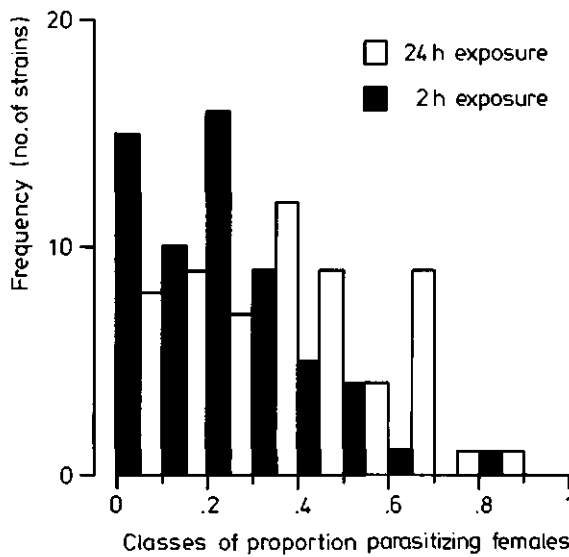
Figure 4.6. Frequency distribution of proportional activity for females of 60 Trichogramma spp. strains during 2 h and 24 h exposure at 12°C.

period of testing. This was probably a source of bias in the counts, since dissections were not made at fixed intervals. However, dissected females contained at least 10 mature eggs, the means per strain varying from 15 to 35. Thus, the fact that certain females did not parasitize was probably not due to an insufficient number of mature eggs in the ovaria or oviduct (Pak et al. 1985). A correlation between the proportional activity and the mean oviposition rate per strain was not apparent. Strains with a high oviposition rate occurred among those with a high activity (e.g. strains 16, 18, 22) as well as among those with a low activity (e.g. strains 11, 15, 55). The mean number of mature eggs in the ovaria of dissected females did not appear to relate to the activity of strains.

The oviposition behaviour of the different strains was similar. Durations of handling time per host egg, measured in some twenty strains, varied significantly ($P < 0.05$) among strains, averaging from 235 s (strain 14) to 573 s (strain 46). The total time spent in drumming, drilling and oviposition was rather consistent among the strains, being about 20%, 35% and 45%, respectively. The mean numbers of eggs laid at 2 h exposure did not appear to be related to the mean handling times in these strains.

DISCUSSION

Activity of a Trichogramma female can be considered as a behavioural response to her physiological state (Connolly 1967). A female's searching behaviour probably is a consequence of an innate "appetite to parasitize" or "ovipositional drive", which may be dependent on the number of eggs in her oviducts (Hassell & Southwood 1978). In the present study, searching activity of females rapidly resulted in encounters with host eggs (due to the relatively small size of the experimental units) and subsequent parasitization activity. The proportional activity of females and the parasitization rate of the active females were determined as two different measures of parasitization activity. These two measures were differently affected by temperature.

The temperature dependent increases in activity and parasitization rate in strains of T. maidis, T. pretiosum and T. semblidis, with maximum values between 20°C and 30°C, are in agreement with results from the literature. Biever (1972) studied the searching activity of five Trichogramma spp. strains at 20–40°C and found that in each strain all females were active at 25–35°C. At 20°C, the proportional locomotory activity varied between strains from 0.3 to 1 and from 0 to 0.7 at 40°C. Boldt (1974) studied searching activity of a T. evanescens and a T. minutum strain at different temperatures (20–35°C) and relative humidities (30–85%). The walking speed was not influenced by relative humidity, whereas with temperature a positive linear regression was determined. The latter is different from the presently observed curvilinear relationship between handling time and temperature.

The highest mean parasitization rate for strains 11, 23 and 48 was between 20° and 25°C. Pak & Oatman (1982b) and Russo & Voegelé (1982) studied the fecundity of several Trichogramma species at various temperatures and found maximum values at 25°C and 20–30°C, respectively. Shchepetilnikova (1939) reported that fecundity in T. evanescens was optimal at 20°C.

The absence of a correlation between proportional activity and parasitization rate at 12°C among strains suggests that locomotion and oviposition are controlled by different temperature dependent physiological processes. This might, for instance, be due to different temperature

optimums of enzymes playing a role in these processes. The suggested
difference in temperature compensation between locomotion and oviposition
activity may be the result of two different strategies in Trichogramma to
overcome the limiting effect of low temperature on reproduction and
fitness. In one strategy, a high proportional activity is maintained when
the temperature decreases (with a variable parasitization rate), whereas in
the other a relatively high parasitization rate in active females is
maintained irrespective of their proportional activity. The latter strategy
may compensate for a low proportional activity. Strains with the best
performance at low temperature have maximized both strategies (e.g. strains
14, 22 and 57). If the overall performance of two strains of different
strategy is similar, the strain with a high proportional activity probably
will be more useful for inundative release. Such a strain will have
relatively more females searching for hosts, which at low host density may
increase the probability of host finding.

A female's physiological state is determined by her genotype and the
environmental conditions of rearing and testing. The variability in
activity at 12°C among strains represents the phenotypic variation for this
trait. Therefore, the data are an expression of the adaptability of each
strain for activity at low temperature. However, this is an indirect way to
determine the effect of genetic variability. It might be advantageous to
study the variation among strains at the enzyme level, since these are a
direct expression of the gene code. For example, Van Dijken et al. (1982)
found a 25% higher NADH-oxidase activity in Drosophila melanogaster strains
selected for high locomotory activity, compared with that in low activity
strains. Locomotory activity was correlated with the activity of the energy
supplying process in the mitochondria. In similar studies, Connolly et al.
(1971) and Tunnicliff et al. (1969) found differences in concentrations of
biogenic amines between selected high- and low-activity strains.

In the present study, a relationship between the activity of strains
(at 12°C) and the climate at their place of origin cannot be established.
The strains with highest activity originate from a continental or subtropical
climate. The low activity of native strains (from a coastal climate) may
render them unsuitable for inundative release in countries like the
Netherlands. This is contrary to the common practice of Trichogramma
workers to mass produce a local strain, because of its supposed optimal

adaptation to the indigenous host species and abiotic environment
(chapter 2). Immature stages of all native strains entered diapause if
development took place at 15°C. Thus, diapause may be induced in field
populations toward the end of the summer, and adults consequently will not
be exposed to low temperatures for long periods of time. Periods of low
temperature during the summer are variable in the Netherlands, and possibly
too brief or infrequent to cause selection for high activity at low
temperature. Under subtropical conditions Trichogramma and host species may
be active year-round and overwinter without diapause (Pak & Oatman 1982b),
although winter temperatures sometimes may drop below 10°C. In this way,
plasticity for temperature-controlled activity in females is adaptive and
will be favoured by natural selection.

It is unknown whether prolongued laboratory rearing of parasites
affects their activity response to low temperature. Many of the tested
strains have been cultured for several years in the laboratories from which
they were obtained, usually at room temperature (20-25°C). Nonetheless,
activity at 12°C was high in several laboratory strains, whereas activity
in some newly field-collected strains was low. A variability in
proportional female activity, as well as in fecundity, was reported by
Sorokina (1983) for successive generations of T. evanescens and
T. euproctidis at 25°C. She also suggested a gradual deterioration of these
traits over successive generations. The present results do not confirm
this. Activity of females in the three strains tested at 25°C invariably
approached unity.

The present study makes it possible to select several candidate
strains of Trichogramma with a relatively high parasitization activity at
low temperature. Whether the anticipated adaptability of these strains to
low temperature will be translated into a higher performance under cool
weather conditions must be validated in field experiments (chapter 9).
However, parasitization activity is a "conditio sine qua non" for host
location and parasitization. The results of this study therefore seem to be
useful as a first criterion to select strains for studies on subsequent
pre-introductory selection criteria.

Chapter 5. HOST-AGE SELECTION

ABSTRACT

The relationship between different ages of host eggs and parasitism by Trichogramma varies between species of the host and the parasite. Six basic types describing the possible relationships between host-age and parasitism are apparent from the literature on host-age selection by Trichogramma. Furthermore, a sequence of different aspects of the parasitization process, from host contact to successful parasitism, is identified: (1) contact, (2) attack, (3) oviposition, (4) mortality and (5) development. In nearly all relationship types, young hosts are more frequently accepted for parasitism than older hosts. Relationship types are generally not consistent for different host species of the same family, and they also appear to vary between Trichogramma species for the same host species.

Host-age selection was examined in laboratory choice-tests for three laboratory strains of Trichogramma (T. buesi, T. evanescens and T. maidis). The behaviour of individual females was directly observed, while they were offered host eggs of two different ages of one of the host species Mamestra brassicae, Pieris brassicae or P. rapae. Host age hardly appeared to affect contact or acceptance of eggs of either host species for any of the strains. Clutch size of the progeny tended to decrease with host age for M. brassicae eggs. The duration of the oviposition behaviour was sometimes influenced by host age. The mean clutch size per host species was not proportional to the mean host size. Females of each strain showed the ability to discriminate between parasitized and unparasitized hosts by external or internal examination.

The lack of variation in host-age selection among the investigated strains suggests that for a given host-Trichogramma combination the selection of hosts of different ages is predominantly dependent on the host species. The experimental results of this study impede the use of host-age selection as a criterion to select candidate strains for the present pre-introductory evaluation program.

INTRODUCTION

The foraging process by which parasitic Hymenoptera locate and parasitize
their hosts has classically been depicted as a stepwise response to
successive levels of host containing areas (Salt 1935, Doutt 1959, Vinson
1977). Vinson (1976) has divided this process into five steps: host-habitat
location, host location, host acceptance, host suitability and host
regulation. He considered the first three steps as aspects of the host-
selection process. Host selection is the selection between hosts of
different ages and/or species (Van Lenteren 1981). It therefore seems more
appropriate to restrict its definition to the process by which a parasite,
upon contact with a host, assesses its acceptability (Bragg 1974). Habitat
and host location are consequently viewed as aspects of the parasite's host
finding or searching behaviour, i.e. the chain of behaviours prior to
contact with the host (Weseloh 1981).
    Host age and species may have a significant effect on whether a host
is succesfully attacked and parasitized by parasitic Hymenoptera. This
chapter deals with the selection between host eggs of different ages by the
egg parasite Trichogramma, in order to select strains for an inundative
biological control program. Host-age selection is studied in laboratory
experiments for three Trichogramma strains, from a collection of laboratory
strains (chapter 2), toward eggs of the host species Mamestra brassicae,
Pieris brassicae and P. rapae. In biological control, the host-specificity
properties of candidate natural enemies are generally considered among the
factors determining their effectiveness as control agents (chapter 2). The
effectiveness is likely to be diminished if some host ages or species are
preferred over others, since the latter have an increased probability of
remaining unparasitized (Ehler & Van den Bosch 1974). The host-age
acceptance behaviour of Trichogramma therefore may be a critcal factor in
the pre-introductory selection of species or strains for biological control
(Marston & Ertle 1969, Schmidt 1970, Van Dijken et al. 1986).
    Many egg parasites only lay eggs into the very early stages of their
hosts (Clausen 1940, Vinson 1976). Austin (1984) suggested that the
development of egg parasites depends on the ingestion of yolk, which
renders hosts increasingly unsuitable with the advance of embryonic
development. Females may use physical (e.g. size, shape, texture, movement)

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and/or chemical cues to recognize (accept) their hosts (Arthur 1981). Size of the host is sometimes related to host-age selection by larval parasites (Vinson 1976). In the egg stage, however, age and size are not related, so that any preference for a particular age of egg must be based on other physical or on chemical differences, internal or external. Schmidt (1970) listed pH, osmotic pressure, immune response and chemical constitution among the factors related to host development or species differences which may be responsible for preventing attack by Trichogramma or inhibiting its development.

The preferred ages of different host species must also be known for the study of host-species selection (chapter 6), in which a choice of the preferred ages of two host species is offered to the parasites (Van Dijken et al. 1986). Furthermore, selective behaviour of Trichogramma toward ages of hosts used in mass rearing may affect the efficiency of the production system (Brenière 1965b, Benoit & Voegelé 1979). Since various Trichogramma species, reared on several factitious hosts, have been applied against numerous pest species, observations on the host-parasite relationship have been reported frequently for the Trichogramma genus. In this chapter, the literature on host-age selection by Trichogramma is reviewed first, in order to identify possible trends among host species or families.

REVIEW OF THE LITERATURE

Classification of data

Reports on a total of 53 studies were found for review, dealing with various Trichogramma species and 26 Lepidoptera (11 families), one Diptera and one Coleoptera. The reported identifications of the Trichogramma species probably must be regarded with caution, due to extensive recent changes in the taxonomy of the genus (Nagarkatti & Nagaraja 1977, Voegelé & Pintureau 1982). Different relationships between host age and parasitism by Trichogramma spp. have been found. Figure 5.1 shows nine standardized relationship types which were derived from the data. In order to facilitate comparison between the different studies, host ages were converted to three relative ages: young (0-2 d old hosts), medium (intermediate ages), or old (0-2 d before hatching). In all types, except in type VI, the youngest

hosts are parasitized better than the older ones. The types differ in the pattern by which parasitism of aging hosts decreases.

In Table 5.1, all references are classified by host family, and the inferred relationship type is given to each host-parasite study. However, the studies varied in objectives and methodology, so that different aspects of the parasitization process may have been determined. Arranged by progressive order of "success" in parasitizing hosts, these different aspects are: (1) contact, (2) attack, (3) oviposition, (4) host mortality and (5) development. These parasitization characteristics respectively
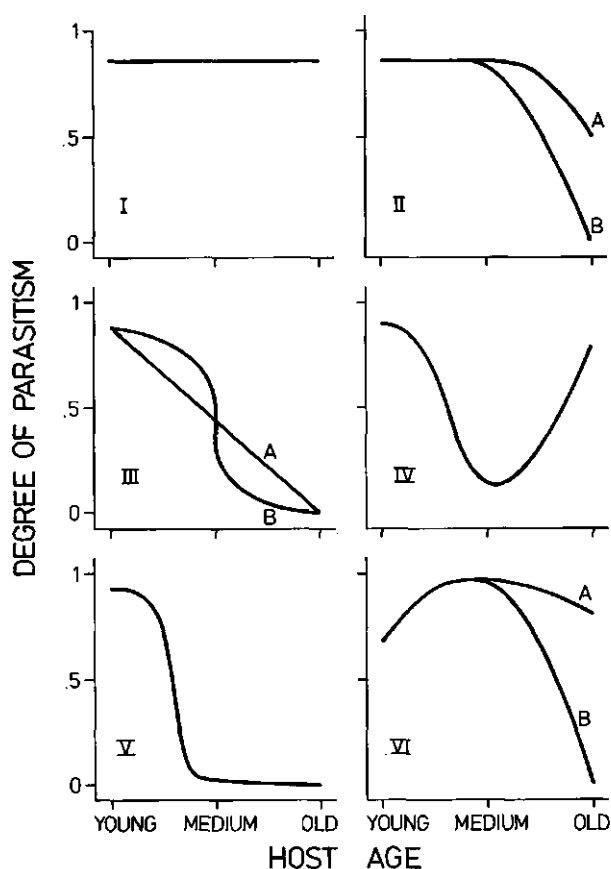
Figure 5.1. Classification from literature data of different types for the relationship between host age and parasitism by Trichogramma spp.
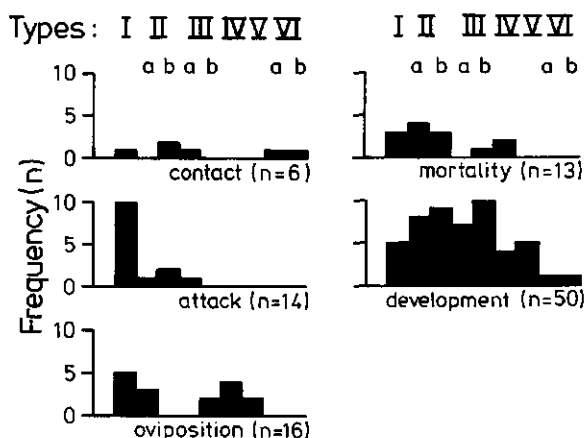
Figure 5.2. Frequencies from literature data of different host-age relationship types (see Figure 5.1) for various aspects of the parasitism process (data in Table 5.1) of **Trichogramma** spp.

indicate (1) examination of a host, (2) drilling a host's chorion, (3) egg laying into a host, (4) killing a host and (5) emergence of wasps.

The frequencies of each host-age relationship type for the different parasitization characteristics are shown in Figure 5.2. Prevalence of a certain type for any of the characteristics is not apparent, except for attack, where type I is most frequent. This suggests that, in general, the host-attack rate of **Trichogramma** females is not influenced by the age of the host egg. Host selection apparently does not occur in this phase of the parasitization behaviour. A proper analysis of the effect of host age on parasitism should encompass the collection of data on all the different aspects of the host-parasite relationship. Table 5.1 shows that only a few studies were comprehensive in this respect (e.g. Marston & Ertle 1969, Lewis & Redlinger 1969). The latter studies demonstrate that, within a given host species, different types may occur for the range of aspects of the host-parasite relationship.

Evidently, most researchers merely studied the effect of host age on successful parasite development, i.e. host suitability (Vinson & Iwantsch 1980a), by recording the number of parasitized (black) eggs. Host selection sensu stricto, however, results from the behavioural actions of the adult

Table 5.1. Review of literature data on relationships between host age and successive traits of the parasitization behaviour and parasitism process for Trichogramma spp. and different host species, arranged by family. See Figure 5.1 for description of relationship types.

Host species	Trichogramma species	Host-age relationship types for traits					Reference
		contact[1]	attack	oviposition	host mortality	development	
Pyralidae (Lep.)							
Cactoblastis cactorum (Berg)	T. semifumatum					II b	Hinckley 1961
Chilo auricilius Dudgn.	T. australicum					II b	Rao & Rao 1980
	T. euproctidis					v	Rao & Rao 1980
	T. fasciatum					III a	Rao & Rao 1980
	T. japonicum					II b	Rao & Rao 1980
	T. perkinsi					III a	Rao & Rao 1980
Corcyra cephalonica Staint.	T. australicum			I	I	II a	Brenière 1965a
	T. minutum				I	II	Krishnamurti 1938
	T. australicum					I	Navarajan 1979
	T. japonicum					III a	Navarajan 1979
Ephestia cautella (Walker)	T. evanescens	I	II a			II b	Lewis & Redlinger 1969
Ephestia kuehniella Zeller	T. evanescens	II b	II b		II a	VI b	Benoit & Voegelé 1979
	T. evanescens					II b	Schulze 1926
Hypsipyla grandella (Zeller)	T. semifumatum	III a[2]	I[2]		II b[2]	II b[2]	Grijpma 1973
Ostrinia nubilalis (Hübner)	T. evanescens	VI b	II b			II b[2]	Benoit & Voegelé 1979
Proceras sacchariphagus Bojer	T. australicum			III b[2]		III a	Moutia & Courtois 1952
Noctuidae (Lep.)							
Earias fabia Fabr.	T. minutum	+				I[2]	Cherian & Margabandhu 1943
Heliothis zea (Boddie)	T. evanescens	VI a		I[2]		II b	Lewis & Young 1972
Mamestra brassicae L.	T. evanescens	+	I[2]	I[2]	II b	III b	Schieferdecker 1965
	T. semblidis					I[2]	Silvestri 1908
Panolis flammea Schiff.	T. cacoeciae				II a	II b[2]	Burzyński & Kot 1963
	T. minutum					I[2]	Eidmann 1934
Trichoplusia ni (Hübner)	T. minutum	II b	I	IV[3]		IV	Marston & Ertle 1969
	T. brevicapillum			IV[3]	IV	IV	Pak & Oatman 1982a
	T. evanescens			IV[3]		IV	Parker & Pinnell 1974
	T. pretiosum			IV[3]		IV	Parker & Pinnell 1974
	T. evanescens		I			III a	Schmidt 1970
	T. semifumatum	+	III a[2]		IV	II a	Taylor & Stern 1971
Tortricidae (Lep.)							
Argyrotaenia velutiana (Walker)	T. minutum				II a	III b	Dolphin et al. 1971

Host	Trichogramma sp.	[1]				Type	Reference
Choristoneura fumiferana (Clem.)	T. minutum	+				III b	Houseweart et al. 1982
Grapholita molesta (Busck)	T. minutum					III b	Peterson 1930
Laspeyresia funebrana Treit.	T. cacoeciae				II a		Wiackowski & Kot 1962
Laspeyresia pomonella L.	T. minutum				II a	III b	Dolphin et al. 1971
	T. minutum					III b	Peterson 1930
	T. minutum	+				III b	Yu et al. 1984
	T. minutum					III b	Flanders 1930a
Tortrix citrana Fern.	T. minutum	+					
Pieridae (Lep.)							
Colias eurytheme Boisd.	T. semifumatum		I[2]		I	I[2]	Stern & Bowen 1963
Pieris rapae (L.)	T. evanescens	+		II a		II a	Parker & Pinnell 1974
	T. pretiosum					none	Parker & Pinnell 1974
	T. evanescens			I		VI a	Schmidt 1970
Gelechiidae (Lep.)							
Sitotroga cerealella Oliv.	T. minutum					II b[2]	Costas 1941
	T. evanescens	+				II a	Kochetova 1969
	T. cacoeciae	+				II a	Kochetova 1969
	T. evanescens		I[2]		I	II a	Salt 1935
	T. cacoeciae	+			II b	III b	Schleferdecker 1965
Papilionidae (Lep.)							
Papilio xuthus L.	T. australicum		I[2]			v	Hiehata et al. 1976
	T. dendrolimi		I[2]	v		v	Hiehata et al. 1976
	T. papilionis		I[2]	v		v	Hiehata et al. 1976
Arctiidae (Lep.)							
Estigmene acrea (Drury)	T. semifumatum	+				III a	Taylor & Stern 1971
Geometridae (Lep.)							
Bupalus piniarius L.	T. embryophagum					III a[2]	Kennel-Heckel 1963
Saturniidae (Lep.)							
Attacus cynthia ricini Boisd.	T. evanescens				III b	III b[2]	Lee 1961
Sciomyzidae (Diptera)							
Sepedon fuscipennis (Loew)	T. julianoi			III b		v	Juliano 1982
Bruchidae (Coleoptera)							
Bruchus obtectus Say	T. evanescens		I[2]	I[2]		nl	Salt 1938

1) A + in this column indicates choice experiment, but no data collected by direct observations.
2) Data scanty to permit proper analysis of host age relationship.
3) Number of eggs laid per host decreases by type III a relationship.

female parasite and therefore had better be studied by directly observing the encounters of individual females with hosts of different ages (Van Lenteren et al. 1978). Moreover, preference for a certain host age can only be assessed by offering a choice of different-aged hosts to the parasites. Several studies included choice situations, but the analysis was usually improper, because acceptance of hosts was not based on the actual number of attacks (Schieferdecker 1965, Taylor & Stern 1971) or on the success of development (Houseweart et al. 1982, Kochetova 1969).

Accessory observations

Several researchers have related the age-dependent changes in host suitability to the embryonic development of the host. Marston & Ertle (1969) found that inhibition of oviposition coincided with blastokinesis of the host. Rotation of the embryo can be followed by a decrease in attack (Benoit & Voegelé 1979) or suitability (Lee 1961). The sharp decrease in parasitism of medium-aged hosts characterizing the type III-b relationship might be related to the rotation of the host embryo (Peterson 1930). Sclerotization of the head capsule of the embryo often appears to mark the end of the period that host eggs can be successfully parasitized (Brenière 1965a, Lewis & Redlinger 1969, Peterson 1930). However, Benoit & Voegelé (1979), Eidmann (1934) and Marston & Ertle (1969) found effective killing of hosts up to the moment of egg hatching.

Eidmann (1934) suggested that success in parasitizing host eggs, which are about to hatch, depends on whether the parasite egg is deposited within the embryo (killing it) or in the amnion cavity (e.g. Benoit & Voegelé 1979, Brenière 1965a). Unfertile or dead eggs appear to be acceptable as a host (Eidmann 1934, Houseweart et al. 1982, Grijpma 1973, Salt 1938, Tothill et al. 1930, Young & Hamm 1967), but Grijpma (1973) often found these to collapse upon parasitization. Mortality of older host eggs, which were unable to sustain parasite development, has been attributed to the stinging of the egg by the wasp (Dolphin et al. 1971) and to the injection of a venom (Eidmann 1934, Lee 1961, Benoit & Voegelé 1979).

Changes in sex ratio or number of eggs laid per host (clutch size) seem to be more subtle expressions of a changing susceptibility of hosts for Trichogramma. A decrease in clutch size with increasing age of the host

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has often been observed (e.g. Dolphin et al. 1971, Grijpma 1973, Juliano 1982, Pak & Oatman 1982a, Taylor & Stern 1971). Kennel-Heckel (1963) however reported a reversed trend. The influence of host age on the sex ratio of the progeny appears to be variable. Stern & Bowen (1963) found no effect, Taylor & Stern (1971) obtained a higher proportion of females from older eggs, and Juliano (1982) and Navarajan (1979) obtained a higher female proportion from younger eggs.

MATERIALS AND METHODS

Three Trichogramma strains of different species were selected from the collection (chapter 2) on the basis of a high parasitization activity at low temperature (chapter 4): strains 38 (T. maidis), 43 (T. buesi) and 57 (T. evanescens). Parasites were maintained by standard procedures on eggs of Ephestia kuehniella (chapter 3). Test females, separated in gelatin capsules, were 1-2 d old and inexperienced with host eggs prior to testing. Host eggs used in the experiments were obtained from laboratory cultures of M. brassicae, P. brassicae and P. rapae (chapter 3). Host eggs were incubated at $25\pm1°C$, $65\pm5\%$ rh and 16L:8D photoperiod; under these conditions they hatched after 3-4 d (chapter 3).

Experiments were conducted in a climate room at $20\pm1°C$ and $65\pm5\%$ rh by exposing combinations of two host ages, 0 vs. 2 and 1 vs. 3 d old (i.e. young vs. late-medium and early-medium vs. old), to individual test females in a choice test. Each test was conducted in a plastic petri dish (5.3 cm diameter) having a piece of graphpaper (2x2 cm) fixed to the center of the bottom. Twenty-five host eggs, 12 or 13 of either age, were placed on the paper (without use of an adhesive) in a draught-board pattern, 2 mm apart. Glas et al. (1981) demonstrated that 2 mm was the maximal distance between host eggs permitting rapid perception of surrounding eggs by Trichogramma females. This arrangement of hosts therefore promoted effective movement from one host to the other. Each test combination of host ages was observed at least 10 times per strain.

At the beginning of a test several females of one strain were introduced into the petri dish. The females were immediately observed and as soon as one of the females showed interest in the host eggs, the other females were removed. Observations were then continued for 1.5 h, recording

Table 5.2. Numbers of contacts and acceptances for M. brassicae, P. brassicae and P. rapae eggs of various ages by three Trichogramma strains in paired-choice tests and statistical analysis of host preference.

Strain no.	Host age (d)	No. tests	No. contacts	No. accept-ances	a/c ratio	Chi-square	P 0.05

Mamestra brassicae

Strain no.	Host age (d)	No. tests	No. contacts	No. accept-ances	a/c ratio	Chi-square	P 0.05
	0	23	68	53	0.78	0.19	NS
	2		43	35	0.81		
38							
	1	16	62	53	0.86	0.20	NS
	3		69	57	0.83		
	0	10	51	49	0.96	0.25	NS
	2		46	45	0.98		
43							
	1	10	39	38	0.97	0.69	NS
	3		30	28	0.93		
	0	10	45	43	0.96	0.58	NS
	2		48	44	0.92		
57							
	1	10	59	56	0.95	6.83	S
	3		61	48	0.79		

Pieris brassicae

Strain no.	Host age (d)	No. tests	No. contacts	No. accept-ances	a/c ratio	Chi-square	P 0.05
	0	20	76	54	0.71	0.14	NS
	2		65	48	0.74		
38							
	1	20	69	37	0.54	0.92	NS
	3		64	29	0.45		
	0	10	35	25	0.71	0.35	NS
	2		46	30	0.65		
43							
	1	10	44	31	0.71	0.21	NS
	3		41	27	0.66		
	0	10	29	24	0.83	1.38	NS
	2		37	26	0.70		
57							
	1	10	23	17	0.74	0.22	NS
	3		28	19	0.68		

Table 5.2 (continued)

Strain no.	Host age (d)	No. tests	No. contacts	No. acceptances	a/c ratio	Chi-square	P 0.05
Pieris rapae							
38	0	14	69	61	0.88	3.10	NS
	2		65	50	0.77		
	1	16	58	42	0.72	0.51	NS
	3		47	31	0.66		
43	0	10	68	54	0.79	0.03	NS
	2		62	50	0.81		
	1	10	81	50	0.62	3.53	NS
	3		63	29	0.46		
57	0	10	17	14	0.82	0.03	NS
	2		17	12	0.80		
	1	10	23	9	0.39	2.17	NS
	3		23	14	0.61		

each host contact made by the test female. A contact is defined as examination of an egg by antennal drumming (chapter 3). Rejection of a host by the female may occur at any time prior to oviposition. Acceptance of a host is defined as oviposition into the host by the parasite female (Arthur 1981). Oviposition was determined after each test by dissecting host eggs that had been attacked (chapter 3).

During observations, the durations of drumming, drilling and ovipositor insertion were recorded. Since parasitized hosts were not removed, recurrent visits to parasitized host eggs were also recorded. Preference for either host age was statistically analyzed by summing the number of acceptances and that of rejections of eggs at the first visit for each of the two ages per test and constructing a contingency table for the distribution of contacts. The acceptance/contact ratios were then evaluated by Chi-square test. Statistical analysis of mean differences in recognition times and number of eggs laid was done by Student t-test.

RESULTS

Host acceptance

Table 5.2 shows that for most tests the acceptance/contact (a/c) ratios
were high (>0.7). First, this indicates that eggs of different ages of the
tested species were contacted and examined at similar rates. Second, eggs
were, upon contact, attacked readily by Trichogramma females of each
strain. Statistical evaluation of the a/c ratios per test revealed a
significant difference ($P < 0.05$) between 1 and 3 d old M. brassicae eggs
for strain 57 only. Thus, females of this strain preferred the younger
eggs. In all other tests, preference for contact and/or acceptance of host

Table 5.3. Clutch size of progeny for three Trichogramma strains in 0-3 d
old eggs of M. brassicae, P. brassicae and P. rapae.

Strain no.	Host age (d)	Clutch size (mean + 95% CL)[1]		
		M. brassicae	P. brassicae	P. rapae
38	0	1.9 + 0.2 a	2.8 + 0.4 a	2.5 + 0.8 a
	1	2.2 + 0.2 b	2.3 + 0.4 b	2.3 + 0.4 a
	2	1.9 + 0.2 a	2.9 + 0.3 a	2.1 + 0.3 a
	3	1.9 + 0.2 a	2.0 + 0.3 b	2.3 + 0.5 a
43	0	2.3 + 0.2 a	2.4 + 0.5 a	1.9 + 0.3 ab
	1	2.7 + 0.3 b	2.6 + 0.5 a	2.1 + 0.2 b
	2	2.5 + 0.2 ab	2.4 + 0.4 a	2.0 + 0.2 ab
	3	1.6 + 0.4 c	2.8 + 0.4 a	1.7 + 0.3 a
57	0	2.6 + 0.2 a	2.2 + 0.4 a	2.5 + 0.4 a
	1	2.3 + 0.1 b	2.2 + 0.3 a	2.3 + 0.3 a
	2	2.1 + 0.1 c	2.7 + 0.4 b	2.5 + 0.4 a
	3	2.0 + 0.1 c	2.0 + 0.3 a	2.5 + 0.6 a

[1] Means per strain and host species followed by the same letter are not
significantly different ($P < 0.05$).

Table 5.4. Mean sizes of eggs of M. brassicae, P. brassicae and
P. rapae (n=10).

Species	Diameter (mm)	Height (mm)	Volume[1] (mm^3)
M. brassicae	0.61	0.58	0.11
P. brassicae	0.55	1.14	0.18
P. rapae	0.44	1.01	0.10

1) Assuming ellipsical shape: $V = 4/3 \cdot pi \cdot (1/2\ D) \cdot (1/2\ H)^2$

eggs of certain ages was not observed in any of the strains. The a/c ratios
for M. brassicae generally are higher than those for P. brassicae and
P. rapae, which suggests that the latter are less acceptable host species.

Clutch sizes for different ages of each host species are presented in
Table 5.3. Between 2 and 3 eggs were on average laid per host. Females of
strains 43 and 57 laid fewer eggs in older M. brassicae eggs, and those of
strain 43 also in older P. rapae eggs. The other host-Trichogramma
combinations did not show significant trends between host age and clutch
size. Strains 38 and 43 had the highest clutch size in P. brassicae eggs
(2.5 eggs/host), but the lowest in M. brassicae and P. rapae eggs,
respectively (2.0 and 1.9 eggs/host). Clutch size of strain 57 did not
differ much between the three host species (2.3-2.5 eggs/host). This shows
that the clutch size was not proportional to the volume of the host, which
is about two times larger for P. brassicae than for P. rapae and
M. brassicae (Table 5.4).

Parasitization behaviour

The durations of the drumming and drilling phases did not appear to be
affected by host age and therefore were averaged per host species (Table
5.5). The mean handling times (the sum of drumming, drilling and
oviposition) are presented for each host age separately in Table 5.6. The
drumming, drilling and oviposition times differed among the strains. For
instance, drumming was shortest on P. brassicae eggs for strain 38, on

Table 5.5. Mean durations (± 95% confidence limits) of drumming, drilling and oviposition behaviour by three _Trichogramma_ strains parasitizing 0-3 d old eggs of _M. brassicae_, _P. brassicae_ and _P. rapae_ at 20°C.

Strain no.	Host species	Duration of behaviour (s)[1]		
		drumming	drilling	oviposition
	M. brassicae	44.8 ± 8.8 de	98.8 ± 7.2 g	213.9 ± 24.7 d
38	_P. brassicae_	27.1 ± 3.1 a	53.9 ± 4.4 d	139.6 ± 10.0 a
	P. rapae	32.0 ± 2.0 b	54.7 ± 3.2 d	140.4 ± 7.4 a
	M. brassicae	34.8 ± 2.1 c	76.4 ± 3.2 f	226.8 ± 27.4 d
43	_P. brassicae_	38.0 ± 2.8 d	39.2 ± 2.7 b	278.4 ± 31.3 c
	P. rapae	31.6 ± 2.0 b	35.0 ± 1.4 a	160.7 ± 9.4 b
	M. brassicae	33.3 ± 1.6 bc	64.0 ± 2.9 e	185.0 ± 11.8 c
57	_P. brassicae_	50.5 ± 4.2 e	46.0 ± 4.3 c	145.7 ± 16.7 ab
	P. rapae	40.7 ± 3.4 d	51.5 ± 5.1 d	194.2 ± 53.8 cd

1) Means per strain within columns followed by the same letter are not
   significantly different (P < 0.05).

P. rapae eggs for strain 43 and on _M. brassicae_ eggs for strain 57. A
correlation between drumming time and host size (Table 5.4) is not
generally apparent. For all strains drilling required most time on
M. brassicae eggs. Strain 38 required more time for drilling, on each host
species, than the other two strains.

Oviposition time required about two thirds of the mean handling time
and had a large variation (Table 5.5). Generally, the duration of
oviposition was shortest on _Pieris_ eggs, except for females of strain 43,
which had an excessively long oviposition time for _P. brassicae_ eggs. In
some cases, there was a relationship between host age and oviposition time
(Table 5.6). For strain 43 the mean handling time increased with increasing
host age in case of _M. brassicae_ and _P. brassicae_ eggs, whereas it
decreased in _P. rapae_ eggs. In strains 38 and 57 the handling time for
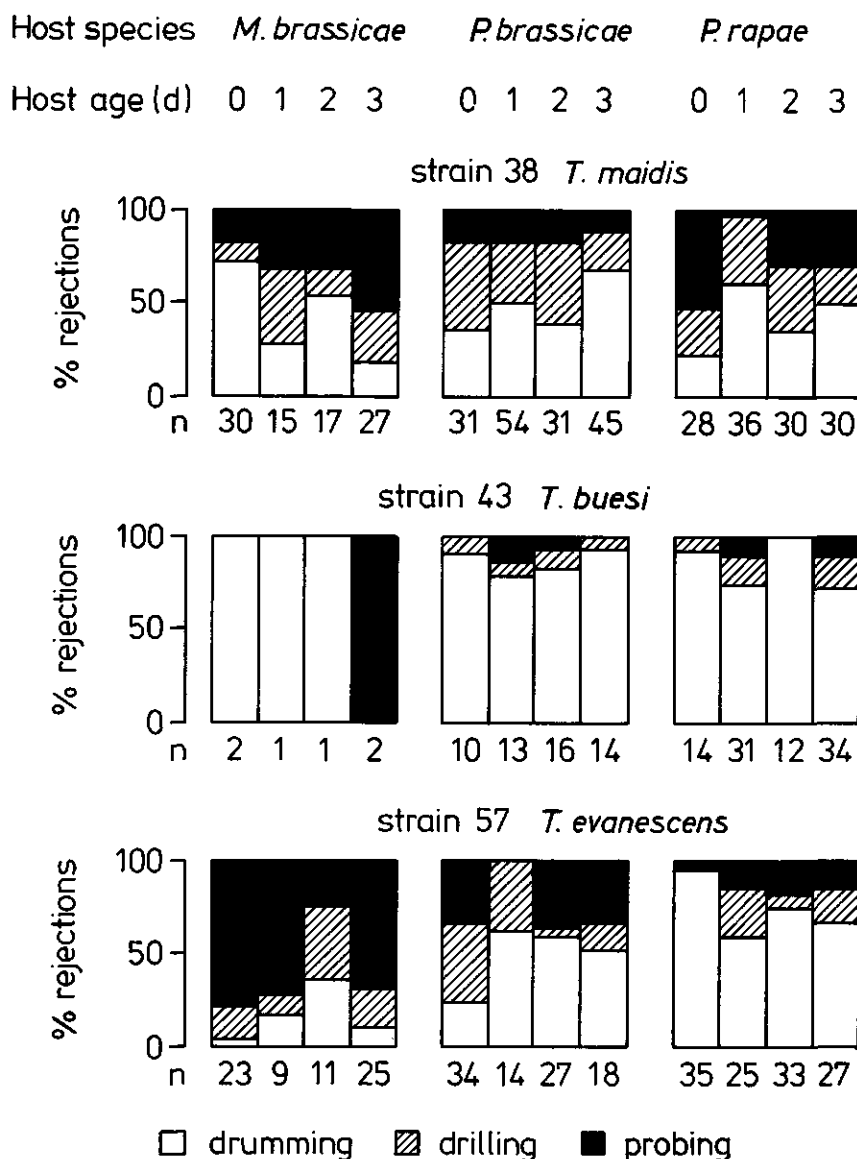_P. rapae_ eggs showed an increasing and a decreasing relationship with

Figure 5.3. Frequency distributions of rejections of unparasitized hosts in the drumming, drilling and probing (= ovipositor insertion) phases of the parasitization behaviour for three Trichogramma spp. strains on 0–3 d old host eggs of M. Brassicae, P. brassicae and P. rapae.

Table 5.6. Mean handling times (+ 95% confidence limits) for three _Trichogramma_ strains parasitizing 0-3 d old eggs of _M. brassicae_, _P. brassicae_ and _P. rapae_ at 20°C.

Strain no.	Host age (d)	Duration of handling (s)		
		M. brassicae	P. brassicae	P. rapae
	0	309.8 + 14.1 c	233.6 + 6.2 b	236.2 + 5.0 c
38	1	408.2 + 22.2 e	195.3 + 8.0 a	235.2 + 7.3 c
	2	301.6 + 14.7 bc	223.6 + 7.1 b	221.5 + 6.0 b
	3	377.6 + 17.8 d	187.6 + 10.4 a	210.1 + 7.5 a
	0	289.9 + 12.9 b	255.4 + 13.2 c	221.8 + 8.0 b
43	1	315.6 + 26.6 c	318.4 + 27.5 d	237.8 + 13.0 cd
	2	368.6 + 23.3 d	367.2 + 35.8 e	237.3 + 11.7 cd
	3	416.9 + 89.1 de	478.8 + 55.3 f	201.0 + 11.4 a
	0	265.2 + 8.0 a	243.2 + 25.3 b	236.6 + 12.6 cd
57	1	297.5 + 7.8 b	257.0 + 12.6 c	228.4 + 10.3 bc
	2	268.6 + 6.3 a	230.3 + 10.1 b	250.4 + 14.1 d
	3	291.2 + 11.2 b	248.5 + 12.0 c	351.3 + 50.2 e

[1] Means per species followed by the same species are not significantly different (P < 0.05).

increasing host age, respectively, while the clutch size remained constant.

Host eggs were rejected during any of the three major phases of the parasitization behaviour. Figure 5.3 shows the frequency distribution of rejections per phase for each host age, where probing indicates insertion of the ovipositor without egg laying. The examination process appeared to be affected by host species, but not by host age. For instance, females of strain 57 rejected _P. brassicae_ and _P. rapae_ eggs mostly in the drumming phase, whereas _M. brassicae_ eggs were usually rejected after the ovipositor had pierced the chorion (rejection by probing). Strain 43 showed a higher proportion of rejections in the drumming phase than strain 57, while strain 38 rejected comparatively many hosts in the drilling phase.

Table 5.7. Host-age relationship types (see Figure 5.1) for host acceptance and oviposition derived for eggs of <u>Mamestra brassicae</u> (Mb), <u>Pieris brassicae</u> (Pb) and <u>P. rapae</u> (Pr) and various <u>Trichogramma</u> strains.

| Strain no. | Trichogramma species | Host-age relationship type | | | | | |
| | | Acceptance | | | Oviposition | | |
		Mb	Pb	Pr	Mb	Pb	Pr
7[1]	<u>T. maidis</u>	I	IV	II-a	II-a	I	I
11[1]	<u>T. maidis</u>	I	I	I	I	I	I
38	<u>T. maidis</u>	I	I	I	VI-a	I	I
43	<u>T. brassicae</u>	I	I	I	II-a	I	VI-a
56[1]	<u>T. evanescens</u>	III-a	I	I	II-a	I	I
57	<u>T. evanescens</u>	II-a	I	I	II-a	I	I

[1] Relationships inferred from data of Van Dijken et al. (1986).

Relationship types

The proportion of hosts contacted (Table 5.2) and the proportion of hosts attacked (Figure 5.3) did not appear to be significantly affected by host age. Thus, in the present hosts and strains, contact and attack of host eggs are generally characterized by a type I relationship (Figure 5.1). Host-age relationship types for host acceptance (i.e. preference; inferred from statistical analysis of a/c ratios in Table 5.2) and oviposition (Table 5.3) are presented in Table 5.7. Beside the present strains, data are included for three other strains from the collection, which were examined by Van Dijken et al. (1986). For <u>P. brassicae</u> and <u>P. rapae</u>, type I relationships are apparent in five out of six strains for host acceptance and for oviposition. For <u>M. brassicae</u>, type I relationships are apparent in four out of six strains, and a type II and III-a in one strain each. Oviposition in <u>M. brassicae</u> eggs is generally characterized by type II-a: a reduction of clutch size for the oldest host eggs.

Table 5.8. Discrimination by three Trichogramma strains between parasitized and unparasitized host eggs of various ages for M. brassicae, P. brassicae, and P. rapae.

Strain no.	Host age(d)	Parasitized hosts			Unpar.[1] hosts	Statistical comparison	
		no. cont.	no. acc.	a/c ratio	a/c ratio	(paras. - unpar. hosts) Chi-square	P (<)
Mamestra brassicae							
	0	27	10	0.37	0.78	14.5	0.001
38	1	71	11	0.16	0.86	64.9	0.0001
	2	34	10	0.29	0.81	21.1	0.0001
	3	76	22	0.29	0.83	42.0	0.0001
43	0-3	28	1	0.04	0.96	146.2	0.0001
57	0-3	47	17	0.36	0.90	379.8	0.0001
Pieris brassicae							
	0	45	12	0.27	0.71	22.5	0.0001
38	1	17	0	0	0.54	16.0	0.0001
	2	92	41	0.45	0.74	13.3	0.001
	3	6	0	0	0.45	4.6	0.05
43	0-3	74	9	0.12	0.68	64.0	0.0001
57	0-3	66	26	0.39	0.74	20.6	0.001
Pieris rapae							
	0	41	0	0	0.88	81.4	0.0001
38	1	23	0	0	0.72	363.2	0.0001
	2	21	0	0	0.77	119.0	0.0001
	3	12	0	0	0.66	16.7	0.0001
43	0-3	146	5	0.03	0.67	154.7	0.0001
57	0-3	26	8	0.31	0.63	8.1	0.01

[1] Data from Table 5.2.

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Host discrimination

From observations of females contacting self-parasitized host eggs it was
evident that females of each strain discriminated between unparasitized and
parasitized hosts of different ages (chapter 3). The statistical
differences between a/c ratios for parasitized and unparasitized hosts of
each age are presented for strain 38 in Table 5.8. Females of strain 38
discriminated against parasitized eggs for each age of the three tested
host species (P < 0.05).
        In some tests with strains 43 and 57, too few contacts with
parasitized hosts occurred to permit statistical comparison with
unparasitized hosts of the same age. Observations for different ages of
each host species were therefore added up and then evaluated (Table 5.8).
Differences between a/c ratios of parasitized and unparasitized hosts were
highly significant for both strains on each of the three host species.
Discrimination by internal ovipositor examination was observed in strain 43
(ca. 20% of rejections), but not in strains 38 and 57. Host age and species
did not appear to affect the examination pattern. The three strains
discriminated mostly during the drilling phase (ca. 50-70%). The remainder
of discriminations occurred by antennal drumming.

Host suitability

Twenty parasitized host eggs of 1, 2 and 3 d old from the tests with
females of strain 57 were incubated at 25°C. A parasitized host egg may
yield one or more adult parasites (successful development in a suitable
host), a host larva (unsuitable host), or none if both the parasite and the
host have died (unsuitable host). Table 5.9 shows that host age of
M. brassicae, P. brassicae or P. rapae did not appear to affect survival of
the parasites. Differences in the percentages of hosts yielding parasites,
host larvae or none were not different between ages per host species.
Mortality of parasites in suitable hosts of various ages is not significant
(no differences between the mean number of eggs laid per host and the mean
number of emerging parasites per host). Adult wasps hardly emerged from
P. brassicae eggs.

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Table 5.9. Suitability of M. brassicae, P. brassicae and P. rapae eggs of various ages
(n=20) as hosts for development of T. evanescens (strain 57).

Host species	Host age (d)	Distribution of yield (%)			Progeny (mean $\pm$ 95% CL)[1]		
		adult wasps	host larvae	none	no. wasps/ suit.host	no. eggs/ host	P 0.05[2]
M. brassicae	1	75 a	5 a	20 a	1.9 $\pm$ 0.3 a	1.8 $\pm$ 0.3 a	NS
	2	75 a	5 a	20 a	2.0 $\pm$ 0.4 a	1.9 $\pm$ 0.2 a	NS
	3	85 a	5 a	10 a	2.3 $\pm$ 0.3 a	2.1 $\pm$ 0.2 a	NS
P. brassicae	1	10 b	40 b	50 b	2.5 $\pm$ 6	1.8 $\pm$ 0.6 a	
	2	5 b	40 b	55 b	2	1.4 $\pm$ 0.6 a	
	3	5 b	65 b	30 ab	1	1.5 $\pm$ 0.6 a	
P. rapae	1	90 a	10 a	0 a	1.9 $\pm$ 0.3 a	2.0 $\pm$ 0.3 a	NS
	2	75 a	5 a	20 a	2.0 $\pm$ 0.2 a	2.1 $\pm$ 0.2 a	NS
	3	75 a	5 a	20 a	2.1 $\pm$ 0.2 a	2.0 $\pm$ 0.6 a	NS

[1] Percentages or means per column followed by the same letter are not significantly
different (P < 0.05).

[2] Statistical comparison between no. wasps/suitable host and no. eggs/host to determine
immature parasite mortality.

DISCUSSION

Host age may have a profound effect on the ability of successful parasitism
for Trichogramma. The present literature review shows that the relationship
between host age and parasitism varies among host species within families,
except perhaps in the Tortricidae. Tortricid host species appear to be
characterized by a type III relationship for development of Trichogramma,
but studies were restricted to the parasite species T. minutum. Some
studies indicate variability among different Trichogramma species in host-
age relationship type for the same host species (e.g. Navarajan 1979,
Rao & Rao 1980). However, the opposite has also been reported, e.g. for

Trichoplusia ni and Papilio xuthus, for which a type IV and type V
relationship, respectively, were found (Hiehata et al. 1976, Parker
& Pinnell 1974). The distinct type IV relationship has as yet only been
found in two studies on T. ni. Types V and VI have also been observed in a
few studies only.

Host-age selection by Trichogramma spp. of lepidopterous species on
cabbage crops was previously studied little (Parker & Pinnell 1974,
Schieferdecker 1965, Schmidt 1970). The present study and that by Van
Dijken et al. (1986) together show that six strains from the present
laboratory collection differ little in host-age selection properties.
Young, medium-aged and old eggs of the three host species generally were
accepted readily as a host by females of each Trichogramma strain, whereby
preference for a certain host age was not observed. Effects of host age
were restricted to a reduced clutch size for older M. brassicae eggs.
Clutch size was not affected by host age in P. brassicae and P. rapae eggs,
except for strain 43 in P. rapae. A similar finding was reported for the
latter species by Parker & Pinnell (1974).

Host age did not affect the duration of the different phases of the
parasitization behaviour. This was also found by Hiehata et al. (1976) for
T. papilionis and T. dendrolimi females parasitizing P. xuthus eggs.
Drumming and drilling times are supposed to depend on the size of the host
and on the thickness or hardness of the chorion, respectively (chapter 3).
Apparently, these factors remain unaltered throughout development of the
host egg. For each strain, drilling was longest on eggs of M. brassicae,
which seem to have a thicker or denser egg shell (chapter 8). Drumming and
oviposition times were not uniform, but varied between strains and host
species. This indicates that clutch size is dependent on the Trichogramma
strain as well as on the host species.

Klomp & Teerink (1962) demonstrated that host size and clutch size in
Trichogramma are related and that the drumming or examining time is
proportional to host size. Schmidt & Smith (1985a) showed that the duration
of host examination is not dependent on the exposed surface area of the
egg, but on its curvature. Subsequently they found out that the wasps use
the duration of the very first part of the examination walk across a host,
the initial transit time, to set the clutch size of their progeny (Schmidt
& Smith 1987a). In the present study the clutch size was not proportional

with the volume of the egg of the different host species. Especially for
P. brassicae eggs the clutch size was lower than expected by the relative
volume. This suggests that the internal compostion of the host egg, which
probably is host specific, influences the process by which the wasps set
the clutch size. Since wasps determine host volume during the examination
walk (Schmidt & Smith 1986, 1987c), the initially determined clutch size
may be adjusted in response to the internal quality of the host, possibly
by means of (chemo)receptors on the ovipositor. For example, Marston
& Ertle (1969) reported that wasps responded to age-dependent differences in
the quality of T. ni eggs after the ovipositor had pierced the chorion of
the egg.

Host age did not appear to affect the ability of wasps to discriminate
between self-parasitized and unparasitized host eggs. Salt (1937a) showed
that discrimination may occur by external examination with the antennae
and/or by internal examination with the ovipositor. Ables et al. (1981)
found that T. pretiosum females only discriminated externally. Internal
discrimination did not occur frequently in the present study, and was only
observed for strain 43. Examinations resulting in external discrimination
were often aborted during the drilling phase, which indicates a possible
use of the ovipositor to perceive external marking pheromones (Van Lenteren
1981).

Females of Trichogramma that are adapted to a certain host (maximal
fitness) should, if they optimize their selection behaviour (Charnov
& Skinner 1985), not accept unsuitable host types when suitable ones are
available (Hubbard et al. 1982). This requires the females to have the
ability to assess the quality of each age of host and recognize unprofitable
types. Similar host-age relationship types for the different aspects of the
parasitism process should then occur within given host and Trichogramma
combinations. This seems to be true for some studies, e.g. parasitism of
Ephestia cautella by T. evanescens (Lewis & Redlinger 1969), but it is
refuted in several others. For instance, in some studies parasites laid
eggs in hosts of unsuitable ages (e.g. Juliano 1982, Parker & Pinnell 1974)
and handling times were relatively long in cases where the unsuitability of
older host eggs was not recognized externally, but only after internal
examination of the host with the ovipositor, requiring drilling through the
chorion of the host (e.g. Marston & Ertle 1969, Hiehata et al. 1976).

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For the one _Trichogramma_ strain investigated, host age did not appear to affect the profitability (progeny per unit of time) of the different host species. Handling times and parasite survival were similar for hosts of different ages. Probably, the available energy and nutrient contents of a host egg remain rather constant during embryonic development. In view of this it may be adaptive for the wasps not to prefer hosts of certain ages over others.

The lack of variability among parasite strains may suggest that the presently studied host-parasite relationship is more determined by the host species than by the _Trichogramma_ species or strain. However, this study is restricted in the number of parasite species tested: five out of six strains belong to the closely related _T. maidis_ or _T. evanescens_ (Pintureau & Voegelé 1980, Pintureau et al. 1982). These findings impede the use of host-age selection as a criterion to select between candidate strains for the present pre-introductory evaluation program. Nevertheless, the results of this study are in favour of inundative releases of _Trichogramma_ against the present host species. The general absence of host-age selection for these species provides the maximal period of time that host eggs in the field are susceptible to parasitism. Insofar as the present criterion is concerned, each tested strain seems to be an acceptable candidate strain.

Chapter 6. HOST-SPECIES SELECTION

ABSTRACT

The host-selection behaviour of nine strains of Trichogramma spp., toward
eggs of Mamestra brassicae, Pieris brassicae and P. rapae, was investigated
in laboratory experiments in order to select candidate strains for
inundative releases against these species. Experiments were carried out by
continuously observing the behaviour of individual females, which were
offered equal numbers of eggs of two host species arranged in a grid.

   M. brassicae is a highly acceptable host species for all strains,
whereas the acceptability of the two Pieris species is similar within
strains, but varies between strains. Considering the variation in
acceptance of Pieris eggs, strains either showed: (1) no preference between
Mamestra and Pieris, (2) a preference for Mamestra, or (3) an aversion for
Pieris. Females of strains from the second group showed a high acceptance
of Pieris eggs if the preferred Mamestra eggs were absent. They contacted
comparatively fewer Pieris eggs in presence of Mamestra eggs, which
indicates selection of hosts at a distance. Strains from the first group
probably are the best candidates for inundative releases.

   For a strain of T. maidis, experiments were conducted with various
ratios of M. brassicae and P. brassicae eggs (15/1, 12/4, 8/8, 4/12, 1/15),
to test whether host selection is frequency dependent. Parasitized hosts
were replaced to keep the ratios constant. Contacts with the two host
species were proportional with the offered ratios, and host selection was
independent of host frequency. Thus there appear to be different, constant
probabilities of acceptance for either of the host species, depending on
the suitability of each species as a host.

INTRODUCTION

The empirical record of (inoculative) biological control programs indicates
that most successes have been achieved with rather specific natural enemies
(Doutt & DeBach 1964, Hokkanen 1985). Due to coevolution with its host, a
host specific parasite is expected to be better adapted, in various
physiological, ecological and behavioural ways, to its host than a

generalist parasite (Huffaker et al. 1971). Furthermore, the occurrence of a hyperparasitic habit, which is a negative trait in the selection of candidate natural enemies (chapter 2), is less likely for a specialist than for a generalist parasite (Rosen & Huffaker 1983).

The notion that specialist natural enemies are better control agents than generalists seems to be coincident with the "regulation" view of biological control, which supposes that control is effected by a specific natural enemy imposing a low and stable pest equilibrium (e.g. Beddington et al. 1978, Hassell 1978). Recent model studies of host-parasite interactions suggest that generalist parasites may also provide regulation of host densities, but by different density dependent mechanisms than specialist parasites (Hassell 1986, Hassel & May 1986).

The regulation hypothesis of biological control has been challenged by field observations, which suggest that local extinctions of host or prey and their enemies may be more commonplace in permanent control situations than low, stable equilibria (Murdoch et al. 1984, 1985, Reeve & Murdoch 1985). Consequently, effective natural enemies are preventing outbreaks of pests rather than reducing their populations. For this goal both the "lying-in-wait" searching strategy of polyphagous predators and the "search-and-destroy" strategy of monophagous parasites appear to be adaptive foraging strategies (Murdoch et al. 1985).

This chapter deals with variability in host specificity among several strains of Trichogramma from a laboratory collection (chapter 2), in order to select candidate strains for inundative biological control of Lepidoptera on cabbage. The host-species selection behaviour of nine strains is investigated in choice experiments with eggs of Mamestra brassicae, Pieris brassicae and P. rapae, which are the major host species in the field. For the present evaluation program, a useful candidate strain should not be specific, but parasitize each host species equally well (chapter 2).

In chapter 5, the definition of host selection was restricted to the process by which parasites, upon host contact, select between hosts of different ages and/or species. Salt (1935) defined this process as a phase of 'psychological selection' within an overall host-selection process from habitat selection to host acceptance (Vinson 1976). The inclusion of host contact within the present definition implies that, while the parasites

select hosts, they are directly perceiving the presence of each host type by means of certain host-related stimuli, e.g. through visual, chemical or tactile cues (Arthur 1981).

Host preference in Trichogramma has been studied especially for natural hosts compared to factitious hosts used in mass production (e.g. Benoit & Voegelé 1979, Brenière 1965c, Taylor & Stern 1971, Yu et al. 1984). Investigations were carried out in the laboratory (e.g. Brower 1983, Fye & Larsen 1969) as well as in the field (e.g. Ashley et al. 1974, Boldt et al. 1973), and preference was usually inferred from the numbers of hosts found parasitized. Hase (1925) and Salt (1935) were the first students of Trichogramma to develop a notion of host preference and, moreover, the only ones to study it by observing the behaviour of the females. Hase concluded that females of T. evanescens are "pantophagous", i.e. do not exhibit host preference, whereas Salt found them to prefer larger hosts over smaller hosts. Apart from the latter idea, generalizations on host-species preference in Trichogramma apparently cannot be made (e.g. Taylor & Stern 1971, Boldt et al. 1973).

For several foraging animals it has been shown that food preference is not consistent at various ratios of the different food types, but depends on the relative frequency of each type available (e.g. Manly 1973, Lawton et al. 1974, Greenwood & Elton 1979). Frequency dependent food selection has interested ecologists as a possible mechanism enhancing the coexistence of different prey species (switching: Murdoch 1969, Murdoch & Oaten 1975) or the maintenance of polymorphisms within a species (apostatic selection: Clarke 1962, Greenwood 1969). Murdoch (1969) suggested that switching is an effect of training (or conditioning) in the predator on the first encountered prey species, and that this will only occur if the predator's innate preference is weak and, moreover, variable among individual predators (Murdoch et al. 1975). Cornell & Pimentel (1978) found that strong preference does not necessarily preclude variability among individuals and then switching may occur as well.

Contrary to switching theory, optimal host selection (a corollary of optimal foraging theory) is mostly concerned with the adaptiveness of the selection behaviour as its ultimate cause, and less with the mechanisms causing the behaviour (Pyke et al. 1977, Pyke 1984, Charnov & Skinner 1985). Optimal host-selection models predict that frequency dependent host

acceptance should be observed for the less profitable host species, in terms of fitness of progeny (Hughes 1979, Houston et al. 1980, Iwasa et al. 1984), which seems contradictory to switching or apostatic selection theory (Van Alphen & Vet 1986). Hubbard et al. (1982) however demonstrated that predictions from optimal host-selection models may resemble the results of apostatic selection experiments.

Frequency dependent host selection has been observed or suggested for some parasitic wasps (e.g. Cornell & Pimentel 1978, Van Alphen & Van Harsel 1982, Van Dijken et al. 1986, Van Strien-Van Liempt & Hofker 1985). Ashley et al. (1974) inferred a frequency independent preference in Trichogramma spp. for Heliothis zea over Trichoplusia ni eggs from field-cage data.

In the present study, host-species selection experiments with Trichogramma were conducted in the laboratory offering equal ratios of two host species to single females. Furthermore, experiments were conducted on frequency dependence of the selection behaviour in a T. maidis strain. In the latter study single females were observed at various ratios of M. brassicae and P. brassicae eggs.

MATERIALS AND METHODS

Experiments

Host eggs and parasites were obtained from laboratory cultures by standard procedures (chapter 3). Test females were reared on eggs of Ephestia kuehniella. Host eggs offered to female wasps in experiments were 0-1 d old, which is the generally preferred age of hosts in Trichogramma (chapter 5). Strains were selected on the basis of a high parasitization activity at 12°C (chapter 4) and are indicated in Table 6.1. Strain 82 was a new strain obtained from S.A. Hassan (Darmstadt), who collected it as strain G'82 near Oberklingen (FRG). This strain showed the best performance among various indigenous, geographical strains tested in cabbage fields in West Germany against M. brassicae and P. brassicae (Hassan & Rost 1985). Present test females were 1-2 d old, mated, fed and unexperienced with host eggs.

Two types of experiments were conducted, viz. preference and switching experiments. In the preference experiments equal densities of two host

Table 6.1. Numbers of paired host-choice experiments conducted with individual females of various _Trichogramma_ strains to study host-species preference between eggs of _Mamestra brassicae_ (Mb), _Pieris brassicae_ (Pb) and _Pieris rapae_ (Pr).

Strain no.	_Trichogramma_ species	Host on which collected	No. of experiments		
			Pb-Pr	Mb-Pb	Mb-Pr
1	T. evanescens Westwood	Pieris rapae	10	10	10
14	T. dendrolimi Matsumara		10		10
18	T. ostriniae Pang & Chen		4		11
24	T. exiguum Pinto & Platner	Heliothis zea	2	8	
38	T. maidis Pint. & Voegelé	Mamestra brassicae	10	10	
43	T. buezi Voegelé		11	10	10
51	T. exiguum Pinto & Platner		2		10
57	T. evanescens Westwood	Chilo sp.	8	10	
82	T. maidis Pint. & Voegelé	Mamestra brassicae	11	10	11

species were presented, without replacing parasitized eggs. In the switching experiments a range of ratios of two host species was kept constant by replacing parasitized eggs.

Preference experiments were conducted to investigate the occurrence of host preference in each strain for either of the three host species. Table 6.1 shows the number of experiments conducted per strain for each of the three pairs of host species. If no preference had been found for eggs of P. brassicae or P. rapae, usually only one of these two species was tested in combination with M. brassicae. If within a certain test combination the eggs of one species were not accepted, testing was discontinued after a few tests. Observations were conducted as in host-age selection experiments (chapter 5), under the same environmental conditions. Thus, hosts were alternatingly arranged in a 5 x 5 grid (2 mm apart, 12 and 13 eggs of either species). A single female was observed per test and observations were continued for ca. 1.5 h after the female had contacted the first egg.

Switching experiments were conducted with females of strain 82 to determine whether host preference is constant, or depends on the relative frequencies of two host species. In each experiment a total of 16 eggs of M. brassicae and P. brassicae was arranged in a 4x4 grid as above. Ratios of the two species tested were 15/1, 12/4, 8/8, 4/12 and 1/15, each of which was tested 10 times. In the 8/8 ratio the two species of eggs were arranged alternatingly, in the 12/4 ratio each row had one egg of the minor species at a different position, and at the 15/1 ratio the single egg of one species was placed in a middle row.

The switching experiments were conducted in an airconditioned room at $20\pm1°C$, $55\pm10\%$ rh and 3200 lux. Observations were carried out as in the preference experiments, except that they were continued for 1 h 15 min upon the first contact with a host egg and were recorded with a computerized event-recorder. Moreover, parasitized hosts were replaced by unparasitized ones of the same species, in order to maintain a constant host ratio in the experiment. Eggs were replaced carefully with a fine, wet brush shortly after they were parasitized, but in order to minimize disturbance this was not done until the female had begun to parasitize an other egg. Each removed egg was dissected to determine the number of parasite eggs laid (chapter 3).

Statistical analysis

The present kind of host-choice tests can be analyzed for the occurrence of host preference in a strain by applying a Chi-square test on the ratios of the sums of acceptances and rejections for the two host species (chapter 5). This method is relatively simple, but it appears to have several limitations. First, the test assumes that preference will not vary with the relative rate at which host species are encountered. Thus, two possible causes of preference are confounded: (1) different rates of encounter for different host species and (2) different rates of rejection following contact. Second, the test ignores variability in response between females of a strain. Third, if parasitized hosts are not replaced, host preference might change as unparasitized eggs of the preferred host species are gradually depleted.

According to Hassell (1978) host preference is usually measured as the

deviation of the proportion (successfully) attacked hosts to the proportion of available hosts, and may be caused by various behaviours such as differential searching rates, different times spent in various habitats, active rejection of host types and differing abilities of hosts to escape. Obviously, the first two causes are a consequence of habitat and host-finding behaviour and, therefore, are not presently considered as causes of host preference. Furthermore, eggs cannot actively escape wasp attacks, so that in the present study host preference is observed as the result of differential rejections of host species by the parasites.

Cock (1978) reviewed eight methods used to measure preference in the past, mainly in studies of predation. The empirically derived method by Manly et al. (1972) is the only one which allows for the exploitation of hosts, but it can also be applied if exploitation is negligible or if hosts are replaced as they are parasitized. Manly's index of preference B_i is the probability that the next host to be parasitized would be of type i, assuming that all types are offered in equal proportions. Index B has a scale from 0 to 1 with a value of 0.5 for no preference, values of >0.5 for preference, and values of <0.5 for aversion (i.e. preference for the other host type(s)). Chesson (1978) derived the probability distribution function of B_i, $P(\underline{B}_i=b)$ and provided maximum likelihood estimates. For the present preference experiments, the following estimator for B_i can be applied:

$$b_i = \log\ (r_i/A_i)\ \sum_{s=1}^{k}\ \log\ (r_s/A_s) \qquad (1)$$

In this equation r is the number of unparasitized hosts remaining at the end of an experiment, A_i is the number of hosts of type i available at the beginning of an experiment, and k is the number of types of host (Manly 1974). In the present experiments two host species were offered simultaneously, so that k=2 and $N=A_1+A_2=25$, which meets the restrictive condition of $N \geq 10$. For estimation of standard errors, Manly (1972) suggested to obtain at least 10 estimates (i.e. 10 experiments) of b. In order to compare the two statistical methods, preference experiments have presently been analyzed by Manly's method and by test of a/c ratios.

For the analysis of frequency dependent host-selection experiments,

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Manly (1973) proposed a model which assumes that the value of B_i is linearly related to the relative frequency of host types offered. Manly's model has been criticised by Greenwood & Elton (1979) for being unrealistic, especially in its assumption that the value of B remains constant throughout the course of each experiment. If, for instance, frequency dependent foraging behaviour is affected by prior experience, then B is likely to change as the forager learns. As an alternative, the following empirical model was proposed to analyse frequency-dependent selection experiments:

$$e_i / \sum_{j=1}^{m} e_j = (V_i \cdot A_i)^b / \sum_{j=1}^{m} (V_j \cdot A_j)^b \qquad (2)$$

In this equation V is a measure of basic relative preference (termed visibility) and b is a measure of frequency dependence; e and A are the number of hosts parasitized and the number available, respectively. Selection is independent of frequency if $b=1$, otherwise it is positively $(b > 1)$ or negatively $(b < 1)$ frequency dependent. Parameters can be estimated by logarithmic transformation of equation (2) and linear regression of log $e=e1/e2$ on log $A=A1/A2$, or by a more complicated iterative method fitting a relationship between A and $p=e_1/(e_1+e_2)$ (Greenwood & Elton 1979, 1983, Fullick & Greenwood 1979).

RESULTS

Host acceptance

Median acceptance/contact (a/c) ratios of **Trichogramma** strains for host eggs in preference experiments are presented in Figure 6.1. If a certain host egg was repeatedly contacted and rejected, only the results of the first and second encounters were included in the female's a/c ratio. No female had parasitized all eggs of one species before the exposure time had lapsed.

In all strains the a/c ratio for M. brassicae eggs was 0.7 or higher. For eggs of P. brassicae and P. rapae the a/c ratio was usually lower than

this, especially when Pieris eggs were offered in combination with Mamestra
eggs. In some strains (e.g. 1, 14, 57) this effect coincided with a low
number of Pieris eggs contacted per female. This may indicate that females
of these strains selected host eggs at a close distance without physically
contacting them. Females of strains 24 and 51, however, contacted similar
numbers of Mamestra and Pieris eggs, although the latter were hardly
accepted.

The mean number of host contacts per female varied considerably among
strains, ranging between 2 and 10 for each host species, and was highest
for strains 1, 14 and 18 (Figure 6.1). The variability in the a/c ratio was
generally larger for P. brassicae and P. rapae than for M. brassicae. As a
consequence, few significant differences occur in the a/c ratio for Pieris
between strains. Nevertheless it seems possible to classify the strains
into three groups on the basis of their acceptance of Pieris eggs, relative
to the concurrent presence or absence of M. brassicae eggs:
(1) a constant and high a/c ratio for Pieris (>0.5) - strains 38 and 82;
(2) a variable a/c ratio for Pieris - strains 1, 14, 43 and 57;
(3) a constant and low a/c ratio for Pieris (<0.5) - strains 18, 24 and 51.

Host preference

Assessment of host preference by means of statistical comparison of the
mean a/c ratios for two host species within a test is included in
Figure 6.1. A/c ratios for P. brassicae and P. rapae are not different
between strains. However, M. brassicae eggs are preferred over P. brassicae
and P. rapae eggs in all strains, except 38 and 82.

Estimates of Manly's index of preference b for the data are presented
in Table 6.2. The complement of b values for two host species in a test is
1 and each b value may range between 0 and 1. There is no host preference
if b=0.5, which was assessed statistically. For the P. brassicae - P. rapae
tests a preference is apparent for P. brassicae in strains 1 and 14, and no
preference in the other strains. For tests of M. brassicae with
P. brassicae or P. rapae, preference for M. brassicae is apparent for
nearly all strains. No preference was found in strain 38 (with
P. brassicae) and strain 82 (with P. rapae). The preference for
M. brassicae generally appears to be strong (b > 0.85), except in strain 82
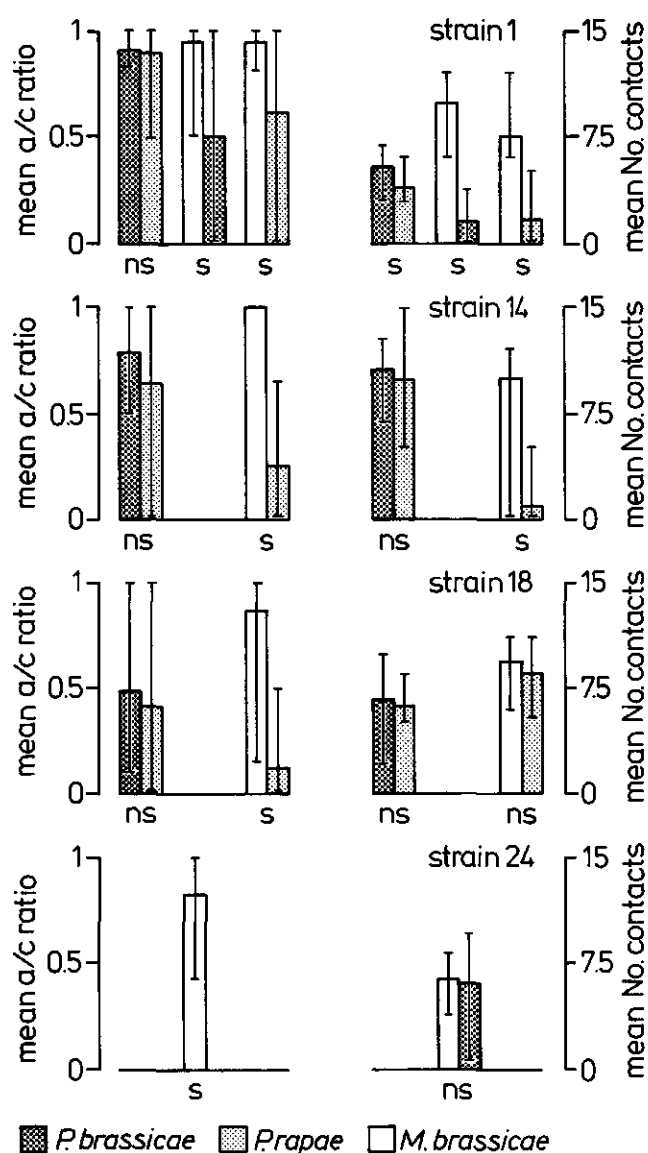
Figure 6.1. Histograms of the mean numbers of hosts contacted and the mean acceptance contact ratios (with ranges) for various strains of Trichogramma spp. in two-choice experiments with eggs of M. brassicae, P. brassicae and P. rapae. Statistical analysis of normalized data (arcsine transformation) by Student t-test.

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strain 38
strain 43
strain 51
strain 57
strain 82

mean a/c ratio
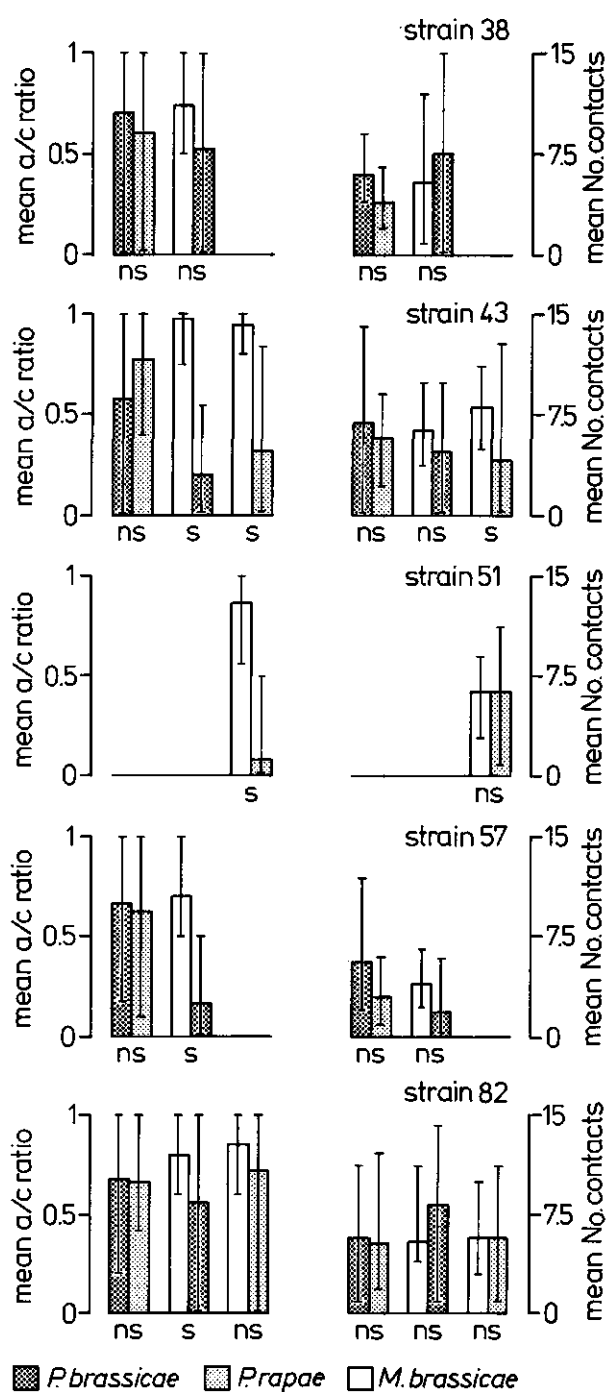mean No. contacts

P.brassicae   P.rapae   M.brassicae

Table 6.2. Manly's index of preference b (means ± 95% confidence limits) for various Trichogramma strains in paired-choice experiments offering eggs of Mamestra brassicae, Pieris brassicae or P. rapae as host species.

Strain no.	P. brassicae vs. P.rapae				M. brassicae vs. P.rapae				M. brassicae vs. P. brassicae			
	index b		95%		index b		95%		index b		95%	
	Pb	Pr	CL	Pref[1]	Mb	Pr	CL	Pref[1]	Mb	Pb	CL	Pref[1]
1	.60	.40	+.07	Pb	.90	.10	+.08	Mb	.93	.07	+.08	Mb
14	.66	.34	+.14	Pb	.95	.05	+.09	Mb				
18	.70	.30	+.26	no	.93	.07	+.08	Mb				
24									1.0	.00	+.00	Mb
38	.66	.34	+.21	no					.55	.45	+.27	no
43	.46	.54	+.16	no	.86	.14	+.11	Mb	.89	.11	+.14	Mb
51					.90	.10	+.20	Mb				
57	.65	.35	+.21	no					.91	.09	+.10	Mb
82	.50	.50	+.11	no	.53	.47	+.02	no	.66	.44	+.14	Mb

[1] Statistical analysis of host preference by means of t-test on significance of deviation of b values from b=0.5 at P <0.05; data normalized by arcsine transformation.

(b=0.66). For all strains except 38 and 82, the b-values for P. brassicae and P. rapae are significantly different (P <0.05) if M. brassicae or one of the Pieris species is the alternative test species.

The two methods used to assess host preference mostly appear to produce similar conclusions. In testing host preference between P. brassicae and P. rapae in strains 1 and 14, however, Manly's analysis produces a weak preference (b <0.7) for P. brassicae, whereas the a/c ratio test does not. Manly's method therefore seems to be more sensitive in the detection of preference than the a/c ratio comparison, which is probably due to the fact that Manly's model takes into account that the ratios of available hosts change during the course of an experiment.

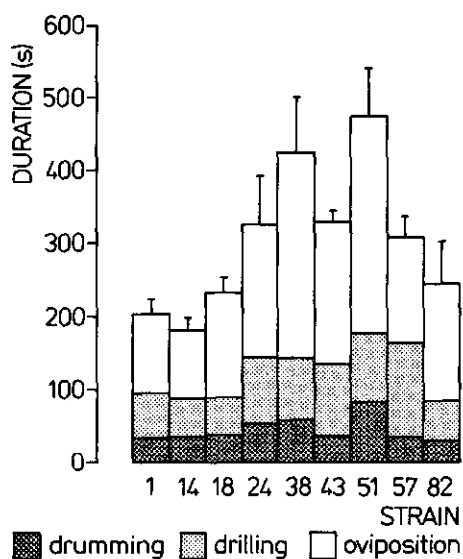Figure 6.2. Histogram of the durations of the drumming, drilling and oviposition phases of the parasitization behaviour (and 95% confidence limits for total handling time) for various strains of Trichogramma spp. on eggs of M. brassicae at 20+1°C.
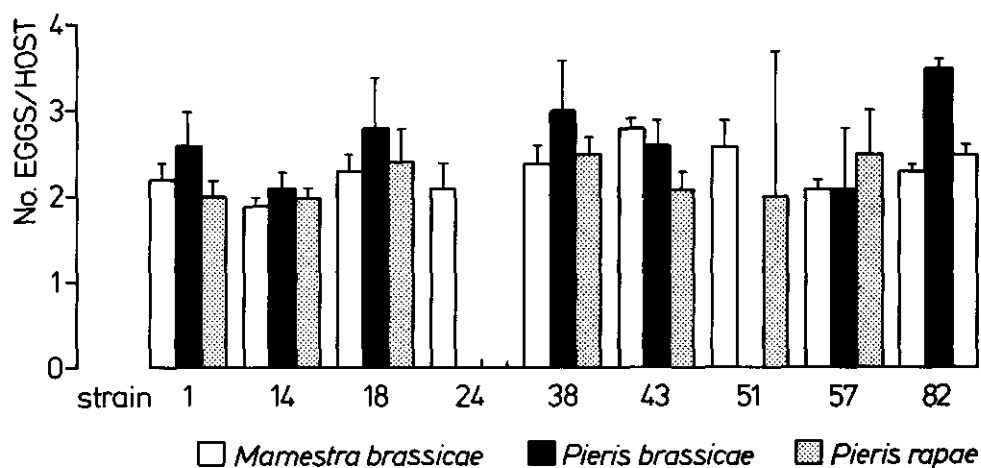

Figure 6.3. Histogram of the mean clutch size per egg of M. brassicae, P. brassicae and P. rapae (and 95% confidence limits) for various strains of Trichogramma spp.

Table 6.3. Durations of the drumming, drilling and oviposition phases of the parasitization behaviour by females of T. evanescens (strain 1) and the clutch size per egg of M. brassicae, P. Brassicae and P. rapae in paired-choice tests on host-species preference at 20+1°C.

Host species	Duration of behaviour (s)[1]						Clutch size no. eggs/ host egg mean+95%CL
	Drumming		Drilling		Oviposition		
	mean+95%CL	n	mean+95%CL	n	mean+95%CL	n	
P. rapae	39.2+5.4c	58	36.5+5.8bc	56	135.4+38.2cd	44	2.1+0.4bc
P. brassicae	48.7+3.8d	140	40.6+2.2c	134	126.7+13.4c	112	3.0+0.5d
P. rapae	26.6+10.8ab	24	30.3+9.8ab	22	86.5+44.2abc	19	1.6+0.4a
M. brassicae	36.6+2.8bc	137	81.3+5.4e	145	114.5+12.0bc	130	2.3+0.2c
P. brassicae	27.8+7.4a	21	30.0+2.8a	20	92.0+17.8a	17	1.7+0.4ab
M. brassicae	33.3+4.4ab	153	62.3+4.4d	156	107.5+12.8abd	136	2.1+0.3bc

[1] Means per column followed by the same letter are not significantly different (t-test, P < 0.05); CL = confidence limit.

Parasitization behaviour

Variability in handling time among strains is demonstrated by the durations of the major phases of the parasitization behaviour for eggs of M. brassicae (Figure 6.2). Females of strains 1 and 14 can be characterized as relatively fast, having the shortest times for each separate phase and ca. 200 s total handling time. Females of these strains also contacted more host eggs in a test than females of the other strains (Figure 6.1). Females of strains 38 and 51 were slowest, requiring more than twice as much time as the former strains to parasitize an egg of M. brassicae. The clutch size generally averaged between 2 and 3 eggs per host (Figure 6.3) and appears to correspond with the size of the host (chapter 5) in strain 82 only.

In strains with a variable acceptance of Pieris, an interaction was apparent between the clutch size for Pieris eggs and the presence of the preferred host species, M. brassicae. For instance, females of strain 1

Table 6.4. Distribution of first contacted host species in paired-choice tests on host preference of various <u>Trichogramma</u> strains for eggs of <u>M. brassicae</u>, <u>P. brassicae</u> or <u>P. rapae</u>.

Strain no.	<u>P. brassicae</u> vs. <u>P. rapae</u>				<u>M. brassicae</u> vs. <u>P. rapae</u>				<u>M. brassicae</u> vs. <u>P. brassicae</u>			
	n	nPb	nPr	P=0.10[1]	n	nMb	nPr	P=0.10[1]	n	nMb	nPb	P=0.10[1]
1	10	5	5	NS	10	9	1	S	10	10	0	S
14	10	8	2	NS	10	9	1	S				
18	4	2	2	NS	10	9	1	S				
24									10	10	0	S
38	10	10	0	S					10	5	5	NS
43	11	5	6	NS	10	9	1	S	10	6	4	NS
51					10	9	1	S				
57	8	7	1	S					10	6	4	NS
82	11	7	4	NS	11	7	4	NS	10	8	2	NS

[1] Fisher exact test.

laid more eggs in <u>P. brassicae</u> and <u>P. rapae</u> eggs when these were offered together, compared to offering one of these species in combination with eggs of <u>M. brassicae</u> (Table 6.3). The durations of the drumming and drilling behaviour were influenced by a similar interaction (Table 6.3). In all strains except 82, a disproportional number of females contacted the preferred host species first in tests presenting a choice between eggs of <u>M. brassicae</u> and <u>P. rapae</u>. By contrast, if the choice was between <u>M. brassicae</u> and <u>P. brassicae</u>, this phenomenon occurred in strains 1 and 24 only (Table 6.4). This behaviour of singling out the preferred species apparently continued throughout the test in strains with a variable acceptance of <u>Pieris</u> eggs (1, 14 and 43), but not in the strains with a low acceptance of <u>Pieris</u> eggs (18, 24 and 51; Figure 6.1). Ratios of the first contacted host species in the <u>P. brassicae</u> – <u>P. rapae</u> tests were either unbiased or, for strains 38 and 57, in favour of <u>P. brassicae</u>. Females of the latter strains possibly were initially attracted to the larger host

Table 6.5. Acceptance/contact ratios of T. maidis (strain 82) for the
first contact with eggs of M. brassicae, P. brassicae and P. rapae in
paired-choice host preference tests and for recurrent contacts with
eggs which were rejected at the first or second encounter.

| Host species | Encounters | | | | | |
| | First | | Second | | third and more | |
	n	a/c ratio	n	a/c ratio	n	a/c ratio
M. brassicae	96	0.91 a	10	0.80 a	2	0 b
P. brassicae	53	0.71 a	33	0.33 b	18	0.16 b
P. rapae	80	0.70 a	13	0.54 ab	9	0.11 b

Ratios within rows followed by the same letter are not significantly
different (Chi-square test, $P < 0.05$).

eggs, but in subsequent encounters they made similar numbers of contacts
with the different host species (Figure 6.1).

Females of most strains rejected unparasitized hosts during short
antennal contact. The percentage of rejections made during ovipositor
contact was usually less than 30, except for strain 43 on eggs of
P. brassicae. Females of the latter strain did not reject any eggs of
P. rapae in the drilling or oviposition phase.

Females of all strains showed the ability to discriminate between
parasitized and unparasitized hosts (chapter 3). For each of the three host
species, the a/c ratios for recurrent visits to self-parasitized hosts were
lower than 0.1 for most strains and below 0.3 for all strains. Parasitized
M. brassicae eggs were usually rejected during antennal contact. Rejection
during ovipositor contact was comparatively more common for parasitized
eggs of P. brassicae and P. rapae.

Host eggs that were rejected at first encounter, and thus remained
unparasitized, could be accepted at subsequent encounters. For instance,
females of strain 82 had a similar acceptance of hosts at first encounter
and at second encounter (with initially rejected hosts) for eggs of

Table 6.6. Numbers of hosts contacted and the acceptance contact ratio (a/c) per female of T. maidis strain 82 (means ± 95% confidence limits) for eggs of M. brassicae (Mb) and P. brassicae (Pb) offered in various ratios (N=16) to individual females (n=10).

Ratio Mb/Pb	No. hosts contacted	Mamestra brassicae		Pieris brassicae		Comparison a/c ratio
		Contacts	a/c ratio	Contacts	a/c ratio	
1/15	8.9+2.3a	0.5+0.7a	.66+.41ab[1]	8.4+2.1d	.52+0.20a	NS
4/12	11.5+2.4b	4.0+1.8b	.76+.25ab	7.5+1.7d	.43+0.22a	S
8/8	10.3+1.2a	4.8+1.0b	.82+.15b	5.5+1.2c	.58+0.12a	S
12/4	11.9+1.8b	9.0+1.7c	.82+.13b	2.9+0.8b	.54+0.16a	S
15/1	12.7+2.3b	12.3+2.1d	.61+.15a	0.4+0.4a	0[2]	

Means per column followed by the same letter are not significantly different (t test, P < 0.05).

[1] 3 females contacted one or more M. brassicae eggs.

[2] 4 females contacted a P. brassicae egg.

M. brassicae and P. rapae (Table 6.5). The acceptance of hosts rejected twice or more was low for each host species. This indicates that the acceptance of (unparasitized) host eggs may be more dependent on the motivation of the parasite to accept a given host for egg laying, than on certain (negative) attributes of the host due to which it is rejected.

Switching

The results of the switching experiments with females of strain 82 are presented in Table 6.6. The variability in the median number of hosts contacted per female between the tested host ratios (8.9-12.7) seems to be unrelated to the host ratio. The regression between the percentage of Mamestra eggs offered and the percentage of contacts with this species at each ratio (Figure 6.4) is highly significant (Y=1.0X+1.37, r=0.99, t=51.3, P< 0.001). This indicates that the distribution of contacts was constant for all ratios and that, due to the positive intercept, it was
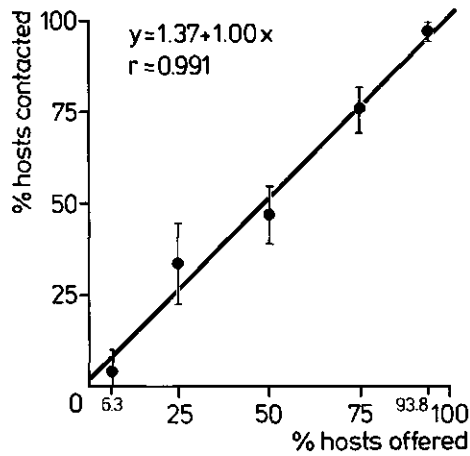
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Figure 6.4. Regression of the percentage of M. brassicae eggs contacted (mean ± 95% confidence limit) on the percentage of these eggs offered to females of T. maidis (strain 82) in host-selection experiments with various ratios of M. brassicae and P. brassicae eggs.
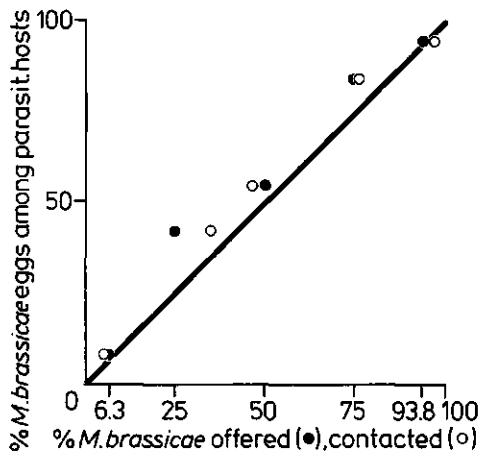

Figure 6.5. Relationship between the percentage M. brassicae eggs of the total number of hosts accepted for parasitism and the percentage M. brassicae eggs offered or contacted. The solid line represents the relationship for constant acceptance and absence of preference.

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Table 6.7. Parameters of Greenwood & Elton's model for analysis of frequency dependent host selection by females of T. maidis (strain 82) offered various ratios of M. brassicae (Mb) and P. brassicae (Pb) eggs (host 1 and 2 respectively).

Ratio Mb:Pb	Ratio of hosts contacted[1]		Ratio of hosts parasitized[2]	
	mean	SD	mean	SD
1:15	0.056	0.103	0.111	0.314
4:12	0.659	0.728	1.136	1.401
8:8	0.989	0.627	1.71	1.90
12:4	3.72	2.11	5.95	3.95
15:1	30.75[3]		70[3,4]	

[1] A=A1/A2; 2) e=e1/e2; 3) data pooled for all females; 4) assuming one Pieris egg parasitized.

slightly biased toward eggs of Mamestra. Since the regression hardly differs from a proportional distribution of contacts, the wasps probably did not select host eggs at a distance prior to making physical contact with an egg.

Figure 6.5 shows the relationship between the percentage M. brassicae eggs among the total number of accepted eggs (of both host species) and the percentage of M. brassicae eggs that was offered, as well as the percentage of eggs that was contacted. The first relationship appears less uniform than the last. To analyse the data for the occurrence of frequency dependence in the process of host selection, parameters were computed for Greenwood & Elton's model (Table 6.7). The probabilities of host encounter were determined from the distribution of contacts with the two host species as observed in the tests. Several females did not contact or accept an egg of the less abundant host species of the two extreme ratios, which would impede evaluation of the model due to divisions by zero in the equations. At the extreme ratios the data for individual females therefore were
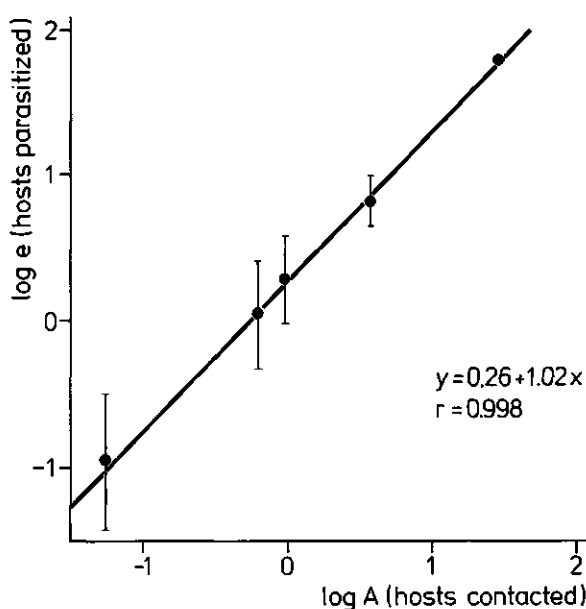
Figure 6.6. Analysis of frequency-dependent host selection in T. maidis (strain 82) by means of Greenwood & Elton's model. Regression of log e (ratio of M. brassicae/P. brassicae eggs parasitized) on log A (ratio of M. brassicae/P. brassicae eggs contacted).

pooled, and at least one Pieris egg was assumed to be accepted at the ratio 15 Mamestra/1 Pieris.

The model is evaluated by logarithmic regression of the ratio of hosts parasitized (M. brassicae/P. brassicae) on the ratio of hosts offered (Figure 6.6), which is highly significant ($r=0.998$, $t=27.3$, $P < 0.001$). The slope of the regression ($b=1.02\pm0.12$), is not significantly different from $b=1.0$, which suggests that host preference was constant at various host ratios, i.e. frequency independent. Thus, no switching occurred in the present series of experiments. This conclusion is supported by the analysis of variability in preference for M. brassicae between females at an equal (8/8) host ratio (Figure 6.7). The frequency distribution appears to be unimodal and normal. By contrast, two separate peaks would be expected if females either strongly preferred M. brassicae eggs or P. brassicae eggs.
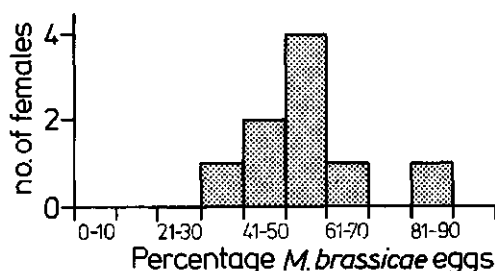
Figure 6.7. Frequency distribution of females of T. maidis (strain 82) for the percentage of M. brassicae eggs among the accepted hosts. The females were offered equal numbers of M. brassicae and P. brassicae eggs.

DISCUSSION

Host preference

The present study demonstrates that M. brassicae eggs are acceptable hosts for females of all tested strains (acceptance/contact ratio > 0.7). Except for strains 38 and 82, all strains showed a preference for eggs of M. brassicae over those of P. brassicae or P. rapae. Among the latter strains there are several (1, 14, 43 and 57), however, that seem to accept Pieris eggs as well as Mamestra eggs if the latter are not present. In two strains (24 and 51) females seem to have a constant aversion for Pieris eggs, which was unaffected by the presence of Mamestra eggs. The behaviour of strain 18 appeared intermediate between a variable acceptance and a constant aversion of Pieris eggs.

Van Dijken et al. (1986) conducted a similar study on the present host species and three other Trichogramma strains (7, 11 and 56) from the present collection of laboratory strains (chapter 2). The behaviour of strain 11 was unselective and is comparable to that of strains 38 and 82. Strains 7 and 56 showed a variable acceptance of Pieris eggs, such as strains 1, 14, 43 and 57 in the present study.

This study suggests that host preference in Trichogramma may be determined by host-specific acceptance levels, which, among other factors, depend on what types of host are available. In phytophagous insects,

selection of host plants (for oviposition) has been explained by a time-dependent acceptance threshold for different host types which is set by: (1) a rank order of specificity, and (2) the specificity of selectivity, which are, respectively, the order and the rate at which host types become acceptable since the last oviposition (Singer 1982, Papaj & Rausher 1983). A similar dual mechanism might be adopted as a model for the presently observed selection behaviour: (1) a rank order of selectivity by which host types are preferred and (2) a certain specificity of selectivity, which determines the acceptance level for each rank (relative to the number and kinds of available ranks). The latter could be fixed for high and low ranking hosts, and vary for medium ranking hosts.

In strains showing a variable acceptance of Pieris eggs, the lower acceptance of the latter if females could choose eggs of the other Pieris species instead of Mamestra eggs, appeared to coincide with a decrease in the number of contacts with Pieris eggs. This was probably due to innate selective behaviour by which Pieris eggs are avoided or rejected at a distance, or by an increased attraction to Mamestra eggs. Selection of hosts without physical contact was not apparent in strains that did not show preference (38 and 82) and those that had an aversion of Pieris eggs (24 and 51). This suggests that short-range host selection may be linked to a variable "specificity of selectivity", and that non-hosts are not recognized from a short distance. There may be a causal relationship between few contacts with Pieris eggs and a low acceptance level in females, if oviposition experience with Pieris increases the tendency of females to accept this species.

Polyphagy

In the past, the Trichogramma genus has been noted for its polyphagy by several authors (e.g. Hase 1925, Clausen 1940, Fulmek 1955, Schieferdecker 1965). Fulmek (1955) recorded 153 host species for the European T. evanescens and 232 species for the American T. minutum. About 80 percent of the host species was from the Lepidoptera, the remainder from the Diptera, Coleoptera, Hymenoptera, Neuroptera and Rhynchota.

Few species of Trichogramma were recognized prior to the seventies (Flanders 1937, Nagarkatti & Nagaraja 1977). Since then, the identification

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of new species has been prolific (Voegelé & Pintureau 1982) and some
species appear to be host selective. For instance, T. nubilale has only
been collected from eggs of Ostrinia nubilalis in corn fields in the
eastern United States (Ertle & Davis 1975). In the laboratory, T. nubilale
preferred O. nubilalis eggs over those of six common American Lepidoptera
(Curl & Burbutis 1978). Especially noctuid and pyralid species were
parasitized under no-choice conditions. These findings and the presently
observed differences in host-selection behaviour between strains and
species of Trichogramma suggest that polyphagy in this genus is a
facultative trait. Alternatively, certain Trichogramma strains or species
may be considered as opportunistic specialist parasites.

The ready acceptance of Mamestra eggs by all strains tested here
corroborates the finding of Kot (1964) that the Noctuidae appear to be
universally acceptable hosts for Trichogramma. Besides, he found that
females of T. evanescens were less selective against eggs of P. brassciae
or P. rapae than females of T. embryophagum or T. cacoeciae. The least
selective strains from the present study, 38 and 82, (and also strain 11)
belong to T. maidis, a species closely related to T. evanescens (Pintureau
& Voegelé 1980, Pintureau et al. 1982). These strains were collected from
M. brassicae eggs. Strains 1, 7 and 56 (also T. maidis/T. evanescens) were
collected from P. rapae eggs and, in contrast, showed a variable acceptance
of Pieris eggs. Thus, a strain does not necessarily prefer the host it was
collected on. Contrary to Pieris eggs, eggs of M. brassicae, and perhaps
any noctuid species, generally seem to be an acceptable alternative (novel)
host to Trichogramma.

Frequency dependence

Switching (sensu Murdoch) was not apparent in females of strain 82 offered
various ratios of M. brassciae and P. brassciae eggs. The observed
preference for M. brassicae eggs in this study seems weak and not highly
variable between females. A low variability in host preference between
females would correspond with the conditions that supposedly prevent the
occurrence of switching (Murdoch et al. 1975, Cornell & Pimentel 1978).

The selection behaviour of strain 82 seems to be adapted to the
difference in host suitability between M. brassicae and P. brassicae

(chapter 8) in a relatively simple way, by means of different acceptance levels, but not in a more complex way by which the frequencies of the hosts are also taken into account (e.g. Houston et al. 1980). In the preference experiment, strain 82 showed a constant (and high) acceptance of Pieris eggs. By contrast, strains with a variable acceptance of Pieris eggs might show frequency dependent selection when tested similarly.

Experimental constraints influencing parasite behaviour may have confounded the results of this switching study. For example, Morrison & Lewis (1981) studied patch-time allocation in T. pretiosum and suggested that the decision to leave a patch is dependent on the ratio of encounters with parasitized and unparasitized hosts. In the present experiments, females did not encounter parasitized hosts, which may have given them a notion of an unlimited host supply for both host species at each ratio tested.

In the present experimental set-up the foraging process probably did not involve decisions by the wasps on what patch to forage in, but was reduced to decisions on what hosts to accept. Foraging might be frequency dependent at the patch level (Hassell & Southwood 1978), which should be investigated in experiments involving spaced out patches of different hosts. Van Dijken et al. (1986) found that, in comparison with a grid arrangement of host eggs, clustering may increase the variability in host-species acceptance between females, because they tend to predominantly parasitize eggs in the first encountered cluster. Clustering of host eggs has also been found to influence the clutch size allocated per host egg by Trichogramma (Schmidt & Smith 1985b, 1987b).

Evaluation of candidate strains

Variability in host-species selection between Trichogramma spp. strains was studied as a criterion to select strains for inundative biological control. The common occurrence of more than one host species in a given field crop probably demands use of a strain which will accept any of the three species. In this respect, strains 38 and 82 are most suitable for field release, since females of these two strains hardly showed preference and had a constantly high acceptance rate for all host species. Strains with a constant aversion of Pieris eggs (24 and 51) cannot be considered as

control agents for this situation. However, the strains with a variable acceptance of Pieris eggs may have good control capabilities despite their preference for Mamestra eggs. Females of these strains accept Pieris eggs as a host if the preferred species is not present. Other than in our experimental patches, host species usually occur in different patches in the field and this distribution pattern might result in high acceptance levels for Pieris eggs.

In females preferring Mamestra eggs over Pieris eggs, two types of behaviour seem to be possible for encounters with an egg cluster of the latter. In the one type, the female leaves the patch after the first contacted egg (of Pieris) is rejected. If thereafter she does not return to the patch, e.g. due to finding a preferred egg cluster, the Pieris cluster will remain unparasitized (Van Dijken et al. 1986). Alternatively, the first encountered host may be accepted, or the female may return to the cluster and then accept an egg. If the second type of behaviour prevails among females of a strain, it is possible that egg clusters of Pieris become parasitized. However, among the strains studied by Van Dijken et al. (1986), this behaviour was only apparent for females of the one strain (no. 11), which did not show a preference for M. brassicae eggs if host eggs were arranged in a grid. Strains with a variable acceptance of Pieris eggs should be studied in tests involving a natural host distribution, with different host species on different leaves, to predict their potential propensity for parasitism of Pieris eggs under field conditions more accurately.

Chapter 7. HOST RECOGNITION

ABSTRACT

Acceptance of eggs of Pieris brassicae varies among strains of
Trichogramma, whereas Mamestra brassicae eggs appear to be highly
acceptable as a host to most strains. In order to explain how Trichogramma
females distinguish between these host species, a strain of T. buesi
(no. 4) and one of T. maidis (no. 11), with a low and a high degree of
accepting P. brassicae eggs, respectively, were selected to study the
factors which determine host recognition. The behaviour of individual
females was continuously observed in an arena containing one kind of natural
hosts, or glass-model hosts which have received various treatments.

   A recognition kairomone from the host-egg surface, possibly a
substance from the host's accessory-gland secretion, as well as the size,
colour and texture of the host, but not its shape, were found to play a
role in the host-recognition process. Females of the two strains showed
different acceptance criteria for these factors, in accordance with their
respective host-species acceptance behaviour. For T. buesi, the
P. brassicae recognition kairomone, a yellow host colour and a large host
size each had a negative influence on host acceptance, whereas these
features were positive or neutral for T. maidis. This difference in host-
recognition behaviour seems adaptive in light of the difference in
suitability of P. brassicae eggs for development of these two parasite
strains. The apparent generalist or opportunistic host-selection strategy
found in Trichogramma is discussed in comparison with the specialist
strategy occurring in the egg-parasite genus Telenomus (Scelionidae).

INTRODUCTION

Host recognition by parsitic wasps is the process whereby, upon contact
with a host, a female accepts or rejects the host for oviposition (Arthur
1981, Weseloh 1974). Several physical factors have been found to play a
role in the recognition process, such as the shape, size, texture and
colour of the host and also movement and sound made by the host (Vinson
1976). Arthur (1981) stressed the importance of volatile and nonvolatile

chemicals as recognition cues. Salt (1934, 1935) was the first researcher who conducted well-planned experiments, on the egg parasite Trichogramma, to investigate the host-examination behaviour of parasitic Hymentoptera. Up until then, ovipositing females were supposed to choose their hosts by some kind of instinct, and not by definable and measurable criteria. Salt found that for females of T. evanescens size was the most important criterion for host acceptance.

This chapter deals with host-recognition factors involved in acceptance and rejection of eggs of Mamestra brassicae and Pieris brassicae as a host by Trichogramma. In the previous chapter it is shown that several Trichogramma strains from the collection of laboratory strains (chapter 2) have a preference for the white and spherical M. brassicae eggs over the yellow and spindle-shaped eggs of P. brassicae or P. rapae. This raises the question of how the wasps distinguish between these two host species. Since M. brassicae and P. rapae eggs are of similar size, it appears that more factors should be involved in the recognition process than size alone (Salt 1935).

For egg parasites, hosts may vary greatly in size and shape. Hosts therefore differ in the quantity of nutrients and possibly also in nutritive quality (Strand 1986). Various host-selection (monophagous – polyphagous) and reproduction strategies (solitary – gregarious) occur among egg parasites (Bin et al. 1984). Depending on these strategies, ovipositing females can be faced with two questions concerning the allocation of progeny: first, is this the right kind of host, and second how many eggs to lay into this host (Charnov & Skinner 1985). Optimal foraging theory assumes that foragers can make decisions on such questions, and that their behaviour is optimized toward maximal reproductive fitness (Pyke et al. 1977, Pyke 1984).

For Trichogramma, the problem of optimal progeny allocation, and its conseqences for the behaviour of the wasps, was studied by Klomp & Teerink (1967, 1978). Reproduction in this genus is facultatively gregarious, and progeny of greatest fecundity and longevity is obtained if a female lays one egg per host. However, laying few eggs per host may give rise to a risk for a female of not finding a sufficient number of hosts for all of her progeny. On the other hand, laying many eggs per host may result in larval mortality and reduced fecundity of emergents. The authors concluded that a

female should allocate her progeny in such a way as to maximize the number of progeny per host and minimize competitive losses among the progeny. This strategy may explain the occurrence of a mechanism in Trichogramma to measure the size of host eggs, which these authors had proven previously (Klomp & Teerink 1962).

Parasites searching for hosts encounter various objects that require examination to determine their acceptability as a host. Both a female's accuracy in the recognition of a host type (i.e. the probability to be mistaken) and the time she needs for the examination process (i.e. the host-recognition or handling time) may influence her foraging efficiency (Hughes 1979, Livdahl 1979). Optimal foraging models predict that the host-selection strategy of a parasite will be affected by the recognition accuracies and the recognition times for different host types, since these, among others, determine the ranking of each host type on the host-profitability scale for the parasite (Hughes 1979, Pyke et al. 1977).

Hirose (1982) demonstrated the effect of recognition time on foraging efficiency for females of T. papilionis, which only parasitize fresh eggs of Papilio xuthus. Older eggs are recognized internally, after repeated drilling of the chorion, and a considerable amount of time seems to be spent on unsuitable hosts. Factors affecting internal host recognition by Trichogramma are especially investigated for the development of acceptable media for in vitro rearing, with the aim to decrease the costs of mass production (e.g. Hoffman et al. 1975, Liu & Wu 1982, Nettles et al. 1983, 1985, Rajendram 1978, Strand & Vinson 1985).

This chapter is primarily concerned with factors affecting the external recognition of hosts by Trichogramma. Size, shape and colour of the host are obvious physical differences between Mamestra and Pieris eggs (chapter 2). The influence of these factors on host acceptance was investigated for two Trichogramma strains differing in acceptance of P. brassicae eggs. In addition to this, the influence of egg-surface chemicals of the two host species as possible host-recognition kairomones (e.g. Vinson 1975, Du et al. 1982, Strand & Vinson 1982a, Leonard et al. 1987) was investigated. The findings on the different host-recognition factors are discussed in relation to the generalist host-selection strategy of Trichogramma, which is compared with the specialist strategy found in the egg-parasite genus Telenomus (Scelionidae) (Strand & Vinson 1983b,c).

MATERIALS AND METHODS

Parasites and hosts

Initial experiments were conducted with strains 1 (T. evanescens), 4
(T. buesi), 11 (T. maidis) and 27 (T. minutum) from the collection
(chapter 2). Test females were 0-1 d old, mated and fed with honey. They
were experienced with hosts by providing a few of them together with
several host eggs for 30-60 min prior to the start of a test. Unless stated
otherwise, test females were experienced on the same host species as they
were tested on afterwards. The number of females per test usually was 40,
otherwise it varied between 30-60. Females were used once in a test.
    Natural hosts used in the experiments were eggs of M. brassicae and of
P. brassicae from laboratory cultures (chapter 3). Glass beads of various
sizes were used as model hosts. The kinds of host objects used in the
different experiments on specific host-recognition factors (viz. accessory-
gland secretion, size, shape and colour), their sizes and treatments, as
well as the Trichogramma strains tested are presented in Table 7.1.
    Ovarial eggs were dissected from the paired oviducts of adult host
females. Such eggs are not coated with the accessory-gland secretion, which
supposedly is deposited when the eggs are passing through the common
oviduct (e.g. Berry 1968, Callahan & Cassio 1963, Strand & Vinson 1983a).
Solutions of accessory-gland secretion of both species were obtained as an
egg-wash by rinsing 500 eggs in 2 ml methanol for 5 min (Klijnstra 1985).
Glass beads were immersed in an egg-wash solution for 30 min and then air-
dried for 1 h in order to coat them with the secretion. For coloration,
glass beads were coated with Humbrol enamel paint no. 22 or 66 to make them
white or yellow, respectively. Coloured beads were not used for at least a
month after painting to let paint volatiles evaporate.

Observations

Experiments were conducted in a climate room at $20\pm1°C$ and $70\pm5\%$ rh. Each
test began by introducing a test female into an arena of 2 cm diameter
(Munger cell) containing 9 host objects of one kind (eggs or model hosts).
One object was placed in the center of the arena, while the other eight

Table 7.1. Features of host objects in experiments on different host-recognition factors for various strains of the egg parasite _Trichogramma_.

| Experiment number | Factor or process studied | Host objects[1] | | | | Strains |
		Nature and treatment	Height (mm)	Width (mm)	Volume[2] (mm^3)	
1	Acceptance of natural host eggs	eggs of _M.b._	0.60	0.68	0.16	
		eggs of _P.b._	1.16	0.52	0.25	1,4,11,27
2	Accessory-gland secretion	normally laid and ovarial eggs of _P.b._ and _M.b._				4, 11
3	Host size	glass beads	0.58	0.58	0.10	
			0.97	0.97	0.46	
			1.21	1.21	0.93	4, 11
			2.55	2.55	8.68	
4	Host colour	yellow and white beads	0.58	0.58	0.10	4, 11
5	Host shape	glass beads	0.58	0.58	0.10	
		glass spindles	1.04	0.43	0.15	4
6	Egg laying	half-embedded and fully ex-posed _P.b._ eggs			0.25	4
7	Host suitability	eggs of _M.b._ and _P.b._			0.16	
					0.25	4, 11

[1] _M.b._ = _Mamestra brassicae_, _P.b._ = _Pieris brassicae_.

[2] Volume _M. brassicae_ = V_{sphere} = $4/3 \cdot pi \cdot r^3$ (r = 1/2 . width)

Volume _P. brassicae_ = $V_{cylinder}$ = $pi \cdot r^2 \cdot h$

were placed around it on a circle, 3 mm apart. The female was observed continuously from the start under a dissecting microscope and 10000 lux

Table 7.2. Time boundaries for sequential levels of host acceptance in host-recognition experiments with the egg parasite _Trichogramma_; see text for explanation.

Acceptance level	Time boundaries for the observed phases of the host-examination behaviour
contact	physical contact – drumming ≤ 5 s
drumming	drumming > 5 s – drilling ≤ 10 s
drilling	drilling > 10 s – oviposition ≤ 10 s
oviposition	oviposition > 10 s or
	drilling > 60 s (on glass model hosts)

illumination, and her behaviour toward the first encountered host was recorded. The duration of the different phases of host examination was recorded (chapter 3) and if this first host was accepted observations were finished. Otherwise, they were continued until a host was accepted. The maximum number of observations was 10 encounters per host type.

Host acceptance is here referred to as acceptance for oviposition, i.e. the occurrence of egg laying into the host (Arthur 1981). Table 7.2 shows the time boundaries between the major phases of the parasitization behaviour, which were chosen as criteria to distinguish between different hierarchical levels of host acceptance: contact, drumming, drilling and oviposition. After mounting a host egg, it takes a female about 5 s (on a host of ca. 1 mm) to walk drumming across the egg in a straight line over the top. If the host is accepted at this first examination, the actual drumming period begins. If the host then is accepted further, this period includes the first 10 s of drilling, since during this time the ovipositor is positioned. Likewise, the actual drilling period includes the first 10 s that the ovipositor is inserted into the host. Dissections showed that egg laying does not appear to occur in this 10 s period.

Parasitized hosts were dissected to determine the number of eggs laid (chapter 3). _Trichogramma_ females were observed to attempt drilling into glass beads for a considerable amount of time, but it was considered unlikely that oviposition would occur. Therefore, the acceptance criterion

for glass beads was set at a duration of the drilling of 60 s, which was
the approximate duration of the drilling phase on accepted host eggs.

Statistical analysis

Differences in the distribution of acceptances in the contact, drumming,
drilling or oviposition phase of the parasitization behaviour were tested
for significance between strains by means of the Kolmagorov-Smirnov test
(Campbell 1974). The K-S test does not make assumptions about the shape of
the distributions underlying the data of different tests, such as normality
and similarity. Moreover, this test does not have certain conditions which
limit the use of the Chi-square test, viz. the numerical value of all cells
should be $\neq$ 0 and for at least 80% of the cells it should be > 5. Mean
differences for handling times (drumming, drilling and oviposition) were
tested by Fisher-Behrens test, due to heterogeneity of the data (Wardlaw
1985). If a certain behavioural phase was observed more than once for a
given test female, only the longest duration was included in the evaluation
of the mean value for the experiment. Analysis of the duration of different
phases only included observations on completed behaviours, i.e. those which
resulted in host acceptance.

RESULTS

Results of the different experiments are presented in figures of equal
lay-out. For each test of a strain on a given host object the numerical
data on wasp-host encounters resulting in either rejection (in contact,
drumming or drilling phase, respectively) or acceptance (in oviposition
phase, or drilling > 60 sec on beads) were transformed to proportional
distributions. The distributions are shown in histograms in the following
bottom-to-top order: (1) rejection - contact phase (black), (2) rejection -
drumming phase (striped), (3) rejection - drilling phase (dotted),
(4) acceptance - oviposition or continued drilling (white).
    Rejection of hosts in the oviposition phase was not apparent, and
therefore not recorded as a separate phase in the figures. The figures also
indicate the number (n) of test replicates that was carried out and the
statistical significance (P) of differences between tests at α = 0.05.
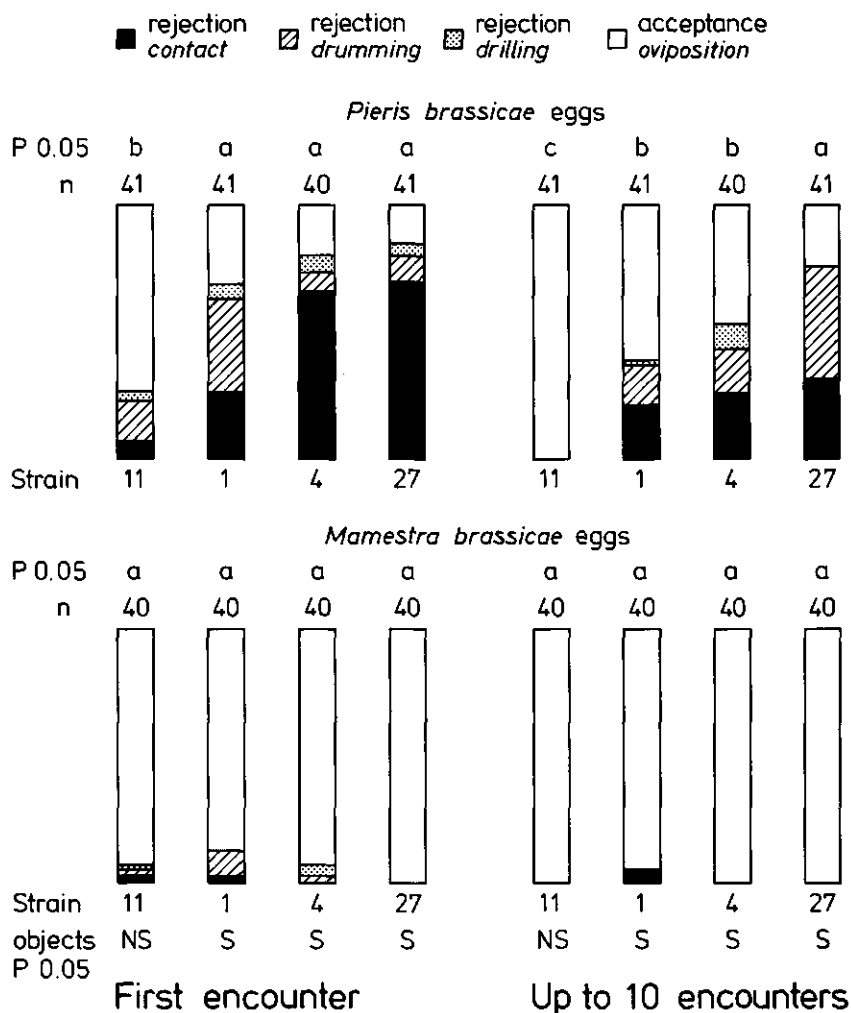
Figure 7.1. Experiment 1; proportional distributions of acceptances and rejections by females of <u>Trichogramma</u> strains 1, 4, 11 and 27 at first host-encounter and after up to ten encounters with eggs of <u>P. brassicae</u> and <u>M. brassicae</u>.

Experiment 1: acceptance of host eggs

Figure 7.1 shows the acceptance of <u>M. brassicae</u> and <u>P. brassicae</u> eggs by females of strains 1, 4, 11 and 27. Results are shown for the first

encounter with a host by the females, as well as for subsequent encounters up to the possible maximum of 10 host encounters per female. The bars are arranged by decreasing order in accepting Pieris eggs among the strains.

Females of strain 11 showed a significantly higher degree of accepting P. brassicae eggs than females of the other strains (72 vs. ca. 25%). All strains accepted over 85% of the M. brassicae eggs at first encounter. Only for strain 11 the acceptance of Pieris and Mamestra eggs was not significantly different. Most rejections occurred in the contact or in the drumming phase.

Compared to the data for first encountered hosts, those for "up to 10 encounters" showed an increase in the number of accepted hosts, especially when acceptance at first encounter was low (Figure 7.1). For strains 1, 4 and 11, the acceptance of P. brassicae eggs increased significantly. However, for all strains the statistical difference in acceptance of the two host species remained the same for 10 host encounters as for the first encounter.

Strains 11 and 4 were selected as strains with a high and a low level of accepting P. brassicae eggs, respectively, for further experiments on the role of specific differences between Pieris and Mamestra eggs as factors affecting the recognition of hosts by Trichogramma. In the following experiments only the data on the first host encounter are presented, except for experiment 4 on host colour.

Experiment 2: accessory-gland secretion

The influence of the secretion of the accessory glands of M. brassicae and P. brassicae as a host-recognition factor was studied in two ways. In one experiment, the acceptance of ovarial eggs, which lack the secretion, was compared with the acceptance of normally laid eggs of the same host species. In the other experiment, the acceptance of glass beads coated with the secretion (egg-wash) of either host species was compared with the acceptance of untreated beads. The size of the beads averaged 0.58 mm, which is nearly the same size as M. brassicae eggs, but smaller than P. brassicae eggs (Table 7.1).

Females of strain 4 accepted more ovarial than normally laid eggs of P. brassicae, whereas egg-wash coated beads were accepted less than

Pieris   *Mamestra*

| Pb laid eggs | Pb ovarial eggs | beads ∅ 0.58mm | egg-wash coated beads | Mb laid eggs | Mb ovarial eggs | beads ∅ 0.58mm | egg-wash coated beads |

Strain 4

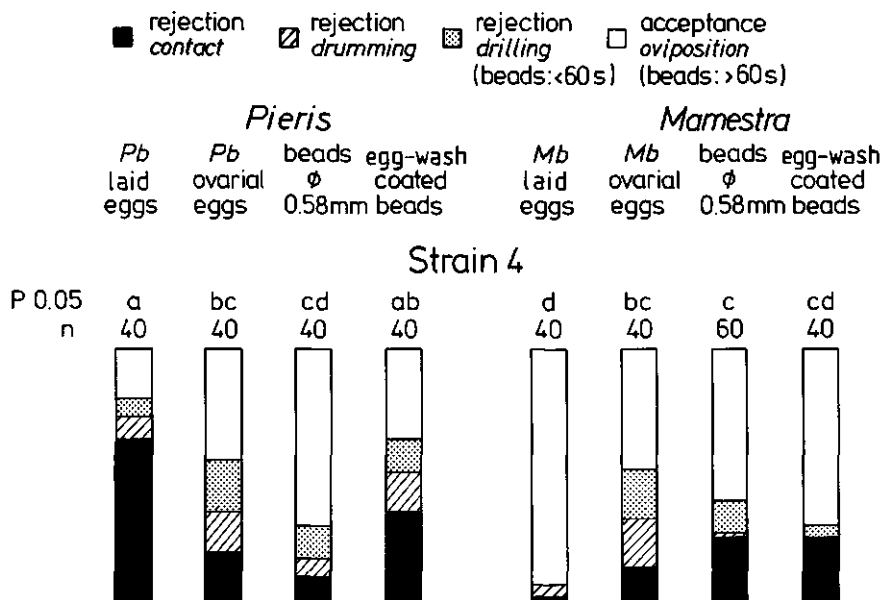| P 0.05 | a | bc | cd | ab | d | bc | c | cd |
| n | 40 | 40 | 40 | 40 | 40 | 40 | 60 | 40 |

Figure 7.2. Experiment 2; proportional distributions of acceptances and rejections by females of T. buesi (strain 4) at first encounter with normally laid and ovarial eggs of P. brassicae and M. brassicae and with untreated and egg-wash coated glass beads.

untreated beads (Figure 7.2). Untreated beads were accepted better than normally laid P. brassicae eggs. This shows that the accessory-gland secretion of Pieris is a negative host-recognition factor for strain 4, promoting rejection of hosts. By contrast, untreated beads and ovarial eggs of M. brassicae were accepted less than normally laid eggs. This indicates that for strain 4 the accessory-gland secretion of Mamestra is a positive host-recognition factor, which is also demonstrated by the equal acceptance of normally laid eggs and egg-wash coated beads. However, acceptance of egg-wash coated beads was not significantly higher than that of untreated beads or ovarial eggs.

It is possible that the present bead treatments were inadequate, for example due to a low concentration of gland secretion in the egg-wash. Furthermore, the influence of the gland secretion may have been confounded by the influence of the surface texture of the hosts (see experiment 5).
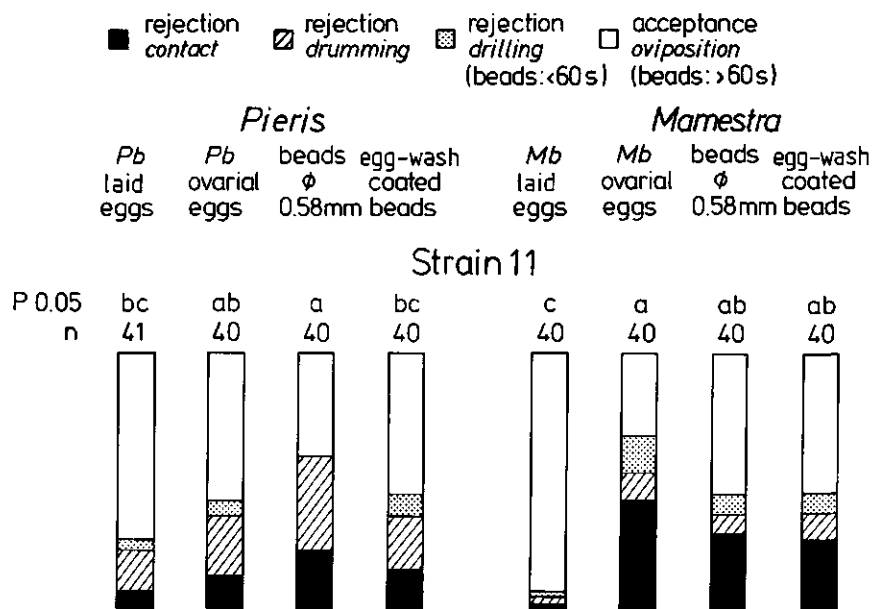
Figure 7.3. Experiment 2; proportional distributions of acceptances and rejections by females of T. maidis (strain 11) at first encounter with normally laid and ovarial eggs of P. brassicae and M. brassicae and with untreated and egg-wash coated glass beads.

The smooth surface of the beads lacks the ribs and texture of the natural hosts and may therefore be less acceptable.

Females of strain 11 accepted ovarial eggs of P. brassicae equally well as normally laid eggs of this species (Figure 7.3). Egg-wash coated beads were accepted more than untreated beads. Untreated glass beads were accepted less than the normally laid eggs, but this comparison includes objects that differed in size and shape as well. Nevertheless, these results indicate that for strain 11 the accessory-gland secretion of Pieris may be a positive recognition factor. At any rate, the secretion is not a negative recognition factor, as for females of strain 4.

Ovarial eggs of M. brassicae were accepted less than the normally laid eggs by the females of strain 11. This indicates that the accessory-gland secretion of Mamestra is a positive host-recognition factor for this strain. As in the experiment with strain 4, the acceptance of egg-wash coated beads was not higher than that of untreated beads.
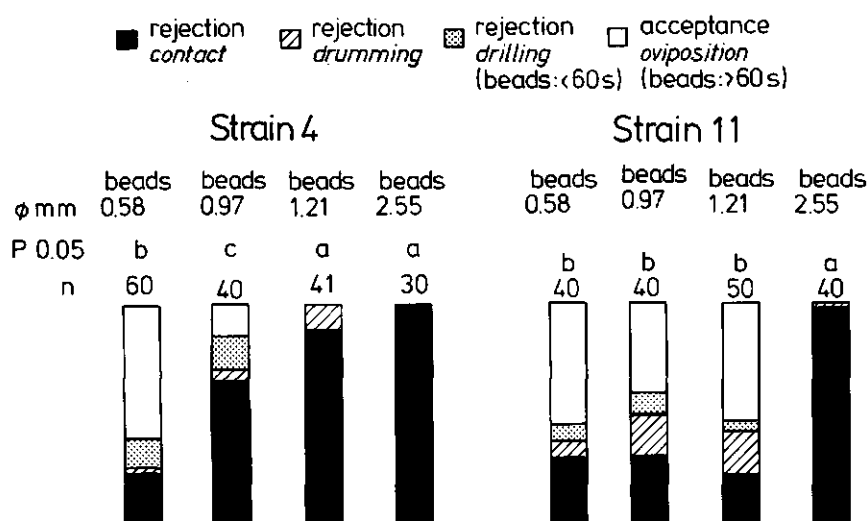
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rejection **contact** rejection *drumming* rejection *drilling* (beads:<60s) acceptance *oviposition* (beads:>60s)

Strain 4   ## Strain 11

φ mm	beads 0.58	beads 0.97	beads 1.21	beads 2.55	beads 0.58	beads 0.97	beads 1.21	beads 2.55
P 0.05	b	c	a	a	b	b	b	a
n	60	40	41	30	40	40	50	40

Figure 7.4. Experiment 3; proportional distributions of acceptances and rejections by females of T. buesi (strain 4) and T. maidis (strain 11) at first encounter with untreated glass beads of various sizes.

Experiment 3: host size

The acceptance of a range of differently sized glass beads, averaging 0.58, 0.97, 1.21 and 2.55 mm in diameter, respectively, was tested for strains 4 and 11. All test females were experienced on eggs of M. brassicae. In both strains, acceptance of beads decreased with increasing size of the beads (Figure 7.4). The largest beads were not accepted by any female, compared to 55-60% acceptance of small beads. The strains differed, however, in acceptance of medium-sized beads. Females of strain 11 accepted 0.97 and 1.21 mm beads equally well as the smallest beads. By contrast, females of strain 4 accepted 0.97 mm beads less than the smallest beads, but more than 1.21 mm beads, which were not accepted at all.

These data demonstrate that for both strains the size of the host is a factor in the host-recognition process. Small hosts are accepted better than large hosts. In addition, criteria for the acceptability of different host sizes seem to be variable among strains of Trichogramma.

Regression analysis was carried out on the relationship between the

Table 7.3. Experiment 3; correlations between the size of model hosts and
the duration (means and confidence limits) of drumming behaviour
(on "accepted" hosts) for females of T. buesi (strain 4) and T. maidis
(strain 11); see text for explanation.

Data

Duration of drumming (s)

Host diameter (mm)	Strain 4			Strain 11		
	n	mean	95% CL	n	mean	95% CL
0.58	54	18.0	2.4	36	41.9	7.4
0.97	17	48.4	7.8	27	92.0	17.7
1.21	1	39.0		37	128.6	19.5
2.55	0			0		

Regression analysis

Regression model[1]	Strain 4		Strain 11	
	equation $D_r =$	correlation coefficient	equation $D_r =$	correlation coefficient
linear	1.39R-21.25	0.74	2.70R-36.31	0.63
curvilin.	$0.03 \cdot (R/100)^{1.85}$	0.63	$1.65 \cdot (R/100)^{1.20}$	0.68

[1] Linear model: $D_r = a + bR$; Curvilinear model: $D_r = a \cdot (R/100)^b$;
   D_r = drumming time (s); R = radius of host (mm).

duration of the drumming behaviour and host size (Table 7.3). For both
strains the drumming time increased with increasing diameter (radius) of
the host. The correlation coefficients of the linear equation are 0.74 and
0.63, respectively, for strains 4 and 11. The data were also fitted to a
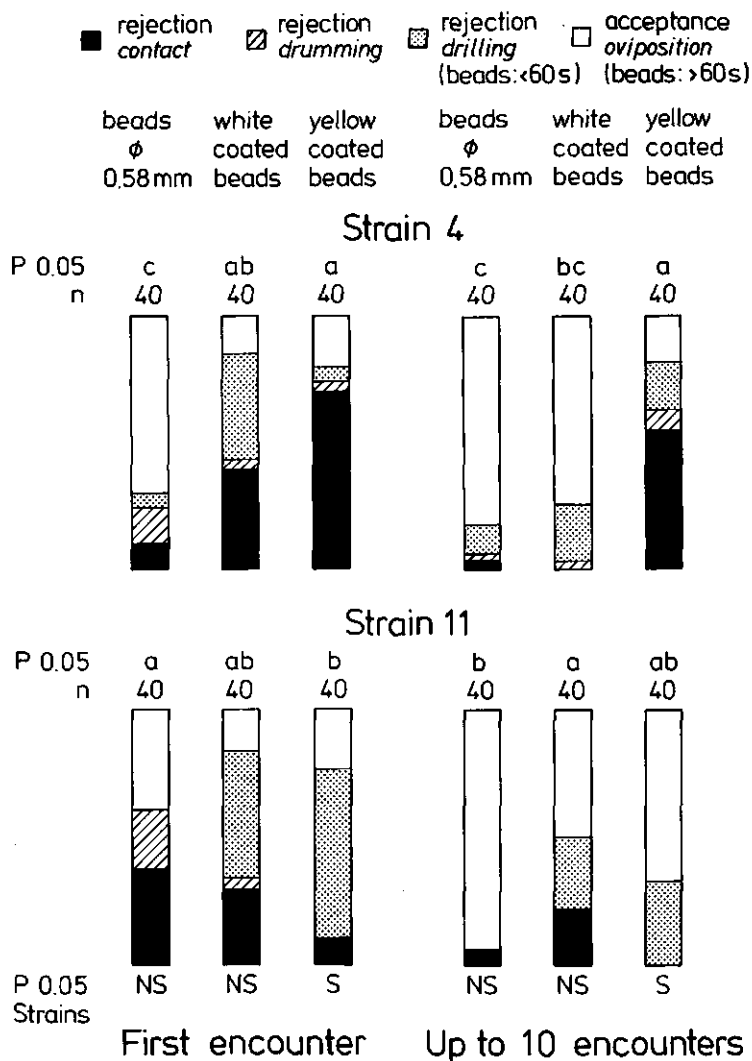curvilinear equation. For strain 11, the correlation coefficient of the

Figure 7.5. Experiment 4; proportional distributions of acceptances and rejections by females of T. buesi (strain 4) and T. maidis (strain 11) at first and after up to ten encounters with untreated, white-coated and yellow-coated glass beads.

latter is slightly higher than that of the linear equation (0.68 vs. 0.63). For strain 4 the curvilinear equation could not be expected to give a better fit, since here the analysis included data for the two smallest bead

sizes only; larger beads were not accepted. The relationship between host size and drumming time is further analyzed in experiment 6.

Experiment 4: host colour

The acceptance of glass beads (0.58 mm), painted white or yellow to mimic the colour of M. brassicae and P. brassicae eggs, respectively, was compared with that of untreated, transparent beads. Females of strains 4 and 11 were experienced on eggs of P. brassicae. Figure 7.5 shows the results for the first host encounter, as well as for 10 encounters. The reason for this is that the observations on the first encounter were biased. A comparatively high number of rejections of paint-coated beads occurred in the drilling phase, especially if rejection in the contact phase was low. Observations showed that many of the females drilling in the coating withdrew their ovipositors at the moment that these appeared to make contact with the glass surface underneath. These beads were then rejected, perhaps as a consequence to this.

At first encounter, females of strain 4 accepted white or yellow beads less than transparent beads. Females of strain 11 accepted yellow beads, but not the white beads, less than transparent beads. The major difference in this experiment between the two strains was the significantly higher acceptance of yellow beads by females of strains 11, whereas acceptance of transparant or white beads was similar. This indicates that the two strains may have different criteria for the acceptability of yellow coloured hosts.

Data for 10 host encounters indicate that females appeared to adapt to the paint/glass boudary-layer effect. As a result, strain 4 accepted white beads equally well as transparant beads. Acceptance of yellow beads did not increase, and therefore was less than that of other beads. By contrast, strain 11 accepted yellow beads equally well as transparant beads, whereas white beads were accepted less than these. More than the results for the first host encounter, those on 10 host encounters demonstrate that Trichogramma females are able to distinguish between hosts of different colour. Thus, colour appears to be another factor in the recognition of hosts.
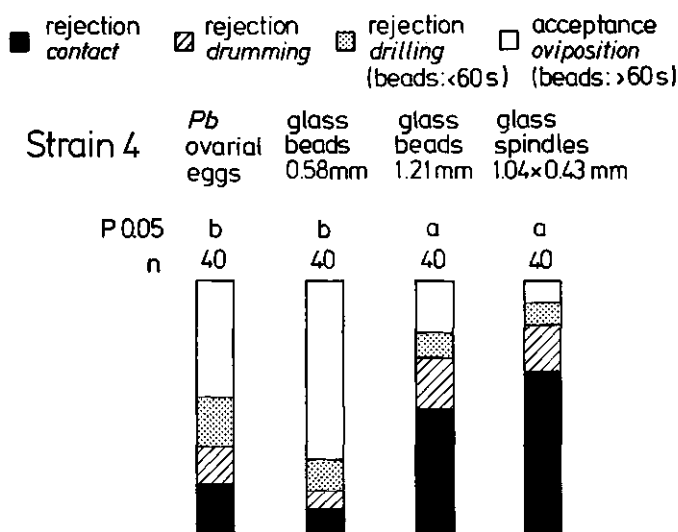
Figure 7.6. Experiment 5; proportional distributions of acceptances and rejections by females of T. buesi (strain 4) at first encounter with glass beads of 0.58 and 1.21 mm, with glass spindles and with ovarial eggs of P. brassicae.

Experiment 5: host shape

Acceptance of differently shaped host objects was studied for strain 4 only. The aim of this experiment was to find out whether the spindle shape of Pieris eggs is as acceptable as the spherical shape of Mamestra eggs. For this, the acceptance of glass objects, similar in size and shape to Pieris or Mamestra eggs, was tested in comparison with the acceptance of ovarial P. brassicae eggs. The latter were included to compare the acceptance of glass spindles with that of hosts with a natural surface texture, but without an accessory-gland secretion. All females were experienced on Pieris eggs. The results of this experiment are shown in Figure 7.6.

   No difference was found between the acceptance of glass spindles (1.04x0.43 mm) and 1.21 mm beads, which are of similar height as P. brassicae eggs. Acceptance of ovarial eggs and 0.58 mm beads (size of M. brassicae eggs) was similar, and higher than for the spindles and larger

beads. These data suggest that host shape is not a critical factor in the process of host recognition. This experiment does not rule out, however, that certain untested host shapes, such as flat eggs, might be less acceptable than the present ones.

The influence of shape can also be evaluated by comparing the volumes of differently shaped objects, since the shape is determined by the three-dimensional configuration of an object. The volumes of the spindles, 1.21 and 0.58 mm beads are 0.15, 0.93 and 0.10 mm^3, respectively, when approximated by cylinder or sphere (Table 7.2). This shows that females did not accept hosts on the basis of volume, because the large beads were six times more voluminous than the spindles and yet were accepted equal to the latter. Rather a one-dimensional measure of host size, for example height or mean radius, appears to be the critical factor in the determination of the acceptability of a certain host shape and/or volume (see experiment 6).

The present data demonstrate that host texture probably is another factor in the host-recognition process, because the acceptance of ovarial eggs was higher than that of glass spindles and small beads. Due to their yellow colour, ovarial eggs should be accepted less than glass spindles; due to their size they should be accepted less than small beads.

Experiment 6: egg laying

For a further analysis of the relationship between examination time, size and other qualities of the host, and the number of eggs laid into a host, females of strain 4 were tested on fully exposed and half-embedded P. brassicae eggs. The latter were embedded in a paraplast substrate, such that the top half of the egg protruded above the surface of the substrate. The results of this experiment are presented in Table 7.4, together with data on the same parameters for ovarial and normally laid eggs of P. brassicae and M. brassicae from experiment 2.

The duration of the drumming phase was similar for normally laid and ovarial eggs within both species and in Pieris for half-embedded eggs also. Thus, drumming time was not influenced by the presence or absence of an accessory-gland secretion, nor by the exposed volume of the hosts. This suggests that chemosensory information does not play an important role in the mechanism of host-volume measurement during external examination.

Table 7.4. Experiment 6; mean numbers of eggs laid by females of T. buesi
(strain 4) into different kinds of hosts and the duration of the drumming,
drilling and oviposition behaviour for these hosts.

| Host object[1] | n | Duration (s)[2] | | | Number[2] of eggs laid/host |
		drumming	drilling	ovipositing	
Pieris brassicae					
laid eggs	19	33.7 $\pm$ 5.5 b	68.5 $\pm$ 18.7 a	226.4 $\pm$ 67.5 b	2.3 $\pm$ 0.3 c
ovarial eggs	29	40.0 $\pm$ 10.0 b	81.5 $\pm$ 14.4 ab	161.2 $\pm$ 29.6 b	1.5 $\pm$ 0.2 b
embedded eggs	23	35.6 $\pm$ 10.0 ab	97.7 $\pm$ 18.5 b	187.3 $\pm$ 40.3 b	1.2 $\pm$ 0.1 a
Mamestra brassicae					
laid eggs	36	32.3 $\pm$ 8.6 ab	93.6 $\pm$ 15.1 b	202.3 $\pm$ 49.6 b	1.5 $\pm$ 0.3 b
ovarial eggs	25	27.9 $\pm$ 5.1 a	74.1 $\pm$ 14.0 a	104.3 $\pm$ 23.1 a	1.0 $\pm$ 0.4 a

[1] See table 7.2 for sizes; laid eggs are normally laid, ovarial eggs are dis-
sected from females, embedded eggs are partially exposed, normally laid eggs.

[2] Means $\pm$ 95% confidence limits; means within columns followed by the same
letter are not significantly different (P < 0.05).

Oviposition times tended to be shorter on ovarial eggs than on
normally laid eggs (significant for M. brassicae). Differences in drilling
times were not consistent between species or kind of host egg. A direct
relationship between drumming time and clutch size was not apparent. Fewer
eggs were laid in M. brassicae eggs than in P. brassicae eggs (1.5 vs. 2.3
eggs per host egg). The mean number of eggs laid per half-embedded egg of
P. brassicae was about half the mean number per normally laid egg (1.2 vs.
2.3). This demonstrates a correlation between exposed host volume and
clutch size per host.

For both host species, fewer eggs were laid in ovarial eggs than in
normally laid eggs. This indicates that females responded to differences
between ovarial and normally laid eggs, which might be due to stimuli
related to the external (e.g. accessory-gland secretion) and/or the
internal quality (e.g. fertilization) of the eggs.

Table 7.5. Experiment 7; suitability of M. brassicae and P. brassicae eggs as hosts for the development of progeny of T. buesi (strain 4) and T. maidis (strain 11).

Strain no.	No. hosts exposed	Distribution of yield (%)[1]			Progeny (mean ± 95% CL)[2]	
		parasite progeny	host larvae	mortality host and parasites	no. eggs/ host (n=10)	no. wasps/ suit. host
Mamestra brassicae						
4	60	96.7 a	3.3 b	0 b	2.7 ± 0.2 a	2.6 ± 0.4 a
11	60	96.7 a	0 b	3.3 b	2.6 ± 0.6 a	2.2 ± 0.6 a
Control	60		100 a	0 b		
Pieris brassicae						
4	57	38.6 c	21.1 b	40.3 a	2.9 ± 0.6 a	2.6 ± 0.6 a
11	67	65.7 b	11.9 b	22.4 ab	3.2 ± 0.7 a	3.1 ± 0.6 a
Control	60		81.7 a	18.3 b		

[1,2] Percentages or means followed by the same letter are not significantly different (P < 0.05) by Chi-square or t test, respectively.

Experiment 7: host suitability.

Eggs of M. brassicae and P. brassicae, parasitized by strain 4 or 11, were incubated until wasp emergence in order to determine host-egg suitability. For each treatment and control about 60 eggs were kept on leaves of white cabbage to maintain natural substrate conditions. In each treatment, 10 parasitized eggs were dissected shortly after egg laying in order to determine the number of eggs laid per host egg.

The suitability of parasitized M. brassicae eggs was equally high (97%) for both Trichogramma strains (Table 7.5). In contrast, a considerable proportion of P. brassicae eggs was unsuitable as a host for both strains. Unsuitable eggs either yielded larvae of the host, shrivelled or appeared to remain unchanged for the duration of incubation. The

suitability of P. brassicae eggs was lower for strain 4 than for strain 11
(65.7 and 38.6% successful development, respectively).

Females of the two strains laid similar numbers of eggs in both host
species. For all treatments, the mean number of wasps emerging from
successfully parasitized eggs was not significantly different from the mean
number of eggs laid per host. Thus, contrary to unsuitable host eggs,
parasite mortality hardly occurred in eggs that were suitable and sustained
development of the parasites. This suggests that the observed unsuitability
of hosts is either complete or absent in individual host eggs. Host larvae
emerged from 10-20% of parasitized P. brassicae eggs. Most of the
unsuitable hosts desiccated, compared to 18% for control eggs. Desiccation
of control eggs was not apparent for M. brassicae. These data suggest that
eggs of P. brassicae are less suitable as a host for strain 4 than for
strain 11, which corresponds to the better acceptance of P. brassicae eggs
by females of strain 11.

DISCUSSION

Host-recognition factors

Trichogramma species are generally believed to be polyphagous wasps,
attacking the eggs of numerous host species from several insect orders
(Anderson 1976, Strand 1986, Bin et al. 1984). For this reason, Salt (1935)
concluded that size is the only criterion by which Trichogramma recognize
their hosts, and that within certain limits any object with dimensions of
less than four times difference will be examined and attacked. In contrast
with the gregarious Trichogramma, species of the egg-parasite genus
Telenomus (Scelionidae) are mostly solitary and in general appear to have
an oligophagous host range (Bin & Johnson 1982, Bin et al. 1984).

Females of Telenomus heliothidis have been shown to use a combination
of a narrowly defined physical cue (host size) and a specific chemical cue
(accessory-gland secretion) in the host-recognition process (Strand
& Vinson 1982a). T. heliothidis apparently is a specialist parasite of the
eggs of Heliothis spp. (Noctuidae), since it readily accepted appropriately
sized eggs coated with accessory-gland secretion from H. virescens or
H. zea as a host, whereas uncoated model hosts or non-host eggs of the

right size (e.g. the noctuid Spodoptera frugiperda) hardly received examination and were not recognized as a host (Strand & Vinson 1982b, 1983a,c).

This study shows that beside host size, several other factors appear to play a role in the host-recognition process of Trichogramma. A role is demonstrated for all external physical host characteristics, except shape. Thus, host recognition in these egg parasites appears to be a much more complicated sensory process than it was thought in the past. Beside mechanosensory or tactile stimuli (Salt 1935, 1940), the process also involves visual (optical) and chemosensory stimuli.

Salt (1935) reported that various regular (e.g. spherical, cubical) and irregular (e.g. glass fragments) shapes of objects are acceptable as a host to T. evanescens. The present data indicate that host shape is not a separate acceptance measure, but seems to be incorporated within the mechanism by which wasps determine host volume (see below). The suggested roles of colour and texture as recognition cues are the first accounts of this nature for Trichogramma or any other egg parasite. The reason for Salt's failure to identify any other recognition factor, beside host size, might be due to the fact that he did not always carry out continuous observations of individual females. In this way, the presently observed subtle differences among individuals may have been obscured.

Host-recognition kairomone

The role of one or more chemical compounds in the host's accessory-gland secretion in host recognition by Trichogramma has also been demonstrated for T. pretiosum on its host H. zea and the non-host S. frugiperda (Nordlund et al. 1987). Strand and Vinson (1982a) applied the term egg-recognition kairomone to this kind of host-produced chemical(s). The chemical is regarded as a stimulus evoking a response in the parasite that is adaptively favourable to the latter, but unfavourable to the host (Nordlund 1981). The present study suggests that the stimulus helps the wasps to assess the acceptability of hosts and that it may promote acceptance of suitable hosts, as well as rejection of unsuitable hosts (see below). In the latter case the response could be favourable to both the host and the wasp and, according to Nordlund's terminology, the

function of the material would be a synomone rather than a kairomone.

Strand & Vinson (1983a) isolated the recognition kairomone from the accessory-gland secretion of H. virescens and showed a chemical similarity between the secretion and the egg-adhesive material on H. virescens eggs. The material containing the kairomone activity consists of two large proteins. The authors claimed this case as the first proof of the kairomonal activity of a non-pheromonal product.

A similar case exists in the present study for the accessory-gland secretion and egg-adhesive of P. brassicae. Schoonhoven et al. (1981) and Klijnstra & Schoonhoven (1987) demonstrated the long persistence (over seven weeks) and the function of the material obtained from the eggs as an oviposition-deterring pheromone (ODP) for gravid females of P. brassicae. The same material was tested for its presumed kairomonal activity in Trichogramma by Noldus & Van Lenteren (1983, 1985b). They conducted their experiments with females of the present strain 11, but reportedly identified as T. evanescens. No reaction to the material was found in an airflow olfactometer (Noldus & Van Lenteren 1983). However, in a flight-landing experiment the parasites showed an arrestment response after landing on a surface treated with the P. brassicae egg-wash material (Noldus & Van Lenteren 1985b). Noldus & Van Lenteren suggested that the P. brassicae ODP, or another substance from the egg coating, plays a role as a contact kairomone in short-range host searching by Trichogramma. In light of the present findings it seems that, by placing the egg-wash material on a flat surface, it may have been presented out of place to the parasites. The kairomonal function of the pheromone should primarily be considered as a host-recognition compound, influencing host examination rather than intensifying local searching behaviour.

Generalism versus specialism

This study shows that, as for Telenomus, host recognition by Trichogramma females probably results from the neural processing of sensory stimuli from chemical and physical cues. Texture and colour of the host, however, were not found to affect host recognition by Telenomus (Strand & Vinson 1983c). Thus, the generalist Trichogramma apparently uses more criteria to assess the acceptability of host eggs than the specialist Telenomus. However, in

T. heliothidis the criteria were very strictly defined and also showed a
conditional dependence. The Heliothis accessory-gland secretion only
elicited examination if applied to a host object, while glass beads only
were given extensive examination if coated with the secretion.

Rigid recognition criteria enable females of T. heliothidis to have a
highly specific host-acceptance behaviour and a short recognition time for
unacceptable hosts. These are important assets for a specialist forager and
hint, according to optimal-foraging theory, to adaptiveness of this
behaviour (Charnov & Skinner 1985, Greenwood & Elton 1983, Hubbard et al.
1982). The latter is exemplified by a decreasing acceptability of
H. virescens eggs older than 48 h, which is coincident with a decreasing
survival of T. heliothidis in older eggs (Strand & Vinson 1983c). The older
eggs probably become less acceptable due to a progressive change in the
shape of aging eggs, from sperical to conical. Strand & Vinson (1982b) also
showed that the survival of T. heliothidis in non-hosts (for example
S. frugiperda), accepted after external application of Heliothis accessory-
gland secretion, was lower than for their natural hosts.

The present study shows that Trichogramma has a much more flexible use
of criteria for host acceptance than Telenomus, which may allow
Trichogramma to be polyphagous. No single recognition factor seems to act
by itself as a condition for acceptance, but several factors actually
affect the acceptability of hosts. The only condition appears to be set for
the size of hosts, which apparently should be large enough to permit
development of at least one individual (Salt 1935), but not be larger than
a certain maximum size (present observations). However, even size probably
is not always an absolute condition, since Morrison et al. (1983) elicited
T. pretiosum to oviposit into a salt solution (Nettles et al. 1983) through
a flat synthetic membrane, apparently without the usual sequence of host-
examination behaviour taking place. Similarly, Rajendram & Hagen (1974)
observed that females of T. californicum laid all their eggs into the first
accepted artificial paraffin egg containing an oviposition-stimulant
solution.

Host volume and clutch size

The presently observed correlations between drumming time and host size and between exposed host volume and clutch size per host are in agreement with the findings on host-volume measurement by females of T. minutum as reported by Schmidt & Smith (1985a). They found that fully exposed Manduca sexta eggs yielded 1.8 times more progeny than partially embedded eggs of which 70% of the surface was exposed. The drumming time on model hosts of different diameter was proportional to the host diameter. Furthermore, the drumming times on partially and fully exposed model hosts were similar for a range of diameters. The authors concluded that drumming time in Trichogramma is dependent on host curvature (radius), and independent of exposed surface area or volume of the host.

     In subsequent experiments Schmidt and Smith (1987a) showed that host curvature determines the total duration of the drumming or examination walk on the host surface and that this measure is used to set an upper limit to the clutch size. Clutch size was found to be set in response to the absolute length of the initial transit walk across the host (Schmidt & Smith 1987c). Wasps seem to measure the initial transit as the time lapse between the moment of mounting the host and, completing a straight route across the egg, the first turn in the examination path when the substrate is contacted (Schmidt & Smith 1988). The host curvature is probably measured by mechanoreceptors which respond to the angles between the head and the scape and flagellum of the antennae (Schmidt & Smith 1986).

     Salt (1935) observed an apparent preference in T. evanescens for the largest host when females could choose between several combinations of a large and a small host species. Subsequent experiments on criteria used in host-size selection were carried out with small host eggs (S. cerealella, E. kuehniella) in combination with various objects ranging in volume from 0.003 to 0.049 mm^3. In the present study, females of strain 4 accepted model hosts inversely proportional to their size, whereas females of strain 11 accepted bead volumes between 0.10 and 0.93 mm^3 equally well. The largest beads (8.7 mm^3) were rejected in both strains. The difference with Salts' findings might be due to the difference in volume ranges studied, differences between strains in host-size acceptance criteria, and/or the difference in experimental methodology. Salt (1940) demonstrated that wasp

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size may also influence the criteria for host-size acceptance. Small hosts, accepted by small wasps, were not accepted by large wasps.

Fewer eggs were laid in ovarial eggs than in normally laid eggs by females of strain 4. This suggests that there might be differences in the internal composition between the two kinds of egg. Rajendram & Hagen (1974) reported that T. californicum laid eggs into various solutions encapsulated in paraffin droplets, but not into distilled water. Nettles et al. (1982, 1983) found that oviposition by T. pretiosum into aqueous solutions in wax eggs was determined by cations, anions and salt concentrations. A certain mixture of potassium chloride and magnesium sulfate appeared the best ovipositional stimulant, whereas calcium ions were shown to inhibit oviposition. Artificial diet media were inferior to the salt mixture as an oviposition stimulant, while glucose-protein hydrolyzate and free amino acids had no effect or were inhibitors of oviposition, depending on their concentrations (Nettles et al. 1985).

Host selection

The present findings on the factors involved in the host-recognition process of Trichogramma help to explain how the females distinguish between different types of host as a means of host selection. Recognition factors may either have a positive or a negative effect on host acceptance, depending on the feature of the factor and on the parasite strain.

Females of strain 4 are able to recognize P. brassicae eggs as unpreferred hosts by means of the specific coating with the accessory-gland secretion (recognition kairomone), the comparatively large size (height) of the eggs and their yellow colour. For eggs of M. brassicae the features of these recognition factors are such that they render the eggs highly acceptable as a host for strain 4.

The host-acceptance behaviour of strain 11 was affected by the same recognition factors as strain 4. However, the two strains appear to have different acceptability criteria for these factors. Consequently, eggs of P. brassicae are as acceptable as eggs of M. brassicae for strain 11. These findings suggest that strain 11 has a wider host range than strain 4, especially in light of the apparently general preference of Trichogramma species for noctuid host eggs (chapter 6). The difference in acceptance of

<u>Pieris</u> eggs between the two strains corresponds with the difference in suitability of <u>Pieris</u> eggs for development of the parasites. One might conclude, that strain 4 is more specific, or less polyphagous, than strain 11. Alternatively, strain 11 might be a specialist strain, which has adapted to parasitizing pierid eggs without a diminished ability to parasitize other host species also.

Does this study help to select candidate <u>Trichogramma</u> strains for inundative biological control of the present host species? The results indicate that an effective strain is either characterized by widely ranging criteria for the different host-recognition cues, especially the recognition kairomone(s), size and colour of the host, or by specific, narrow criteria. Rigid criteria for these factors might, however, make a strain too selective for control of a complex of host species. The most suitable exploration areas to collect generalist strains seem either to be diverse habitats with a great diversity in Lepidoptera, or uniform habitats with a high abundance of Pieridae or species with eggs very similar to <u>Pieris</u> eggs.

Chapter 8. HOST SUITABILITY

ABSTRACT

The suitability of eggs of Mamestra brassicae as a host for development of
Trichogramma is generally high, whereas that of Pieris brassicae eggs is
variable between strains of the parasite. In order to elucidate which
factors might cause this difference in host suitability, comparative
developmental studies were conducted, involving the two host species and
several Trichogramma spp. strains. Host eggs are probably killed by the
injection, during oviposition, of a toxic substance causing dissociation of
the host contents. For both host species, the frequency of killing host
eggs increased linearly with the duration of the oviposition phase.
Survival of immature Trichogramma in P. brassicae eggs was related to the
number of eggs laid per host by an optimum curve.

A comparative histological study of host pathology was made for strains
33 (T. maidis) and 46 (T. pintoi). Parasite development was consistently
longer (1 d at 25°C) in P. brassicae eggs than in M. brassicae eggs. No
progeny of T. pintoi emerged from P. brassicae eggs, due to inhibition of
development in the egg stage or in the third instar larval stage, depending
on humidity conditions during incubation. Larvae died prior to complete
ingestion of the host contents. These findings suggest that parasite
development in Pieris eggs may be retarded or inhibited by noxious physical
conditions, e.g. a low osmotic pressure, and/or the action of one or more
toxic chemicals in the host egg.

Humidity conditions did not affect the suitability of M. brassicae
eggs, whereas the success of parasite development in P. brassicae eggs
increased with increasing humidity (40-80% rh). In P. brassicae eggs,
resistance to desiccation imparted by the egg shell was easily impaired by
direct handling of the eggs. Scanning and transmission electron microscopy
studies were conducted to determine differences in the external and
internal structure of the egg shells of M. brassicae and P. brassicae. The
chorions of the two species have the same thickness, but M. brassicae eggs
have a denser internal chorion layer than P. brassicae eggs. Ovipositor-
puncture wounds are of the same diameter for the two host species and
become sealed after parasitization.

INTRODUCTION

Host suitability is concerned with factors affecting the development of a
parasite within potential hosts (Vinson & Iwantsch 1980a). Following the
host-finding and host-selection process, host suitability is a final step
in the host-parasite relationship toward successful parasitism (Vinson
1976). The success of parasite development may depend on factors as the
nutritional adequacy of the host, its immune system, toxins and competition
(Vinson & Iwantsch 1980a). Active alteration of host physiology by the
parasitizing female or her progeny has been referred to by Vinson
& Iwantsch (1980b) as host regulation. Alterations of host endocrine
functions and development are common in larval parasites (Beckage 1985),
but can hardly play a role in egg parasites (Strand 1986). Besides,
pathological effects of host suitability and host regulation may be
difficult to separate (Thompson 1983, Strand 1986) and is therefore not
attempted in this study.

This chapter deals with the suitability of eggs of Mamestra brassicae,
Pieris brassicae and P. rapae as hosts for the egg parasite Trichogramma.
In previous chapters (5, 6 and 7) it was apparent that Mamestra and Pieris
eggs differ in suitability for development of Trichogramma, which
corresponds with the host-selection behaviour of female wasps. M. brassicae
eggs generally seem to be a suitable and acceptable host for various
Trichogramma spp. strains. The acceptance of Pieris eggs, however, differs
among strains, which might correspond with variability in host suitability
for this species. The purpose of the present study therefore is to compare
the developmental process of different Trichogramma strains in Mamestra and
Pieris eggs and to investigate the influence of certain external and
internal conditions, such as humidity and the number of parasites
developing per host egg, on the success of development.

Trichogramma parasitize eggs of many species that may differ in several
respects, e.g. size, degree of clustering, hardness of the chorion and
development time (Salt 1940). Salt (1937a) suggested that eggs may be
protected from parasitization by a hard shell (e.g. eggs of Orgyia antiqua
(Lymantriidae) and Smerinthus populi (Shingidae) and by chemicals which
inhibit egg laying (e.g. the sawfly Selandria sixi). These factors do not
pertain, however, to the present definition of host suitability. Salt

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(1937a) suggested that the developmental unsuitability of eggs of Tenebrio molitor and Bruchus obtectus (Coleoptera) and of Sialis lutaria (Neuroptera) for T. evanescens was due to nutritional inadequacy. The latter species is a suitable host, however, for T. semblidis (Salt 1937b), which demonstrates the occurrence of interspecific variation in host suitability for Trichogramma. Inter- and intraspecific differences in suitability of one of the present species, P. rapae, as a host for Trichogramma have been reported by Parker & Pinnell (1974).

In addition to biological factors, chemical and physical factors seem to determine host suitability for Trichogramma. Research on in vitro culture of various parasite species has increased insight in their nutritional and other requirements, which seem to be less complex for egg parasites than for larval or pupal parasites (Thompson 1986, Strand et al. 1988). Cells functioning as teratocytes appear to play a role in host decomposition in the Scelionidae, but not in the Trichogrammatidae (Strand 1986, Strand et al. 1988). T. dendrolimi has been reared successfully in a medium devoid of insect additives (Liu & Wu 1982, Wu et al. 1982). In contrast, pupation and adult emergence of T. pretiosum appears to depend on chemical host factors (Irie et al. 1987, Strand & Vinson 1985, Xie et al. 1986b). In addition to the quality of nutrition, control of its quantity and maintenance of proper humidity conditions appear important factors for successful in vitro development of Trichogramma (Strand et al. 1988, Xie et al. 1986a).

In this chapter, the process of parasite development in M. brassicae and P. brassicae eggs is studied by in vivo and in toto dissections and by histological sectioning of parasitized eggs at different times after parasitization for two strains of Trichogramma (one T. maidis and one T. pintoi). The variability among several strains from the laboratory collection (chapter 2) for success in parasitizing M. brassicae and P. brassicae was investigated for possible selection of candidate strains. The requirement for a candidate Trichogramma strain for inundative biological control is that it should be able to kill the hosts (chapter 2).

In the present study, P. brassicae eggs turned out to be sensitive to desiccation in a dry environment. Investigations were therefore carried out to determine the effect of humidity on desiccation of host eggs and its influence on development of immature Trichogramma. In turn, parasitism

might affect the resistance of the host to desiccation, e.g. due to structural desintegration or evaporation through oviposition wounds in the egg shell (Boldt & Ignoffo 1972). Differences in the external morphology of P. brassicae and M. brassicae eggs before and after parasitization were investigated by scanning electron microscopy. The internal morphology of the egg shells of the two species was studied by transmission electron microscopy.

MATERIALS AND METHODS

Various aspects of the development of Trichogramma from egg to adult in eggs of M. brassicae and P. brassicae, and occasionally P. rapae, were investigated for several strains from the laboratory collection (chapter 2). Experiments mainly differed in ways of manipulating host eggs prior to parasite exposure and in conditions under which eggs were incubated, especially humidity (Table 8.1). Host eggs and parasites were obtained from laboratory cultures maintained by standard procedures (chapter 3). Parasites were reared on eggs of Ephestia kuehniella and usually were 1-2 d old, fed, mated and unexperienced with hosts prior to their being used in experiments. Host eggs had to be removed from the substrates they were laid on in the rearings (Mamestra eggs on tissue paper and Pieris eggs on Brussels sprouts leaves). Mamestra eggs were readily removed with a fine brush, but comparatively much more effort was required to remove Pieris eggs (chapter 3).

Parasitization behaviour

Parasitizing females of strain 11 were interrupted in order to determine whether the duration of the oviposition phase (chapter 3) influences the success of development in M. brassicae or P. brassicae eggs. Ovipositing females were removed from host eggs at 5 or 10 s time increments. At each time interval this was done for 10 females from the moment that they pierced the chorion until the moment they withdrew their ovipositors.

To determine the relationship between the number of parasites developing within a host (clutch size) and the success of development, females of strain 34 were manipulated to lay a certain number of eggs into

Table 8.1. Trichogramma strains and host species used in various
experiments on factors influencing host-egg suitability for parasite
development.

Experiment	Host species	Strains
1. Parasitization behaviour: interruption of oviposition	M. brassicae, P. brassicae	11
2. Clutch size, host wounds: 1-5 eggs/host, 1-2 punctures	P. brassicae	34
3. Effect of manipulation: eggs on foliage vs. eggs transferred to filter paper	M. brassicae P. brassicae, P. rapae	11
4. Relative humidity: 40-60-80% rh	M. brassicae, P. brassicae	34
5. Host viability: unfertilized eggs sterilized eggs	M. brassicae M. brassicae, P. brassicae	7 40
6. Host suitability	M. brassicae, P. brassicae	several
7. Host pathology: histological sections	M. brassicae, P. brassicae	33, 46
8. Electron microscopy: egg-shell structure and puncture wounds	M. brassicae, P. brassicae	4, 11

P. brassicae eggs. A wasp usually lays 2 or 3 eggs in a P. brassicae egg.
By offering eggs briefly after parasitization by a first female to a

second, inexperienced female, the latter would superparasitize the egg
(chapter 3). In this way host eggs were obtained containing from 1 up to 5
eggs per host. Due to parasitization by 1 or 2 wasps, hosts with 1 or 2
eggs were punctured once, those with 3 eggs once or twice and those with 4
or 5 eggs always twice. For this experiment, the oviposition behaviour of
Pieris females in the rearing was manipulated so that they laid eggs on a
parafilm substrate. Eggs were much easier removed from parafilm than from
foliage. Exposed hosts were incubated individually in cotton-plugged glass
vials at 25+1°C and 80+5% rh.

Development

Initial experiments showed that Pieris eggs are sensitive to desiccation,
apparently due to damage from the manipulation. Thus manipulation and
desiccation might confound the results of host suitability experiments. The
effect of host manipulation on suitability was studied for eggs of
M. brassicae, P. brassicae and P. rapae parasitized by strain 11
(T. maidis). Manipulated eggs were transferred from the rearing substrate
(Brussels sprouts plant) onto a piece of filter paper in a petri dish.
Unmanipulated eggs remained on the plant. In each experiment, 50 eggs of
one species were exposed to 5 females for 4 h without directly observing
their behaviour. Exposed eggs were incubated (treated eggs on filter paper
in a petri dish, control eggs on a plant) at ca. 25°C and 70% rh. Emerging
caterpillars were removed before they would use the other eggs as a food
source.
    In order to determine the effects of relative humidity and parasitism
on egg desiccation, M. brassicae and P. brassicae eggs parasitized by
females of strain 34 (T. maidis), and unparasitized control eggs, were
incubated at relative humidities of 40, 60 or 80% (+5%) and 25+1°C. Eggs
were obtained by removal from parafilm (see above) and placed individually
in glass vials (55x15 mm) plugged with cotton. Eggs were parasitized by
introducing a female into the vial and removing her immediately after
parasitization had taken place.
    The effect of host viability on the success of parasite development was
studied for strain 7 (T. maidis) in unfertilized eggs of M. brassicae and
for strain 40 in sterilized eggs of M. brassicae and P. brassicae. The

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latter were sterilized by ultra-violet irradiation (6 and 24 h exposure, respectively, at 163.8 $J/m^2 \cdot h$).

The suitability of Mamestra and Pieris eggs for various strains from the collection (chapter 2) was tested under conditions which, in the preceeding experiment, were found least confounding: eggs laid on parafilm for easy removal and incubation in vials at a high humidity (80% rh and 25°C). This test especially included strains of which the host-selection properties had previously been studied (chapter 6). Each test female was observed while she was allowed to freely parasitize up to 5 host eggs. Between 5 and 10 females were tested per strain.

Histology

Histological studies of parasitized M. brassicae and P. brassicae eggs were conducted in cooperation with N. Hawlitzky at the "Station de Zoologie (INRA)" at Versailles, France. Strain 33 (T. maidis) and strain 46 (T. pintoi) were selected for this study, because these strains appeared to have an extremely high and low success of development in Pieris eggs, respectively. Host suitability in these two strains was studied for incubation of hosts transferred onto a dry substrate (index paper card) or onto a moist substrate (white cabbage leaves). Observations of developmental stages were made in vivo by dissecting parasitized hosts in Ringer's solution and in toto by staining hosts with toluidine blue. Developmental stages that appeared of interest for histological sectioning were fixed by immersion in Duboscq Brasil. Fixation required 14 h in Pieris and 48 h in Mamestra. After 5 and 24 h fixation, respectively, the external layer of the chorion was removed to increase the permeability to the fixative. Fixation was terminated by washing the eggs in 70% alcohol and, after rehydration in distilled water, they were enclosed in a drop of gelose on a microscope slide. The gelose was hardened in 70% alcohol, trimmed and then thoroughly dehydrated in 4 baths of 100% alcohol (0.5 h each). Hereafter the piece of gelose was immersed in toluene (1.5 h) and then held in 3 baths (1-1.5 h each) of paraffin at 55°C. Pieces of gelose were enclosed in liquid paraffin in a mould.

Objects fixed in solid paraffin were cut sagitally into sections of 5 microns thickness with a microtome. Sections were placed on an albumized

microscope slide, briefly heated and dried at 35°C. Paraffin was eliminated
from the slides by immersion in toluene (2 baths of 3 min), which was
followed by rehydration and colodinizing (successive dips in 100% and 90%
alcohol, collodion solution, 70% alcohol and water). The sections were
stained in Mayer's glymalun (10 min), dipped for differentiation of
colouring in hydrochloric alcohol (3 s) and washed in running water
(10 min). To increase the intensity of coloration, the slide was immersed
for 5 min in 1% sodium bicarbonate and then washed in running water
(5-60 min).

Objects were stained a second time by immersion in eosin (1 min), which
had to be followed by dehydration (successive dips in 70, 95, 100 and 100%
alcohol). Finally, the slide and a coverslip were dipped in toluene and the
object was mounted in Canada balsam. Mounted sections were studied under a
light microscope and sections showing interesting stages or details were
photographed.

Egg-shell structure

The external structure of M. brassicae and P. brassicae eggs was
investigated with a Jeol JSM-35 C scanning electron microscope. Eggs were
mounted with silver and goldcoated in vacuum for fixation. The effect of
wasp-ovipositor drilling on the structure of the egg-shell surface was
studied for strains 4 and 11. The diameters of the drilling punctures for
different hosts and strains were determined from picture measurements. The
development of a seal covering the puncture wound (Boldt & Ignoffo 1972)
was studied by fixing eggs at 2h and 4d postparasitism. In addition, the
length and width of the wasp ovipositor was determined for slide-mounted
females of strains 4 and 11 under a light microscope.

The internal structure of the egg shells of M. brassicae and
P. brassicae was studied by sectioning eggs that were fixed in Dubosq
Brasil and embedded in paraffin. Semi-thin sections (1 micron) were
observed under a light microscope, after staining in hematoxylin-eosin.
Ultra-thin sections were fixed by standard procedures for transmission
electron microscopic study in cooperation with D. Gerling (Tel Aviv
University, Israel).

RESULTS

Parasitization behaviour

Manipulation of oviposition behaviour in females of strain 11 showed that
the number of eggs laid per host increased gradually with increasing
oviposition time in both M. brassicae and P. brassicae eggs (Figure 8.1).
The maximum number of eggs laid per host and the maximal duration of the
oviposition phase did not differ between the two species. The success
ratio, i.e. the fraction of hosts not yielding a caterpillar, increased
similarly with ovipositing time. Hosts were apparently not killed at the
beginning of the oviposition phase but by some phased process. The
correspondence between the rate of oviposition and the rate of killing
hosts suggests a possible relationship between these two processes, such as
the action of a toxin secreted by the eggs, or the injection of a toxic
substance (venom) concurrently with the deposition of eggs. The fraction of
desiccated or unchanged hosts of both species varied between 0 and 14% and
did not seem to be related to oviposition time.

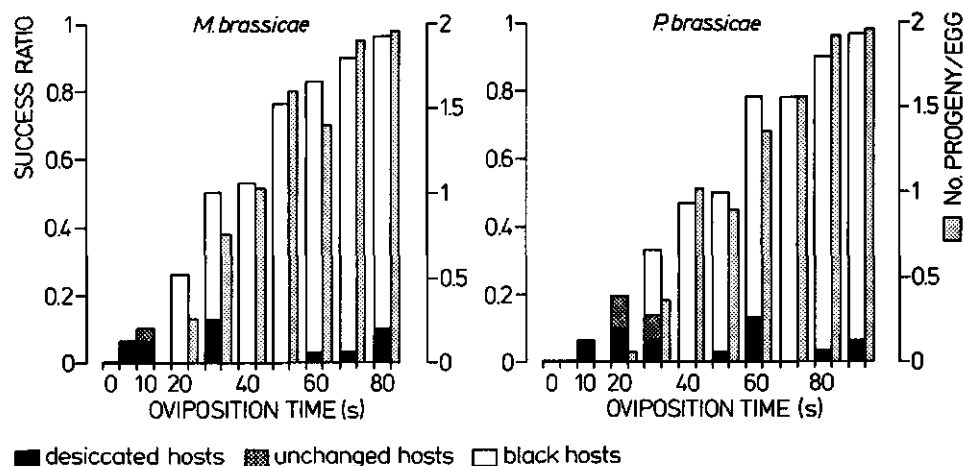■ desiccated hosts ▓ unchanged hosts ☐ black hosts

Figure 8.1. Histograms for the success ratio (no. of hosts killed/total no.
of exposed hosts) and the mean number of parasite progeny per exposed host
(n=30) at incrementing durations of the oviposition-phase for females of
T. maidis (strain 11) and eggs of M. brassicae and P. brassicae, incubated
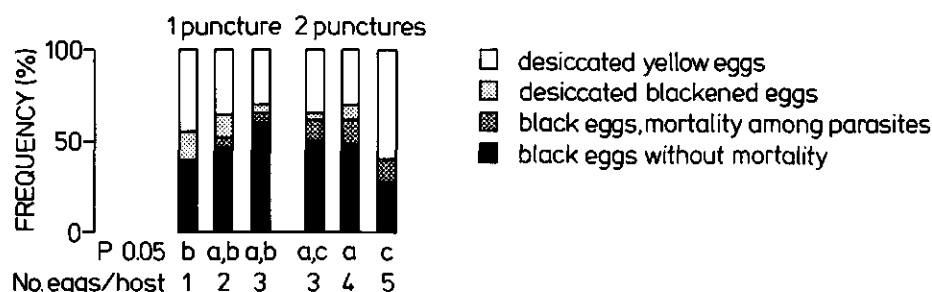at 25±1°C and 65±5% rh.

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Figure 8.2. Frequency distribution of desiccation and progeny emergence from P. brassicae eggs in which 1-5 eggs of T. maidis (strain 34) were laid by 1 or 2 females (1 or 2 punctures) and incubated at 25+1°C and 80+5% rh. Chi-square test across al treatments: χ^2=29.7, df=15, P=0.01.

The frequency of successfully parasitized hosts increased between 1 and 3 parasite eggs per host (strain 34) and then decreased between 3 and 5 eggs per host (Figure 8.2). The difference trend across all treatments is significant (Chi-square test, P=0.01). In most hosts that turned black, all immature parasites developed into adults. However, the fraction of black hosts in which mortality occurred among the progeny increased with increasing clutch size, from 0% for 1 egg/host to an average of 14% for superparasitized hosts. This suggests that in the latter hosts the survival of the parasites was affected by crowding. By contrast, the fraction of black hosts desiccating decreased with increasing clutch size from 15% for 1 egg/host to 0% for 5 eggs/host. A possible relationship between the number of eggs laid per host and the fraction of black hosts desiccating (and/or hosts remaining unchanged) was not apparent in the previous experiment.

The results between 1 and 2 punctures in hosts receiving 3 eggs were not different, which indicates that a second puncture did not affect the parasitism process. The relationship between the survival per immature parasite and the clutch size per host is an optimum curve (Figure 8.3). The survival maximum determined from the polynomial equation is at 2.9 eggs per host. This is similar to the mean clutch size of females that were allowed to oviposit without interruption (3.1+0.2, n=135). Thus the clutch size of the wasps appears to be adapted to the probability of parasite-progeny survival for this host species.
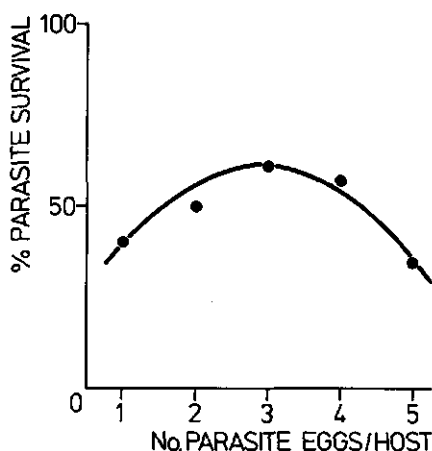
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Figure 8.3. Percentage progeny of T. maidis (strain 34) surviving to adult
stage for different clutch sizes in P. brassicae eggs (obtained by
manipulation of wasp-oviposition behaviour), incubated at $25\pm1°C$ and
$80\pm5\%$ rh. Polynomial regression: $Y=12.1+X(33.7-5.8X)$, optimum $X=2.9$. The
clutch size for wasps parasitizing without interruption averaged 3.1 ± 0.18
($n=135$, $sd=1.1$).

Parasitism process in Mamestra and Pieris

Development of Trichogramma strains 33 and 46 from egg to adult in
M. brassicae eggs was not affected by the kind of substrate on which eggs
were incubated (Figure 8.4). For both strains and substrates wasps emerged
from at least 90% of the eggs. Wasp emergence from eggs of P. brassicae was
similar to that from M. brassicae for strain 33. In contrast, no wasps of
strain 46 emerged from P. brassicae eggs. Nearly all of these eggs
desiccated if they were incubated on paper cards, whereas they appeared to
remain unchanged if incubated on leaves. A similar phenomenon occurred in
the control treatment, which yielded caterpillars from 65% of unparasitized
P. brassicae eggs. The remaining fraction of eggs mostly desiccated
(5% unchanging) if incubated on cards, whereas nonhatching eggs remained
unchanged on leaves. The two treatments are significantly different
(Chi-square test, $P < 0.05$).

The mean number of wasps emerging from successfully parasitized (black)

EGGS ON LEAVES

EGGS ON PAPER CARDS

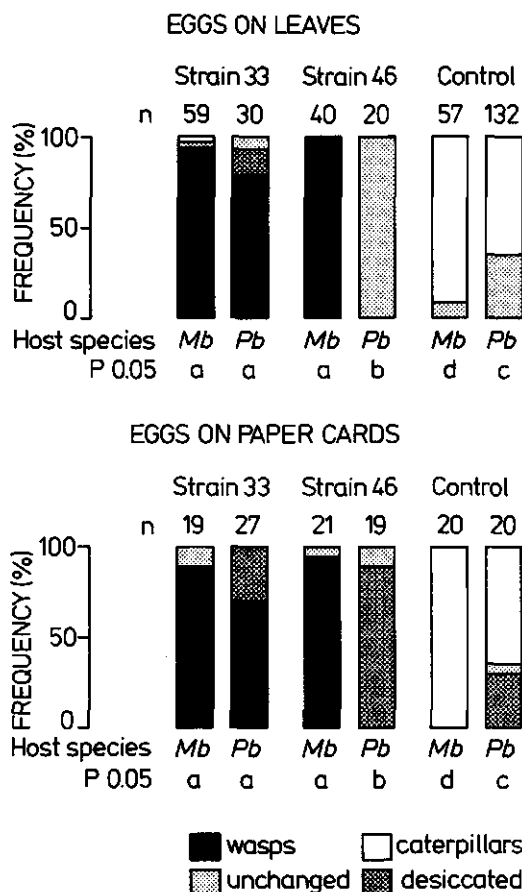■ wasps □ caterpillars
▦ unchanged ▨ desiccated

Figure 8.4. Frequency distribution of various yields from eggs of M. brassicae (Mb) and P. brassicae (Pb) parasitized by T. maidis (strain 33) and T. pintoi (strain 46) and incubated at 25±1°C and 50±5% rh.

host eggs was not different from the mean number of eggs laid per host egg for both strains in M. brassicae and P. brassicae eggs, except for P. brassicae eggs parasitized by strain 46 from which no wasps emerged (Table 8.2). This shows that parasite mortality within a host was either insignificant or complete, i.e. involving the entire clutch. Host eggs in which all parasites died apparently remained unchanged or desiccated.

The different results for the two substrates show that Pieris eggs are vulnerable to damage by the presently conducted manipulations, which for a

Table 8.2. Number of parasite eggs laid per egg of M. brassicae and
P. brassicae and the number of adult progeny per "successfully"
parasitized (black) host egg for T. maidis (strain 33) and T. pintoi
(strain 46).

| Strain no. | M. brassicae[1] | | | | P. brassicae[1] | | | |
| | Eggs / host | | Wasps / host | | Eggs / host | | Wasps / host | |
	n	mean	n	mean	n	mean	n	mean
33	10	2.5+0.7	59	2.3+0.5	10	4.5+0.7	30	4.5+0.7
46	10	2.1+0.7	40	2.1+0.4	10	2.6+1.2	20	0

[1] Means ± 95% confidence limits; eggs/host determined by dissection.

certain proportion of the eggs appears to result in disruption of
embryonic development. These damaged eggs subsequently desiccated if kept
on a dry substrate, which indicates that the permeability of the egg shell
was affected. Handling apparently did not affect Mamestra eggs. Pieris eggs
were not a suitable host for strain 46. Nevertheless, this strain seemed to
be effective in killing Pieris embryos, since caterpillars did not emerge
from parasitized eggs. As in the control treatment, eggs in which the
embryo was killed desiccated on paper cards, but remained unchanged on
leaves. This suggests that, beside damage, also parasitism affected the
permeability of the egg shell in Pieris.

Developmental schemes for strains 33 and 46 in the two host species,
inferred from dissections at various time intervals, are shown in
Figure 8.5. The pear-shaped, translucent parasite eggs hatched about 1 d
after being laid. Between the second and fourth day of development sac-like
third instar larvae were found. The first and second larval instars
probably were not observed due to their relatively short duration, which is
limited to a few hours (N. Hawlitzky pers. comm.). Larvae consumed most of
the host contents as a third instar and rapidly increased in size. Prepupae
appeared on the fourth and fifth day in M. brassicae and P. brassicae eggs,
respectively. At this time host eggs turned black. Urate secretion bodies
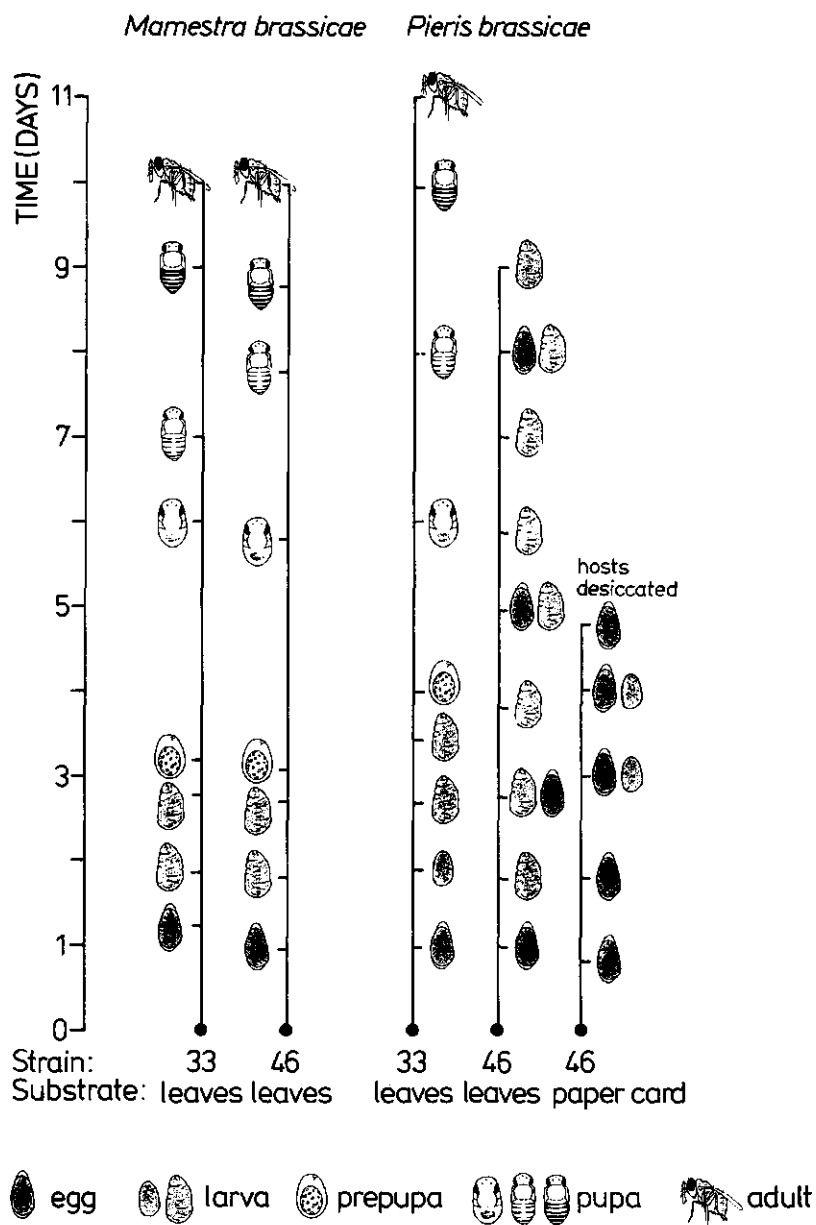are present as white kernels in the prepupal fat body. The pupal stage

Figure 8.5. Developmental stages of T. maidis (strain 33) and T. pintoi (strain 46) in eggs of M. brassicae and P. brassicae dissected in vivo (n=10) at various time intervals. Eggs were parasitized at t=0 and incubated at $25°\pm1°C$ and 50% rh on cabbage leaves or paper cards.

lasted 4-5 d. Pupae first are unpigmented and gradually acquire adult structures and colouring. Pharate adults appeared 1 d before wasp emergence.

Adult-parasite emergence was 1 d earlier for M. brassicae eggs than for P. brassicae eggs (9-10 vs. 10-11 d). Development of strains 33 and 46 progressed similarly in Mamestra eggs, whereas it was different in Pieris eggs. Contrary to strain 33, Pieris eggs parasitized by females of strain 46 did not turn black and there was no emergence of adults. Development of strain 46 appeared to be halted in the third instar larval stage.

If Pieris eggs parasitized by strain 46 were incubated on paper cards instead of foliage, parasite development seemed to be inhibited in the embryo stage rather than in the third instar larval stage (Figure 8.5). A few first instar larvae were found, while most individuals remained in the egg stage for up to 5 d postparasitism. Third instar larvae were not found in this experiment. When host eggs began to desiccate (shrivelling of the egg shell), the parasite eggs inside appeared normal for up to a day afterwards and then shrivelled also. The development of the two strains in P. brassicae eggs was monitored across ca. 1-d time intervals, up to prepupal development, by means of histological sectioning. Table 8.3 shows that about half of the eggs of strain 46 remained in the egg stage, as a blastoderm, while the other half developed into early third instar larvae.

The longer development time of parasites in eggs of P. brassicae than in those of M. brassicae appears to be due to a longer duration of the third instar larval stage. This might indicate a possible difference in nutritional quality and/or quantity for Trichogramma between the two host species. Composition of nutrition may also have played a role in the inhibition of development of the egg and larval stages of strain 46. The effect is influenced, however, by the occurrence of dehydration of the host. Most parasites did not seem to develop at all in hosts incubated on paper cards, whereas they partly developed into early third instar larvae if the hosts were incubated on leaves.

Dissections of parasitized eggs in toto revealed that 4-5 h postparasitism the germ disc of the host appeared to be desintegrating. Histological sections showed a distinct zone of modified yolk and germ cells around the site of the parasite eggs (Figure 8.6). It appears to be a zone of dissociation or lysis: the germ cells are irregular and contain

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Table 8.3. Percentages of different developmental stages of T. maidis (strain 33) and T. pintoi (strain 46) in 1 d old eggs of P. brassicae (n=5) sectioned after fixation at various time intervals from egg laying (t=0) until the prepupal stage (incubated at 25±1°C and 50±5% rh).

Strain no.	n	Time interval (d)	Distribution of parasite stages (%)[1]					
				Larval instars				
			egg	L1	L2	L3 early	L3 late	prepupa
33	14	1.0	100					
	22	1.8	36	64				
	13	2.8					100	
	8	3.7						100
46	14	0.8	100					
	12	1.8	58	42				
	14	3.9	57			43		

[1] Mamestra eggs turned black after 2.85 d (both strains), Pieris eggs after 3.7 d (strain 33).

pycnotic nuclei and the dark nuclei of the yolk cells have faded. Yolk spherules (yolk compartments surrounded by a membrane and one or more vitellophages in the center), which were found in the yolk of unparasitized eggs, were not observed in this zone. The staining outside the cytolytic zone generally was darker than within.

In contrast to the partial dissociation occurring in parasitized Pieris eggs, the entire host contents seemed to lyse in parasitized Mamestra eggs. This might be due to the difference in size between the eggs of the two species (Table 5.4). Cytolysis of the Pieris oocyte appeared more extensive for strain 46 than for strain 33. For Mamestra eggs no differences in the cytolytic process were observed between the two strains.

Larval instars of strains 33 and 46 looked similar in Mamestra eggs and also similar to instars of strain 33 in Pieris eggs (Figure 8.7). Larvae of
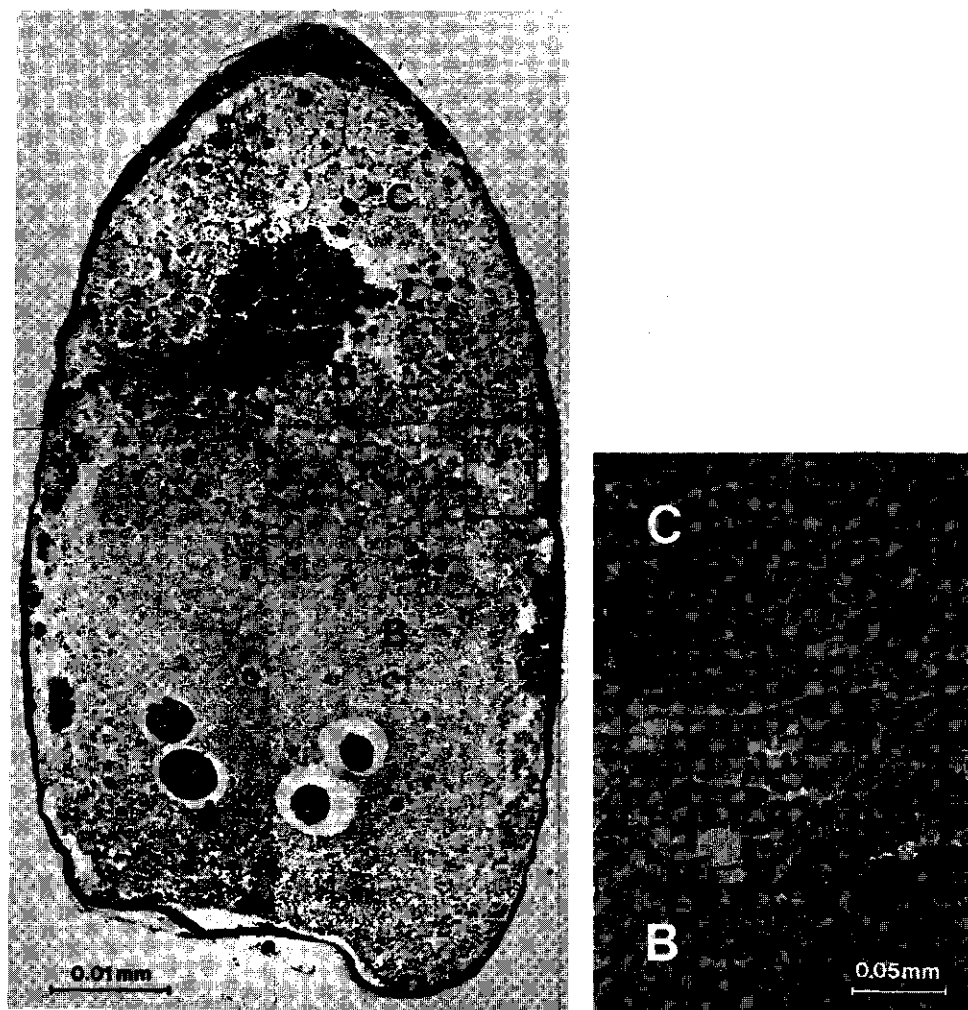
Figure 8.6. Histological section of a P. brassicae egg ca. 5 h after
parasitization by T. maidis (strain 33); A = parasite egg, B = dissociation
zone, C = yolk spherules, D = germ disc. Magnified inset: transition between
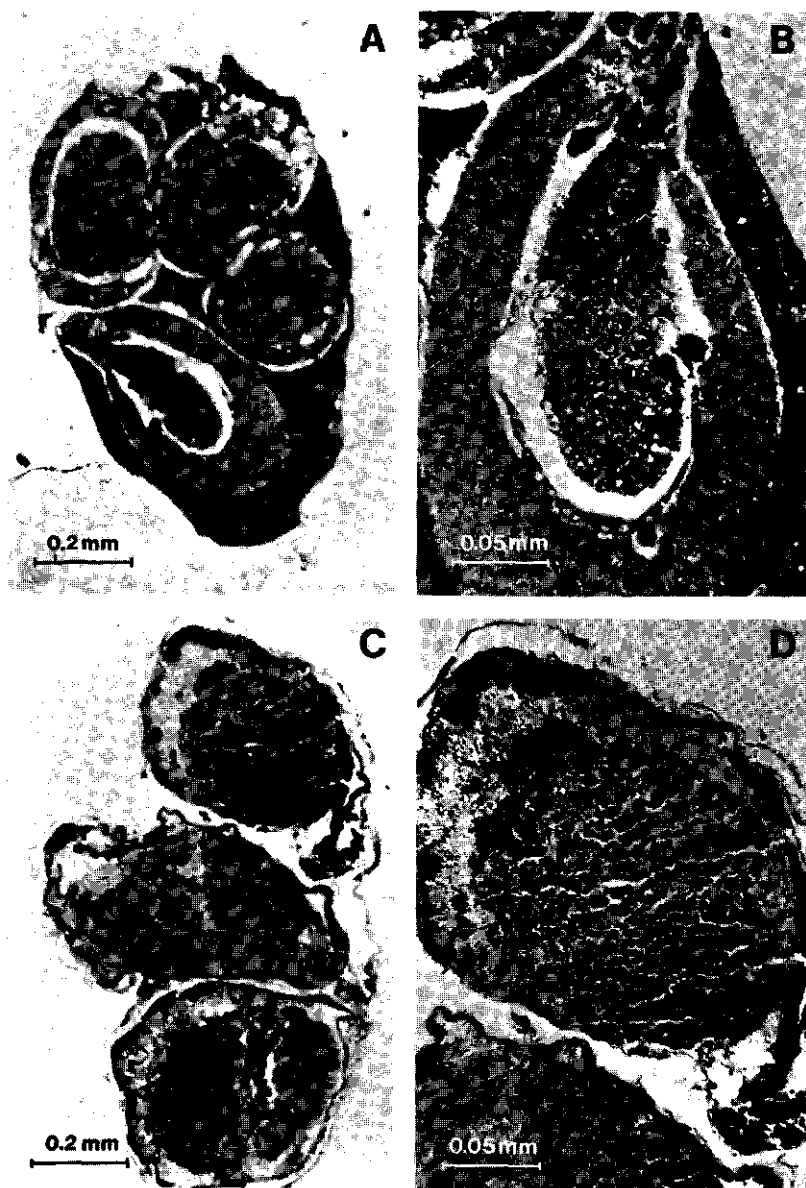dissociation zone (B) and unaffected zone of yolk spherules (C).

Figure 8.7. Histological sections of P. brassicae eggs containing third instar larvae of T. maidis (strain 33) and T. pintoi (strain 46);
A, B = strain 46, 4 d after parasitization, B = detail of larval instar;
C, D = strain 33, 3 d after parasitization, D = detail of larval instar.

Table 8.4. Suitability of fertile and sterile (unfertilized) 2 d old <u>M. brassicae</u> eggs for development of <u>T. maidis</u> (strain 7), incubated at 25±1°C and 50±5% rh.

Host condition	n	Distribution of yield (%)				No. of progeny[1]		
		desic- cated	unchan- ged	cater- pillars	wasps	eggs laid/ host egg	wasps/ black egg	sex ratio (M/M+F)
Parasitized eggs								
fertile	23	0	0	0	100	1.6±1.0	1.7±1.2	0.36
sterile	19	0	0	0	100	2.4±0.7	2.3±0.5	0.48
Unparasitized eggs								
fertile	20	0	0	100				
sterile	20	5	95	0				

[1] Means ± 95% confidence limits.

strain 33 in <u>Pieris</u> eggs seem to contain more host material than those of strain 46. Probably due to this, the ectodermal layer appears to be stretched much thinner in larvae of strain 33 than in those of strain 46. The developmental age of the larvae of strain 46 did not seem to progress beyond the early third instar, because larval cuticle was not deposited. At this stage the larvae had not yet consumed the entire contents of the host. Dark coloured secretory cells were apparent around the gut. Shortly afterwards, third instars were found that appeared to be in dissociation, comparable to the dissociation of germ cells. Irregular cell shapes and pycnotic nuclei were also observed in eggs of strain 46 which had remained for ca. 4 d in the egg stage, as a blastoderm.

Internal host factors

Adult emergence (strain 7) was not different between fertile and sterile host eggs (Table 8.4). Moreover, the mean number of wasps emerging per host corresponded with the mean number of eggs laid per host for both kinds of host. This indicates that sterility of hosts did not affect their
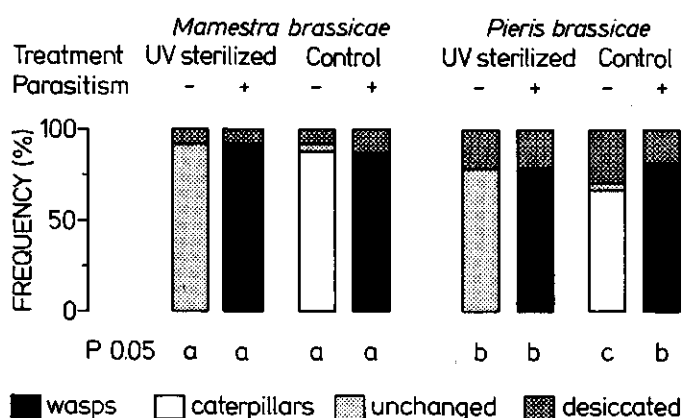
Figure 8.8. Frequency distribution for various yields from U.V. sterilized or untreated eggs (n=500) of M. brassicae and P. brassicae, unparasitized or parasitized by T. maidis (strain 40) and incubated at 25±1°C and 80±5% rh. Statistical comparison for the percentage desiccated eggs per treatment by Chi-square test.

suitability for parasite development. However, the wasps appeared to allocate fewer progeny to fertile than to sterile hosts, which might be a response to possible physiological or embryological differences between the contents of fertile and sterile eggs.

Irradiation effectively sterilized Mamestra and Pieris eggs, since no caterpillars emerged from treated eggs (Figure 8.8). The eggs remained largely unchanged during incubation at 80% rh. The fraction desiccated eggs differed significantly between Mamestra and Pieris (7.8 vs. 21.6%, respectively). This fraction was similar across treatments within the two species, except for untreated Pieris eggs (30% desiccation for the latter vs. ca. 20% for sterilized and/or parasitized eggs, $P < 0.05$). Since host embryos were killed by both sterilization and parasitization, this finding indicates that development of the host embryo may affect the resistance of the egg shell to desiccation.

The proportion of host eggs yielding adult parasites was not different between sterilized and viable eggs for both M. brassicae and P. brassicae. This shows that in both species host suitability for Trichogramma is independent of the host's viability. Compared to other strains, the
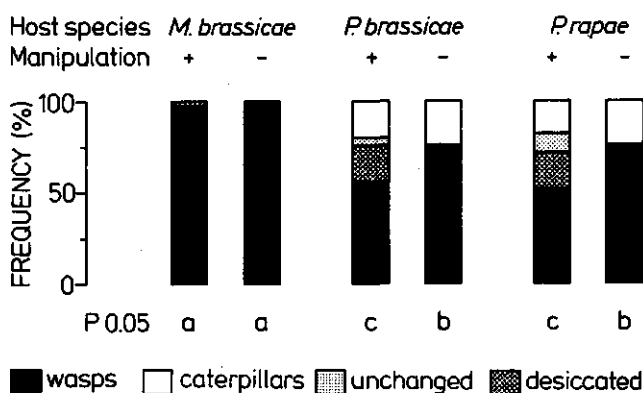
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Figure 8.9. Frequency distribution of various yields from eggs of
M. brassicae, P. brassicae and P. rapae (n=50) exposed for 4 h to 5 females
of T. maidis (strain 11) and incubated on foliage or (after manipulation)
on filter paper at $25\pm1°C$ and $70\pm5\%$ rh.

suitability of P. brassicae eggs seems to be high in the present strain
(ca. 80% of eggs yielding wasps vs. ca. 90% for M. brassicae).

External factors

Results of the host-transferring experiment for strain 11 showed that
Mamestra eggs were always successfully parasitized, whether kept on leaves
or manipulated and incubated on filter paper (Figure 8.9). P. brassicae and
P. rapae eggs on leaves either turned black, yielding wasps (76%) or
yielded caterpillars. The latter probably emerged from unparasitized eggs,
since emergence of caterpillars was not observed in any of the preceeding
experiments. Desiccated or unchanged eggs were not observed. Manipulated
eggs of these two species yielded a similar caterpillar fraction, but a
comparatively lower wasp fraction (ca. 54%). The remaining fraction
(25-30%) largely consisted of desiccated eggs.

    The latter results suggest that the manipulations for transferring eggs
equally impaired the suitability of the two species of Pieris eggs (in
about one third of the eggs). This is similar to the fraction of
unparasitized eggs damaged by a similar way of handling (Figure 8.4) and
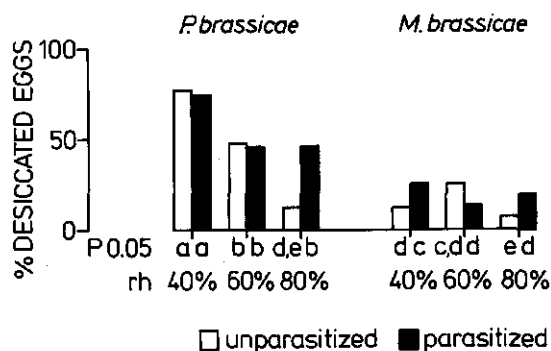confirms the conclusion from the comparison of development of strains 33

Figure 8.10. Percentage desiccation for unparasitized and parasitized
(T. maidis, strain 34) eggs of P. brassicae and M. brassicae (n=90),
incubated at 25±1°C and 40±5%, 60±10% or 80±5% rh.

and 46 on different substrates. Thus especially after manipulation,
P. brassicae and P. rapae eggs seem to be more susceptible to desiccation
than M. brassicae eggs, which phenomenon appears to interfere with the
suitability as a host for Trichogramma.

In the humidity-range experiments it is shown, that the percentage
desiccation of unparasitized P. brassicae eggs decreased considerably with
increasing humidity from 78% at 40% rh to 13% at 80% rh (Figure 8.10).
Desiccation of parasitized (strain 34) and unparasitized eggs did not
differ at 40 and 60% rh. However, at 80% rh it was higher for parasitized
(47%) than for unparasitized eggs of P. brassicae. The percentage
desiccation of unparasitized M. brassicae eggs was relatively low at each
humidity (14-26%) and similar to that of P. brassicae at 80% rh.
Desiccation of parasitized M. brassicae eggs varied within a similar range
(8-26%) as that of unparasitized eggs. At 40 and 80% rh the percentage
desiccation was slightly higher for parasitized than for unparasitized
eggs.

The present findings demonstrate that in Pieris eggs, other than in
Mamestra, a decreasing humidity has an increasing detrimental effect on the
resistance to dehydration. If the relative humidity is sufficiently high
(80%), this effect is reduced to the level of desiccation generally
occurring in Mamestra eggs (the two species of eggs were manipulated in a
similar manner). At a high humidity, parasitism of host eggs may increase

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their sensitivity to desiccation, which is especially apparent in Pieris eggs. This suggests that there may be an interactive effect of relative humidity and parasitism on the resistance of eggs to desiccation.

In this experiment wasps drilled longer on Mamestra than on Pieris eggs (46.6 ± 4.9 s vs. 28.6 ± 2.0 s, respectively). This indicates that there is a difference in the structure and or thickness of one or more of the egg-shell layers between the two species, which might be a cause of the difference in resistance to desiccation (see below).

Egg-shell and puncture structures

Scanning electron microscope pictures of the external structure of M. brassicae and P. brassicae eggs show that in both species the surface is distinctly ribbed (Figure 8.11). In P. brassicae, ten vertical ribs extend from the base of the egg to the micropylar area on the top. Each pair of ribs has an intermediate rib which terminates before the micropylar area. Cross ribs between the vertical ribs divide the smooth surface of the Pieris egg into rows of rectangular areas, giving it an undulating appearence.

In M. brassicae eggs major vertical ribs (about 18) run from the reticulate areas surrounding the micropyle to the base. In between the major ribs, one or two minor ribs extend some distance upwards from the base of the egg. Cross ribs in Mamestra eggs are not as conspicuous as in Pieris eggs, while the surface texture appears wrinkled rather than smooth. In both species, aeropyle openings were only observed in intersecting ribs in or near the micropylar area.

Two hours after parasitization about half of the observed punctures were already found to be sealed in both Mamestra and Pieris eggs. The diameter of unsealed punctures was similar for the two host species and strains, averaging 4.8 microns (Table 8.5). The punctures were somewhat smaller than the width of the wasp ovipositor (4.8 vs. 5.9-6.1). Puncture holes were about 4 times wider than the aeropyle openings. Four days after parasitization all punctures were sealed. This indicates that the difference in host suitability between the two species probably is not due to a difference in puncture diameter or puncture sealing.

Microscopic pictures of egg-shell sections show that the thickness of
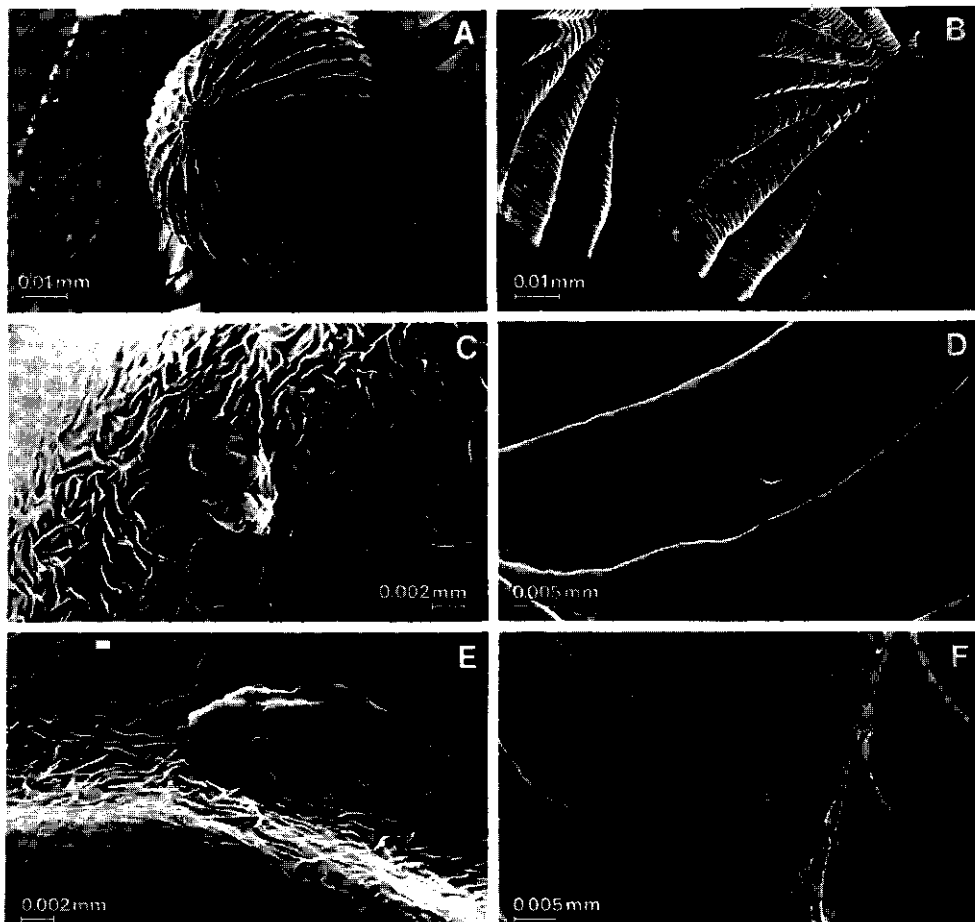
Figure 8.11. Scanning electron microscope pictures of eggs of M. brassicae and P. brassicae, unparasitized (A-B) or parasitized by Trichogramma (C-F). A = unparasitized M. brassicae egg; B = unparasitized P. brassicae egg; C = M. brassicae egg with sealed ovipositor puncture, 2 h after parasitization; D = P. brassicae egg with an unsealed ovipositor puncture, 2 h after parasitization; E = M. brassicae egg with an aeropyle opening and a sealed oviposition puncture 4 d after parasitization; F = P. brassicae egg with a sealed ovipositor puncture 2 h after parasitization.

Table 8.5. Size measurements (means + 95% confidence limits, n=10) of wasp ovipositors for T. buesi (strain 4) and T. maidis (strain 11) and of ovipositor punctures in egg shells of M. brassicae and P. brassicae.

Strain no.	Puncture diameter		Ovipositor size (microns)	
	M. brassicae	P. brassicae	length	width[1]
4	4.8+0.3	4.8+0.3	135.6+1.1	5.9+0.2
11	4.7+0.2	4.8+0.2	138.5+0.8	6.1+0.2

[1] At median length.

the shell is similar for M. brassicae and P. brassicae eggs, varying between 3.5 and 4.5 microns (Figure 8.12). The chorion of M. brassicae eggs appears to consist of two homogeneous layers, which readily parted due to fixation. The inner chorion is thicker and less electron dense than the outer chorion. The chorion of P. brassicae eggs consists of a thick spongy outer layer, a thin homogeneous middle layer and a very thin trabecular inner layer. Thus the eggs of the two species differ especially in the structure of the outer chorion, which might impart a greater hardness and evaporation resistance to M. brassicae eggs than to P. brassicae eggs. Ribs of the egg shell seem to be protrusions of the inner chorion in M. brassicae, but of the middle chorion in P. brassicae. Chorional ribs of P. brassicae seem to be specialized structures.

Variability between strains

Data on the suitability of M. brassicae and P. brassicae eggs as hosts for various Trichogramma spp. strains are shown in Table 8.6. Under the present incubation conditions (high humidity and limited manipulation of eggs) host eggs either yielded wasps (suitable host) or gradually desiccated if they did not turn black during incubation (unsuitable host). Therefore, only the percentages successfully parasitized eggs (suitable hosts) are presented. M. brassicae eggs invariably seemed to be a good host for all strains (> 90% suitable eggs). Suitability of P. brassicae eggs differed between strains, ranging from 0 to 75% successful development. The results suggest
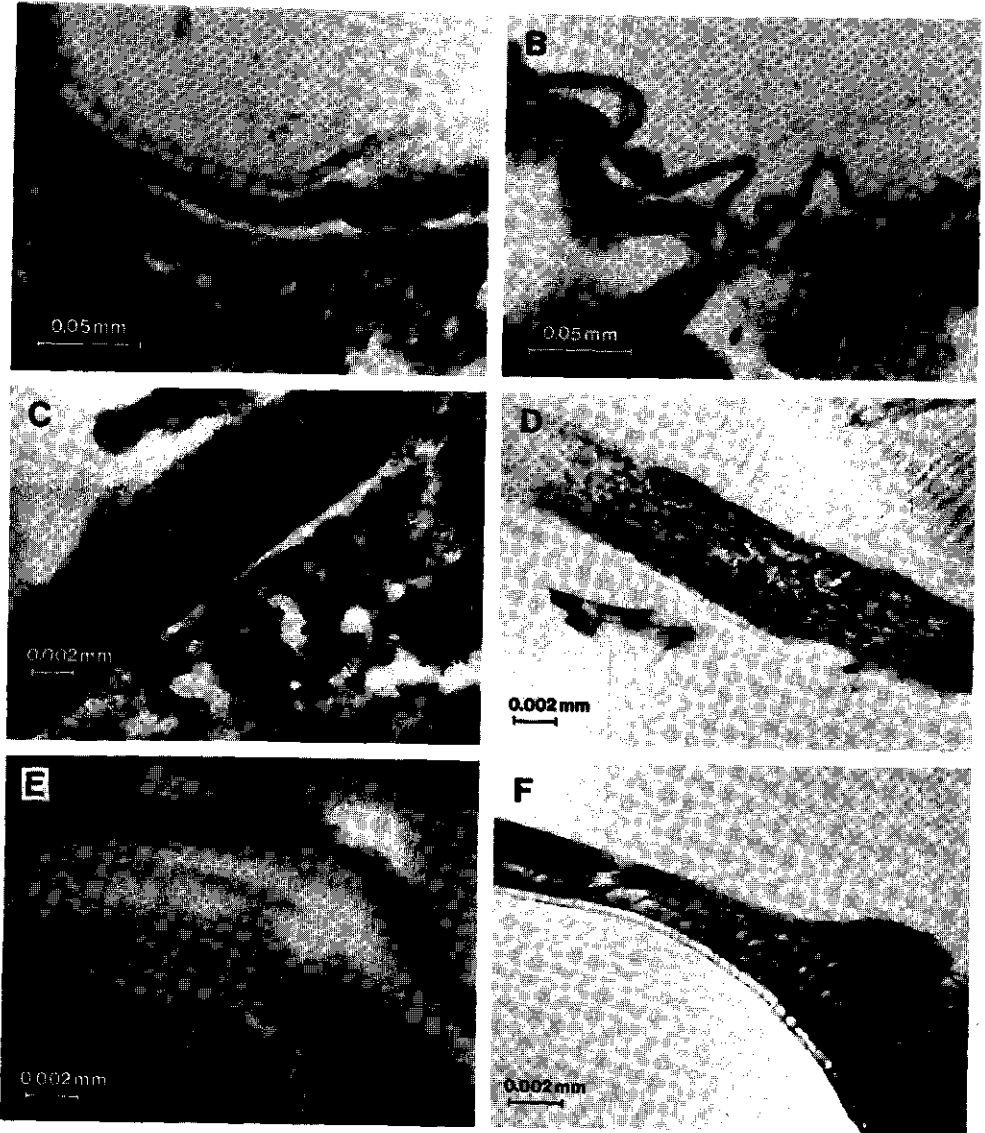
Figure 8.12. Light microscope (LM) and transmission electron microscope pictures (TEM) of egg-shell sections of M. brassicae and P. brassicae.
A = M. brassicae chorion (LM); B = P. brassicae chorion (LM);
C = M. brassicae chorion (TEM); D = P. brassicae chorion (TEM);
E = M. brassicae chorional rib (TEM); F = P. brassicae chorional rib (TEM).

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Table 8.6. Suitability of <u>M. brassicae</u> and <u>P. brassicae</u> eggs for
development of various <u>Trichogramma</u> spp. strains and the clutch size (means
± 95% confidence limits) for suitable (black) and unsuitable (desiccating)
eggs of <u>P. brassicae</u>. Eggs were incubated at 25±1°C and 80±5% rh.

Strain no.	Suitability of eggs (% of n)				Clutch size in <u>P. brassicae</u> eggs		
	<u>M. brassicae</u>		<u>P. brassicae</u>[1]		Suitable	Unsuitable	t-test[2]
	n	%	n	%	mean±95%CL	mean±95%CL	P 0.05
4	50	96.0 a	50	35.7 e	2.6±0.2	2.8±0.3	NS
5	45	93.4 a	40	42.5 de	2.4±0.2	2.7±0.4	NS
11	50	98.0 a	50	64.0 abc	2.3±0.2	2.0±0.3	NS
14	35	97.2 a	35	42.8 de	2.3±0.3	2.6±0.2	NS
17	25	96.0 a	50	56.0 bcd	2.2±0.3	2.8±0.5	S
18	50	98.0 a	50	46.0 cd	2.3±0.4	2.6±0.3	NS
23	50	94.0 a	50	68.0 ab	2.5±0.3	2.4±0.1	NS
33	40	95.0 a	40	75.0 a	3.5±0.5	3.2±0.5	NS
38	35	100 a	50	52.0 bcd	3.1±0.2	3.3±0.3	NS
43	40	90.0 a	40	25.0 e	2.4±0.3	2.2±0.2	NS
45	50	94.0 a	50	48.0 cd	2.5±0.5	3.4±0.4	S
46	50	98.0 a	35	0 f		2.0±0.2	
57	50	98.0 a	50	52.0 bcd	2.5±0.4	2.7±0.5	NS
82	50	98.0 a	50	66.0 abc	3.0±0.3	3.2±0.4	NS

[1] Percentages per host species followed by the same letter are not
significantly different by Chi-square test, P < 0.05; differences between
host species are significant for all strains.

[2] Mean comparison for suitable and unsuitable host eggs.

that a relatively high suitability of <u>Pieris</u> eggs may correspond with a
high host acceptance for this species (chapter 6), e.g. in strains 11, 38,
57 and 82. The result for strain 14 indicates that this is not necessarily
the case.

Females of several strains from the collection refused to accept any
<u>Pieris</u> egg, e.g. strains 9 (<u>T. embryophagum</u>), 24 (<u>T. exiguum</u>),

32 (T. maidis) and 47 (T. sp. near sibiricum). Intraspecific variability in
host suitability of Pieris eggs is demonstrated by the results for
T. maidis strains throughout this study. Variability in host suitability
between females within strains was not apparent. Clutch sizes for
P. brassicae eggs differed among strains, also within the same species
(Table 8.6). The clutch sizes for suitable and unsuitable hosts within
strains were the same for most strains, or higher for unsuitable hosts.
Thus, mortality of parasites seemed largely restricted to desiccating
Pieris eggs.

DISCUSSION

Suitability

Eggs of M. brassicae and P. brassicae differ in suitability for development
of Trichogramma. Parasitism of Pieris eggs appears to be effective for some
strains of Trichogramma, but not for others. None of the individuals of
strain 46 were able to complete development in P. brassicae eggs, although
all parasitized hosts were killed. This indicates that the chemical
substance by which Trichogramma kills and digests its hosts preorally
(Strand 1986) is also effective in decomposing Pieris eggs. Histological
sections indeed showed that a few hours after parasitization the host
content was dissociated in a zone around the parasite eggs.

In Pieris eggs parasitized by strain 46, dissociation of the oocyte
appeared more intensive than in eggs parasitized by strain 33 (N. Hawlitzky
pers. comm.), for which P. brassicae is a suitable host. This suggests a
possible quantitative or qualitative difference in toxicity of the injected
fluid between the two parasite strains, resulting in a detrimentally rapid
desintegration of the host. Hypersensitivity of hosts to wasp venom,
resulting in precocious host death, has been reported for larval parasites
(e.g. Gerling & Rotary 1973, Boulêtreau & Wajnberg 1986). The phenomenon
may be relatively common in host-parasite relationships, but has not been
reported for egg parasites (Vinson & Iwantsch 1980b).

A correspondence between the rates of egg laying and of killing hosts
was shown for both the suitable and the less suitable host species. This
suggests that, other than by injected venom, hosts might also be killed by

a toxic secretion from the parasite eggs. However, this hypothesis is refuted by the nonhatching of T. pintoi eggs (strain 46) in P. brassicae eggs, which were effectively killed. The data suggest that a toxic substance is injected gradually during the oviposition phase, or more likely, during each separate egg-laying sequence. Moreover, the data show that a single or brief injection may not be sufficient to kill hosts which sustain development of two or more parasites. This is contrary to the findings by Strand (1986) for T. pretiosum parasitizing Heliothis virescens eggs. The presently observed phenomenon helps to explain the emergence of caterpillars from (pseudo)parasitized eggs in previous experiments on host suitability (chapters 5, 6 and 7).

Development of strain 33 was 1 d longer in Pieris eggs than in Mamestra eggs, largely due to a longer duration of the third instar larval stage. In this stage most of the food is ingested (Hawlitzky & Boulay 1982), probably by pharyngeal pumping with continuous oral ingestion and swallowing (Thompson 1986). This suggests a physical or chemical difference between the eggs of the two host species (e.g. in viscosity or in substances acting as feeding stimulants), due to which the egg contents of Pieris was ingested slower than that of Mamestra. An increased duration of development was found by Strand & Vinson (1985) for T. pretiosum cultured on an artificial medium which had to contain at least 30% hemolymph to induce pupation. Parasites reared in vitro had a 1 d longer larval stage than in vivo (H. virescens), while all other stages were of equal duration.

Development time of Trichogramma has also been found to increase with decreasing humidity (e.g. Lund 1934, Quednau 1957). Calvin et al. (1984) found that development of T. pretiosum at 30°C increased from 7 d at 80% rh to 10 d at 20% rh. Osmotic pressure, one of the critical factors for in vitro parasite development (Thompson 1986), probably will increase in an egg at low humidity (Biemont et al. 1981). A high osmotic pressure might explain the inhibition of egg hatching of strain 46 in Pieris eggs on a dry substrate. In a medium of high osmolality Trichogramma eggs do not swell (J.M. Schmidt pers. comm.), which they normally do soon after the egg is laid (Klomp & Teerink 1967). Host-fluid absorption through the chorion might be essential as a trigger of parasite embryogenesis (Fisher 1971). Part of the eggs of strain 46 hatched if hosts were incubated on foliage. The larvae subsequently died, apparently full of food, as early third

instars. Lack of a pupation factor (Irie et al. 1987) does therefore not seem to be a cause of host unsuitability in Pieris.

The action of a toxin in Pieris eggs might also provide an explanation for their unsuitability as a host for Trichogramma. However, inferences about the presence of a toxin can hardly be made from this study. Xie et al. (1986a) found that in vitro reared T. pretiosum eggs did not hatch if sorbic acid and methyl-p-hydroxy-benzoate were added as antibiotics to the medium. A salt solution (KCl-MgSO4), used as an ovipositional stimulant for T. pretiosum (Nettles et al. 1982, 1983), killed eggs if exposure lasted longer than 6 h at 27°C (Xie et al. 1986b). Development of T. pretiosum (as a hyperparasite) is also inhibited by a cytolytic toxin associated with T. heliothidis (Strand & Vinson 1984, Strand 1986), but not by the "arrestment factor" injected into the host by T. heliothidis prior to egg laying (Strand et al. 1983).

Host defense and competition probably did not play a role in the present study as factors that may determine host suitability (Vinson & Iwantsch 1980a). Success of development in dead or live Pieris eggs was the same and encapsulation was not observed in any of the histological sections. Furthermore, it is unlikely that hemocytic reactions are possible if the oocyte undergoes lysis shortly after becoming parasitized. Klomp & Teerink (1978) found that competition in Trichogramma may lead to immature death by physiological suppression. Present data show that there is an optimal clutch size for which survival of the progeny is maximal. Freely ovipositing wasps exactly laid the optimal number of eggs into P. brassicae eggs.

Host-egg desiccation

This study shows that eggs of P. brassicae are more sensitive to desiccation than eggs of M. brassicae, which may have attributed to the difference in suitability as a host for Trichogramma between the two host species. Desiccation of eggs seemed to be mostly a consequence of manipulations carried out to remove eggs from the substrate they were laid on. Transferring eggs may have affected the permeability of the egg shell resulting in desintegration of the egg. Alternatively, physical stress may have affected the oocyte, resulting in structural desintegration and

increased permeability of the egg shell.

The water loss across the surface of an egg depends, by Fick's law, on the concentration of water vapor at the surface, the concentration of water in the atmosphere and the resistance of the egg to water loss (Monteith & Campbell 1980, Wright & Cone 1986). Damaged eggs appeared less resistent to water loss and desiccated in a dry environment. If Pieris eggs were removed from parafilm, and incubated at 80% rh, desiccation was reduced to a level (up to 10%) generally occurring in M. brassicae eggs removed from tissue paper. Differences found in the chorion structure of Mamestra and Pieris eggs seem to be related to a difference in permeability of the egg shell between the two species, because wasps drilled longer on Mamestra eggs and fixative solutions penetrated slower into eggs of this species than into Pieris eggs.

The relationship between egg-shell structure and resistance to desiccation has been studied for several Lepidoptera. Chauvin & Barbier (1972) compared the egg-shell structure of two tineid species and found that resistance to desiccation was imparted by characteristics of the chorion. A unidirectional tubular structure of the main inner layer appeared less permeable than a helicoidal structure. A serosal cuticle appeared to impart only a partial resistance. In eggs of the wax moth Galleria melonnella (Pyralidae) resistance to desiccation was attributed to the densification (condensation) of the vitelline membrane (to 10% of its initial thickness) after fertilization, while the serosal cuticle supposedly provides mechanical protection (Barbier & Chauvin 1974). Salkeld (1973) suggested that waterproofing in the egg of the noctuid Amathes c-nigrum is imparted by a thin wax layer between the inner layer of the chorion and the vitelline membrane. A similar resistance mechanism has been reported for E. kuehniella (Cruickshank 1972) and for the micropterigid Micropterix calthella (Chauvin & Chauvin 1980).

The densification process in the vitelline membrane has been investigated by Barbier & Chauvin (1977) for several Lepidoptera and appears to occur just prior to the onset of embryogenesis. The structural change in the membrane is a tanning process, iniated by the oocyte at the moment of egg laying. The oocyte itself may be activated by sperm penetration or by some other chemical or physical stimulus in parthenogenetic eggs. In most lepidopterous species a serosal cuticle is

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deposited about 2 d after egg laying. The external layer of this cuticle has an unpermeable helicoidal structure. In the present study, however, resistance to desiccation of P. brassicae eggs did not appear to increase with age, because desiccation of sterilized, fresh eggs was less than that of viable control eggs.

Parasite desiccation

The present results indicate that host eggs which, due to damage, are desiccating become unsuitable hosts in which the parasites desiccate also. This phenomenon helps to explain why parasite death within a host either appears to affect the entire progeny clutch or is virtually non-existent. Within clutch mortality only appeared to be significant in superparasitized hosts, probably due to crowding competition (Klomp & Teerink 1978). In contrast, clutch survival increased and clutch desiccation decreased with increasing clutch size under non-crowding conditions, which provides another explanation for clutch mortality.

It is not likely that the resistance to water loss of parasitized eggs was affected by wasp-ovipositor punctures in the egg shell, since desiccation of eggs with one or two punctures was similar. Two hours after parasitization about half of the punctures in both Mamestra and Pieris eggs appeared to be sealed. Boldt & Ignoffo (1972) suggested that egg-wound seals might be important in preventing desiccation. However, the origin (e.g. host or parasite induced) and function of the seals are not exactly known.

At a high humidity (80% rh) eggs of P. brassicae parasitized by females of strain 34 were more sensitive to desiccation than unparasitized eggs. This suggests that, beside manipulative physical damage, embryogenesis of the parasite may also interfere with the permeability of the egg shell. The embryonic effect probably is different from the effect of physical damage, since the difference in desiccation between parasitized and unparasitized eggs did not occur at lower humidities. Increased mortality of immature Trichogramma with decreasing humidity has been reported by Calvin et al. (1984) and Lund (1934). In contrast to the findings for P. brassicae eggs, the resistance of M. brassicae eggs to desiccation was presently not found to be influenced by parasitism.

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About one third of unparasitized P. brassicae eggs desiccated after removal from foliage and incubation on paper cards. By comparison, all P. brassicae eggs desiccated if parasitized by females of strain 46. Immature development of strain 46 was inhibited in the egg stage, so that the increased chorion permeability of the host egg appears to be directly related to the deposition of parasite eggs within. However, the duration of egg laying, which was coincident with the number of eggs laid and the percentage of hosts killed, did not influence the percentage of desiccating eggs.

Interaction

The increased desiccation of parasitized eggs, compared to unparasitized eggs, is probably explained best by an interaction between physical damage to the egg and dissociation of the host due to the parasitism process. Strand (1986) reported that Trichogramma are protected from desiccation in young infertile hosts (less than 12 h old) by their pupal case. Infertile eggs lack a serosal cuticle, and if older eggs are parasitized the parasites desiccate prior to becoming a prepupa. In the present study, however, the suitability of 2 d old infertile eggs of M. brassicae as a host for Trichogramma was not different from that of fertile eggs. This suggests that resistance to desiccation in M. brassicae eggs may be imparted by the structure of the chorion rather than by the subchorional layers. In this respect M. brassicae eggs might be different from P. brassicae eggs. Strand's observation apparently may not be generalized, but it demonstrates how survival of the parasite may interact with the resistance to desiccation of the host. Alternatively, parasites developing in fresh host eggs may be less resistant to dehydration than parasites in older eggs having a waterproof vitelline membrane or serosal cuticle.

The longer development time of Trichogramma larvae in P. brassicae eggs may be a factor contributing to the interaction of host and parasite desiccation, because this extends the duration of the interaction. However, data suggest that the interaction does not necessarily depend on whether the host and/or parasite embryo are dead or alive. Thus the interaction might be physical and/or chemical in nature rather than being an active biological process. Interactive effects might, however, be confounded by

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more rapid dehydration in dead than in live stages (Machin 1979). Further research on the effect of manipulation and parasitization of eggs on the structure of the chorional and subchorional layers should be conducted to establish the exact nature of the suggested interaction.

Evaluation

The present study helps to explain the difference in suitability of Pieris eggs between strains of Trichogramma. This difference seems to be largely due to variation between strains in the ability to adequately ingest and/or digest the host, e.g. due to nutritional inadequacy of the host or the action of a toxin. The success of development is further dependent upon dehydration of the host, which may be influenced by several factors determining the permeability of the envelopes protecting immature hosts and parasites. Water-loss resistance in Pieris eggs appears to be low and vulnerable to physical or chemical impairment.

The inter- and intraspecific variability in suitability of P. brassicae eggs among Trichogramma strains appears to correspond to variability in host acceptance (chapter 6). Host suitability data cannot be used as a separate criterion in the present pre-introductory selection program, however, because a high success of development of a candidate strain in a certain host species does not necessarily preclude the occurrence of preference for other suitable species. Females of all tested strains that accepted Pieris eggs as a host were able to kill the embryos of unsuitable eggs. As far as the present study is concerned, each candidate strain meets the requirement for use in an inundative release program.

Chapter 9. EXPERIMENTAL FIELD RELEASES

ABSTRACT

Experiments with inundative releases of Trichogramma against Lepidoptera on
cabbage were carried out from 1982 to 1985 with four candidate strains in
field plots of Brussels sprouts infested by natural host populations. Each
year two strains were selected on the basis of results from pre-
introductory studies in the laboratory, such as the parasitization activity
at low temperature and host-species preference. Mamestra brassicae was the
most abundant host species. Its density usually averaged between 0.5 and
2.0 eggs per plant throughout most of the season (June - September). A very
high peak density of 12 eggs per plant occurred in 1982. In other years the
peak was below 2.5 eggs per plant.

A correspondence between the observed patterns of parasitism and
previously studied behavioural traits of the strains was generally
apparent. Strain 57, with a high parasitization activity at 12°C, performed
best against M. brassicae. However, even its highest seasonal rate of
parasitism (52%) was not sufficient for effective suppression of larval
host populations. Strain 11 performed best against Pieris brassicae and
P. rapae, but parasitism remained low (<30%) and was generally limited to
peak densities (>0.5 eggs per plant) occurring in 1982 and 1985. Plutella
xylostella was an abundant species in some years, but parasitism of its
eggs was never observed. Egg densities of Evergestis forficalis remained
low in all years. The relationship between parasitism and host density and
the influence of the parasite-release rate are discussed.

INTRODUCTION

Experimental evaluation of the performance of natural enemies in the field
is necessary to show their value and shortcomings, to provide insight into
the principles of population ecology and to give proof of their
effectiveness as a control agent (DeBach et al. 1976). Methods to evaluate
the actual and potential importance of natural enemies include their
addition to or exclusion from a crop habitat, or some kind of interference
with their activity (DeBach & Huffaker 1971). The methodology and

feasibility of different evaluation techniques has been reviewed by
Kiritani & Dempster (1973). For inundative biological control the addition
method seems to be the most appropriate evaluation method. In case of
Trichogramma releases this method generally involves a comparison of host
population development, parasitism and crop damage in release fields with
that in control fields (e.g. Oatman & Platner 1978, Hassan et al. 1978,
Hassan 1981b).

This chapter is concerned with field experiments on releases of
candidate Trichogramma strains conducted from 1982 to 1985. Each year two
strains from the laboratory collection (chapter 2) were selected for
inundative releases into plots of a Brussels sprouts field. Natural
populations of the host species (chapter 1) were allowed to develop in the
field. The objective of these releases was to compare the performance of
the strains and to determine whether this performance corresponds to
certain behavioural characteristics which have been investigated previously
in the laboratory. Two criteria thus tested are the parasitization activity
at a low temperature of 12°C (chapter 4) and host-species selection
(chapter 6).

One of the strains released in 1982, T. maidis (no. 8) was used in
West-Germany to control O. nubilalis in corn (Hassan et al. 1978), whereas
the other strain (no. 11) was a local collection of this species. Strain 11
has a high level of acceptance for both Pieris and Mamestra eggs
(chapter 6), but a low parasitization activity at 12°C (chapter 4). Strain
11 was therefore tested together with T. evanescens (no. 57), an Egyptian
strain with a high activity at 12°C, in the experiments of 1983 and 1984.
In 1985 the performance of strain 57 was compared with that of strain 82,
which had shown a superior perfomance among several indigenous strains
tested in cabbage fields in West-Germany (Hassan & Rost 1985). Strain 82
had a higher acceptance level of Pieris eggs in the laboratory than strain
57 (chapter 6).

MATERIALS AND METHODS

Releases

Experiments were conducted in a yearly different field (0.25-0.5 ha) of

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Brussels sprouts (Brassica oleracea c.v. Rampart) near Wageningen. Nine
uniform plots were usually laid out in the field; the size of the plots
varied between years from 35-100 m^2, depending on the size of the field.
Plots were surrounded by two rows of plants and separated by uncultivated
margins (ca. 1 m). Standard cultural practices were carried out, excluding
the application of pesticides. Temperature and other weather recordings
were obtained from a nearby weather station. Details of the experimental
design have been reported by Van Alebeek et al. (1986) and Van der Schaaf
et al. (1984).

Each year two Trichogramma strains were introduced as pupae in three
plots each, whereas no introductions were made in three control plots. The
parasites were mass produced on eggs of the Angoumois grain moth, Sitotroga
cerealella (Gelechiidae), at the Institute for Biological Pest Control in
Darmstadt, FRG, in cooperation with S.A. Hassan. Releases were made using
paper cards carrying Sitotroga eggs containing parasites in three
developmental stages (Hassan 1982). The cards were placed under a
protecting cover between 5-40 cm above the ground, inside plastic tubes
(15x1.5 cm) of which the open sides were covered with fine gauze, or in
bags (3x6 cm) of fine gauze. The number of release sites per plot varied
between years from 1 to 4. About 200,000 parasitized eggs/ha were
introduced at intervals of 14 days in the first three years, whereas this
number was about 450,000 per ha in 1985.

Sampling

All plots were sampled weekly during the release period by means of visual
examination of plants. In 1982 and 1983 fixed plants were sampled, which
were located on concentric circles around the center of each plot. Randomly
selected plants were sampled in the next two years, because in 1983 the
sample plants remained smaller than the other plants in the plots. The
number of plants that could be sampled per plot within a day was limited by
the size of the plants and the number of observers. In 1982 and 1983 the
number of sample plants was held constant throughout the season: 23 (out of
98) and 49 (out of 220) plants per plot, respectively. In 1984 the number
of plants examined per plot of 225 plants was decreased at mid-season from
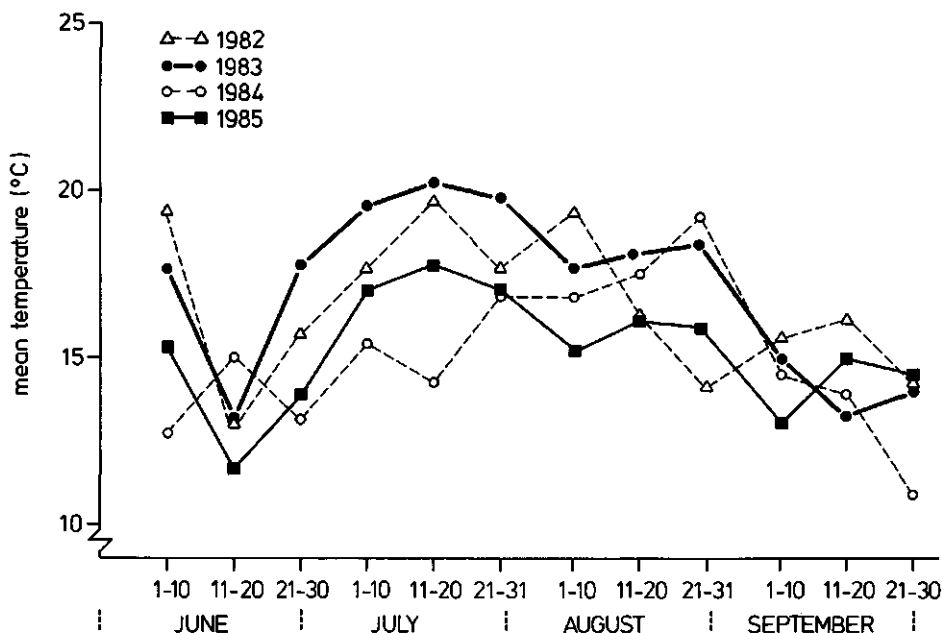100 to 40, in 1985 from 40 to 25.

Figure 9.1. Mean field temperature (°C), for periods of 10 days from June through September in 1982 - 1985.

For each sample plant the numbers of eggs and larvae of the different host species (chapter 1) and the degree of caterpillar feeding-damage (Theunissen & Den Ouden 1983) were recorded. Egg parasitism was determined from the numbers of parasitized and unparasitized eggs (1983, 1985) or by marking sampled leaves on which unparasitized eggs were found and checking these for parasitism a few days afterwards (1982 and 1984). In 1984 the qualitative damage of harvested sprouts was determined.

RESULTS

Temperature conditions

Figure 9.1 shows the weekly mean temperature for each year from June through September. The monthly averages (1951-1980) are 14.9°C (June), 16.4°C (July), 16.5°C (August) and 14.4°C (September). Especially in June, mean daily temperatures below 15°C occur frequently (Figure 4.1).

Figure 9.2. Egg-population density of M. brassicae in 1982 – 1985. Numbers are averages for release and control plots.

Temperatures in mid-June were generally below average. July was relatively warm in 1982 and 1983, but cool in 1984. August was cool in 1985, September in 1984 and 1985. Overall, the weather was fine in 1982 and 1983, wheras it was cool in 1984 and 1985.

Host-egg densities

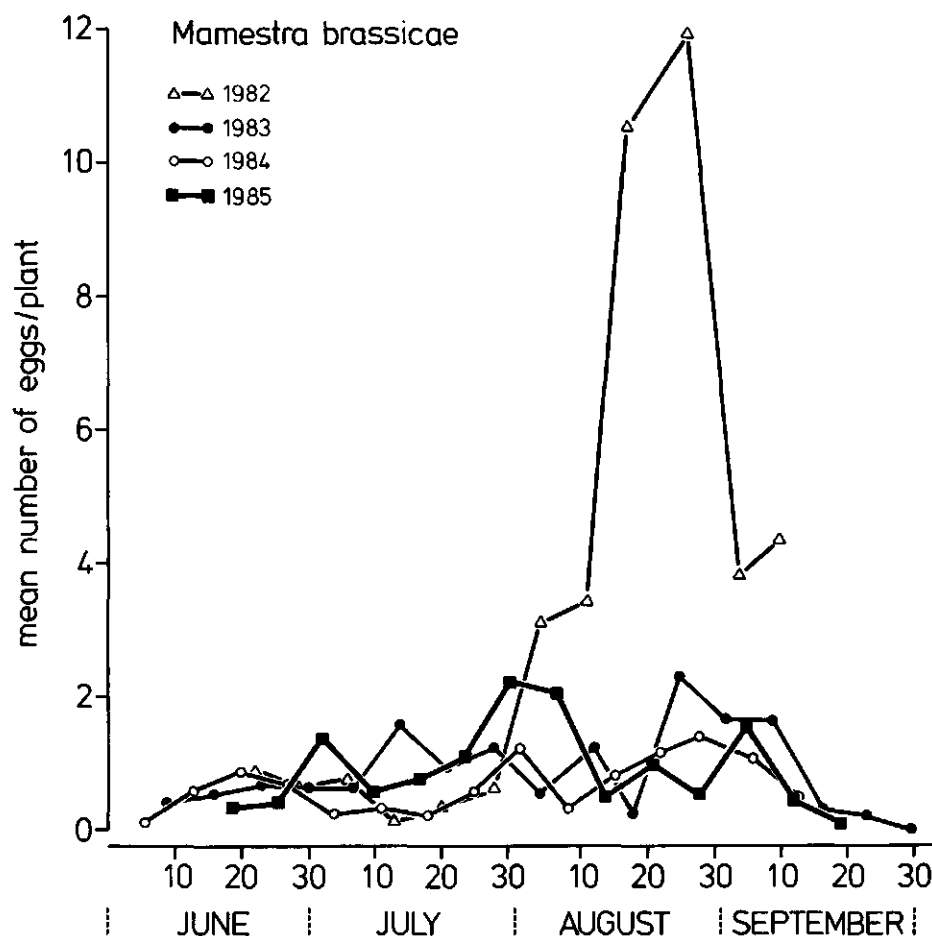Different patterns of egg distribution occur among the host species (chapter 1). M. brassicae, P. brassicae and E. forficalis lay eggs in
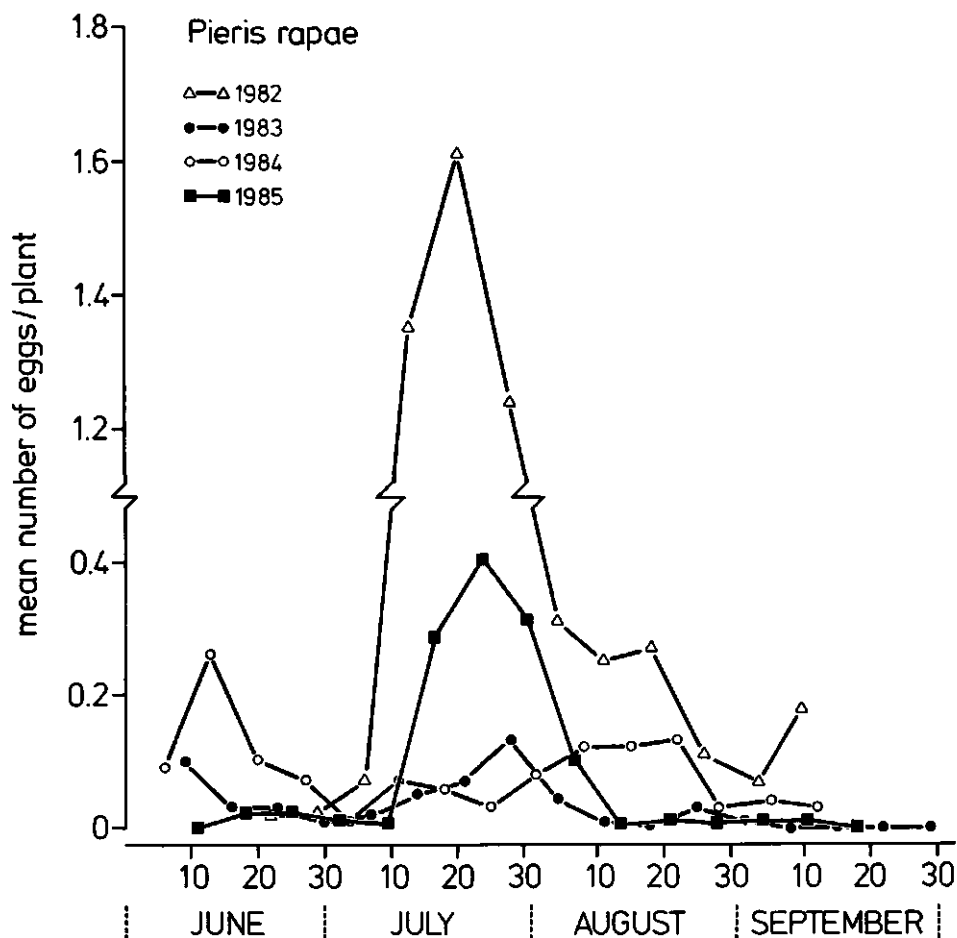
Figure 9.3. Egg-population density of P. rapae in 1982 - 1985. Numbers are averages for release and control plots.

clusters, whereas P. xylostella and P. rapae lay single eggs. For each species, egg densities were determined as the mean number per plant. Extremely high densities occurred in 1982, whereas they remained relatively low in other years. M. Brassicae was the most abundant species in all years. The sampling precision for the mean number of eggs per plant (Cochran 1977) was generally between 10 and 20% of the mean value (α 0.05).

The abundance of M. brassicae eggs for each year is shown in Figure 9.2. Peak densities usually occurred in the second generation, at
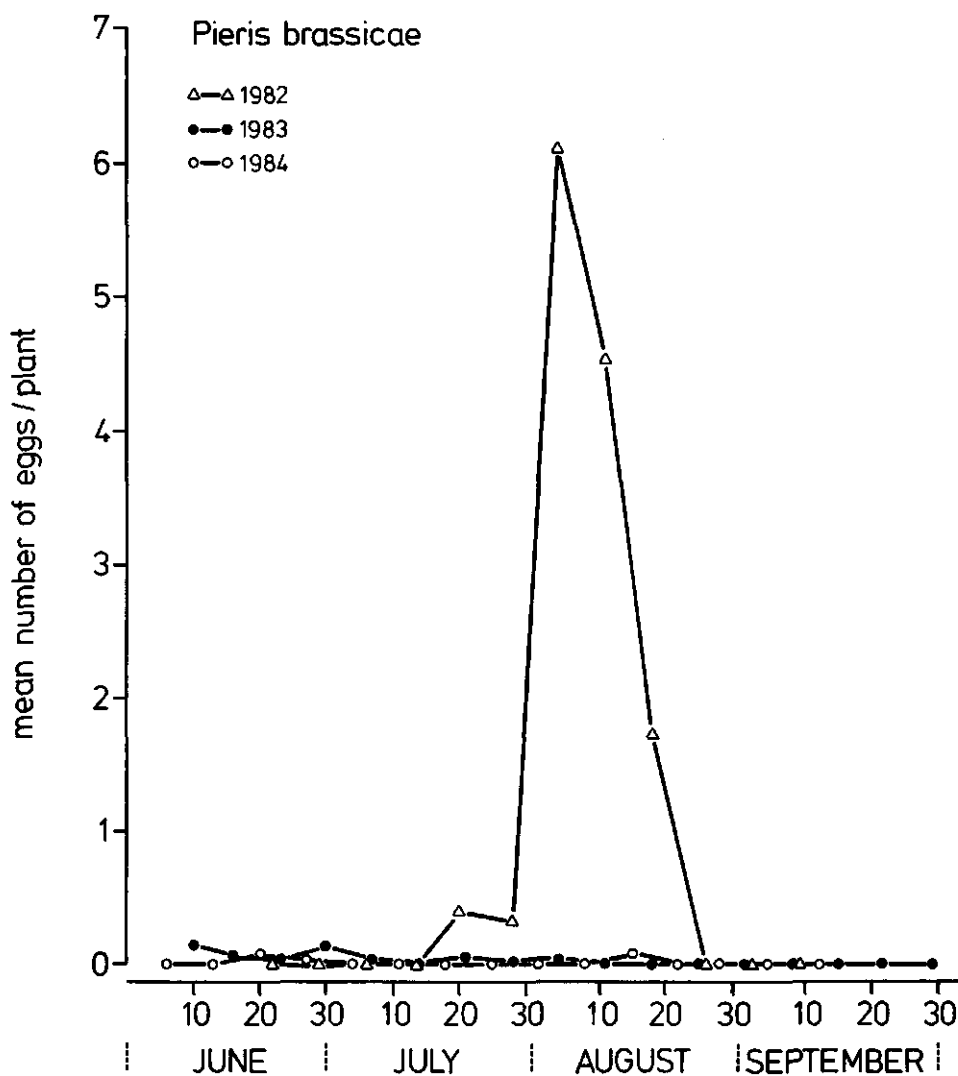
Figure 9.4. Egg-population density of _Pieris brassicae_ in 1982 – 1984.
Numbers are averages for release and control plots.

the end of August. A high peak density of 11.9 eggs/plant occurred in 1982.
In subsequent years the peak was below 2.5 eggs/plant.

P. rapae was the second abundant species, but was much less abundant
than M. brassicae (Figure 9.3). In 1983 and 1984, the P. rapae egg density
varied between 0 and 0.2 eggs/plant throughout the season. However, peak

Figure 9.5. Parasitism of M. brassicae egg clusters for different Trichogramma strains in 1982 - 1985.

Table 9.1. Percentage seasonal parasitism[1] for release and control plots
(means for 3 plots) in four years of experimental releases of different
Trichogramma spp. strains in Brussels sprouts field plots.

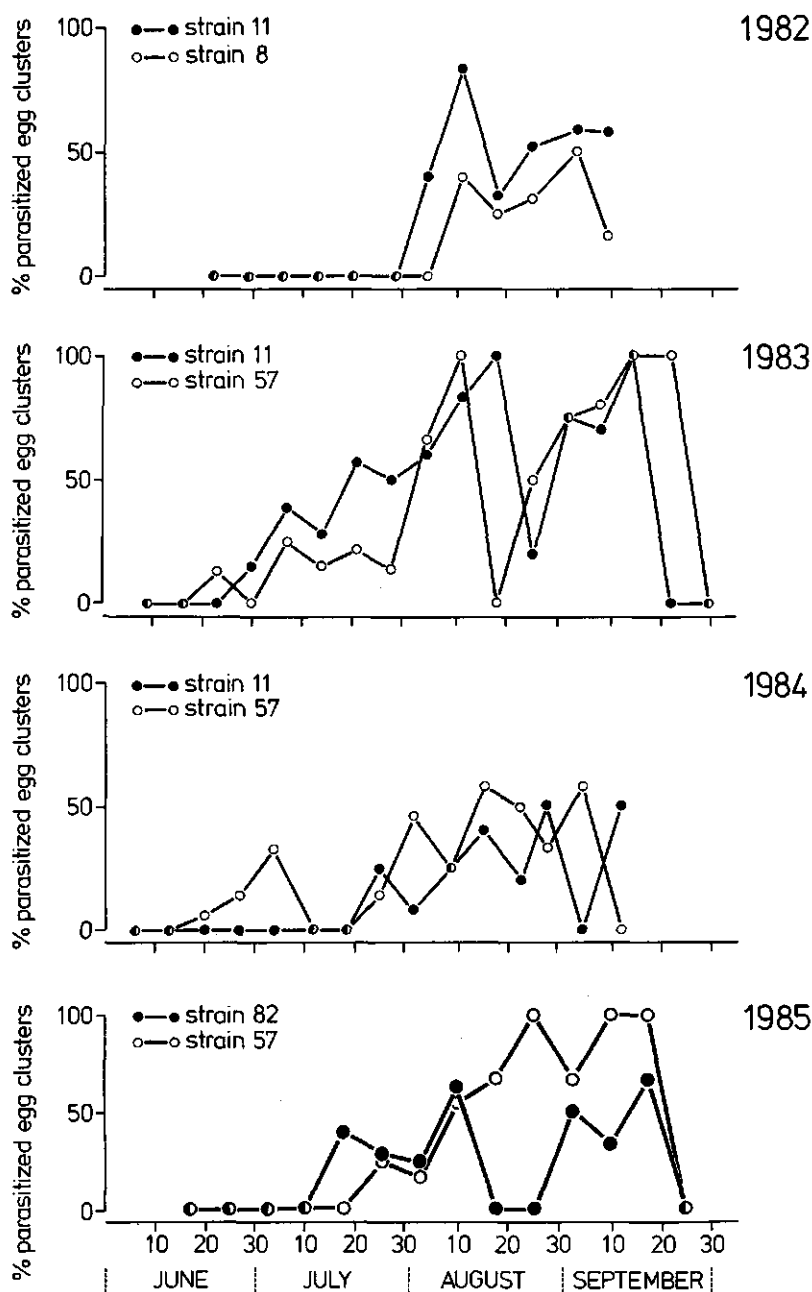| Year	Release plots				Control plots parasitism %
	Strain no.	parasitism %	Strain no.	parasitism %	
1982	8	22.2 b	11	46.8 a	0 c
1983	57	41.6 a	11	35.8 a	34.2 a
1984	57	23.9 a	11	12.1 b	4.2 b
1985	57	52.4 a	82	37.3 ab	22.2 b

[1] % parasitism = 100 (no. parasit. eggs)/(no. unpar. + par. eggs). Means
per row followed by the same letter are not significantly different
($P < 0.05$, Fisher exact test).

densities were observed at the end of July in 1982 and 1985 (1.6 and 0.4
eggs/plant, respectively).

In 1983, 1984 and 1985, only a few _P. brassicae_ egg clusters were
found. Egg densities never exceeded 0.15 eggs/plant (Figure 9.4). By
contrast, an extreme peak density occurred in August 1982 (6.1 eggs/plant).

P.xylostella eggs were only found in 1983. However, eggs of this
species were probably overlooked, due to their small size and greyish
colour, since larvae of this species were regularly found. _E. forficalis_
egg densities were very low in all years.

Parasitism of _Mamestra brassicae_

In 1982 the first parasitized _M. brassicae_ eggs were found in the beginning
of August (Figure 9.5). In June and July the egg density had been low, less
than 3 clusters per 100 plants (Figure 9.2). Except for the first sample
date, the maximum temperature averaged over 20°C during this period. Table
9.1 shows that the mean seasonal parasitism was higher for strain 11 than
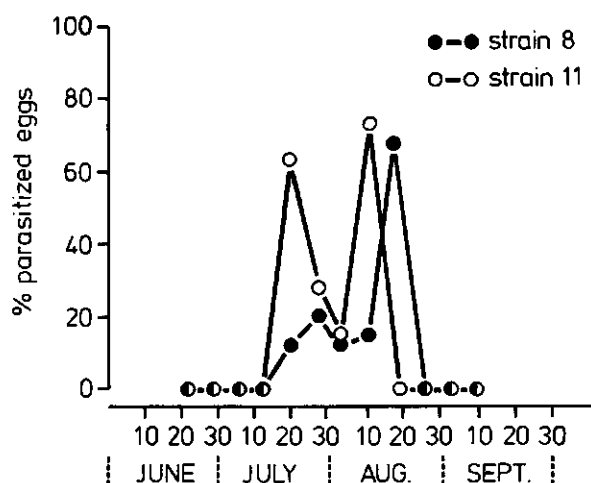for strain 8 (46.8 vs. 22.2%, respectively).

Figure 9.6. Parasitism of P. rapae eggs for two Trichogramma strains in 1982.

In 1983 parasitism started to increase at the end of June for both strains tested, just after the closing of the leaf canopy (Figure 9.5). At the same time the first parasitized eggs were found in the control plots, which indicated that parasites had migrated from release plots into control plots across distances of up to about 20 m. Mean seasonal parasitism was similar for the two strains and the control, averaging between 30 and 40% (Table 9.1).

In 1984 the mean temperature remained below 15°C in June and July (Figure 9.1). During this period parasitized M. brassicae eggs were only found in the plots treated with strain 57 (Figure 9.5). Parasitism for both strains increased in the end of July, which coïncided with the closing of the leaf canopy. Table 9.1 shows that host-egg parasitism was higher for strain 57 (23.9%) than for strain 11 (12.1%).

In 1985 the first parasitized eggs were found in the beginning of July, two weeks after the first parasite release. Parasitism remained low in July, but especially for strain 57 it increased considerably in August (Figure 9.5). Seasonal parasitism was not significantly different between strain 57 and strain 82 (Table 9.1). Parasitism for strain 57 (52.4%) was higher in 1985 than in the previous years.
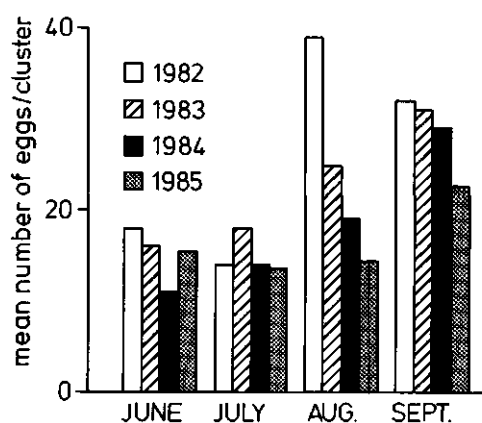
Figure 9.7. M. brassicae egg-cluster size averaged per month in 1982-1985.

Parasitism of other host species

No parasitized P. rapae eggs were found in 1983 and parasitism was low in 1984 (1.6%). In 1982 parasitized eggs were found from the end of June through August (Figure 9.6), which was a period of high egg density (Figure 9.3). Seasonal parasitism for strain 11 and strain 8 was significantly different (28.8 vs. 16.5%, respectively, P < 0.05, Fisher exact test), whereas there was no parasitism in the controls. In 1985 parasitism occurred in the period of peak density only. Seasonal parasitism was not different between strains 57 and 82, averaging 8.9%.

In 1983, 1984 and 1985, parasitism of P. brassicae eggs was not found. In 1982 parasitized P. brassicae eggs were found during a period of high egg density in August (Figure 9.4). Mean seasonal cluster parasitism (i.e. percentage of egg clusters containing parasitized eggs) was significant (control 0%) but not different between the treatments (53 and 36% for strains 11 and 8, respectively; P < 0.05, Fisher exact test).

Not a single parasitized P. xylostella egg was found in all four years. However, due to their greyish colour, parasitized P. xylostella eggs probably are even more difficult to discover than unparasitized eggs of this species. Parasitism of Evergestis forficalis clusters was only found incidentally.

Table 9.2. Parasitism of M. brassicae egg clusters of different sizes in three years of experimental releases of different Trichogramma strains in Brussels sprouts plots.

	Percentage parasitism of egg clusters[1]					
	1982		1983		1984	
Size class (no. eggs /cluster)	Strain 11	Strain 8	Strain 11	Strain 57	Strain 11	Strain 57
	n %	n %	n %	n %	n %	n %
2-10	9 11.1 a	10 10.0 a	47 40.4 a	35 28.5 a	44 6.8 a	58 15.6 a
11-30	41 39.0 ab	23 26.1 a	55 36.4 a	30 36.7 ab	37 10.8 a	51 45.1 b
30	44 50.0 b	21 23.8 a	18 55.5 a	18 61.1 b	10 50.0 b	8 12.5 ab

[1] I.e. egg clusters with one or more parasitized eggs. Means within columns followed by the same letter are not significantly different by Fisher's exact test (P < 0.05).

Egg-cluster size

The probability that an egg in a cluster becomes parasitized is dependent on two conditions: first, the probability of encounter with an egg cluster, and second, the probability of parasitism of eggs in the cluster given such an encounter (Chesson 1982). The two probabilities may be affected differently by the size of the cluster. Figure 9.7 shows that the mean size of M. brassicae egg clusters usually increased with the progression of the season. Cluster size did not correlate with overall egg density.

Percentages parasitism for different size classes of M. brassicae egg clusters are presented in Table 9.2. In some releases there was no effect of cluster size on parasitism, whereas in others parasitsm was highest for the largest clusters. Strains did not appear to behave differently in this respect. These observations suggest that under certain conditions large egg clusters have a higher probability of encounter than small clusters. The probability of parasitism for eggs in the cluster, however, decreased with increasing egg-cluster size (Table 9.3). This might have been due to the limited egg complement of female wasps. However, it may also suggest that females leave egg clusters before parasitizing each egg in the cluster.

Table 9.3. Percentage partly parasitized M. brassicae egg clusters of different sizes; data pooled for 1983 and 1984.

Size class (no. eggs/ cluster)	n	Percentage partly parasitized[1]
2-10	43	4.7 a
11-30	68	17.6 b
30	40	45.0 c

[1] Percentage of total number (n) of clusters with parasitism; if followed by the same letter not significantly different by Fisher exact test (P < 0.05).

Caterpillar densities

In 1982 the number of M. brassicae larvae/plant varied between 0 and 1.2 in the treated plots and between 0.1 and 2.3 in the control plots. In the second half of the season the number of larvae in the strain 11 plots was consistently lower than in the strain 8 or control plots. In 1983, 1984 and 1985 differences in larval densities of M. brassicae between treatments were not apparent. From June through September the density generally varied between 0.2 and 0.4 larvae/plant. For the other species no differences in larval densities between treatments were found in any year.

Theunissen & Den Ouden (1985) developed tolerance levels for the complex of caterpillars in Brussels sprouts (i.e. the percentage of plants that may be infested without occurrence of economic damage), relative to the growing stages of the plants. Tolerance drops sharply when sprout formation begins, 10-12 weeks after planting. Figure 9.8 shows that in 1982 and 1984 the tolerance level was exceeded in the second half of the season.

In 1982 and 1983 no differences in the percentages of plants damaged by caterpillars were found between treatments. In both years leaf-area reduction averaged about 1%. Reductions of this magnitude probably do not result in yield losses for Brussels sprouts (Wit 1982). The assessment of damage to sprouts at harvest in 1984 resulted in a similar percentage
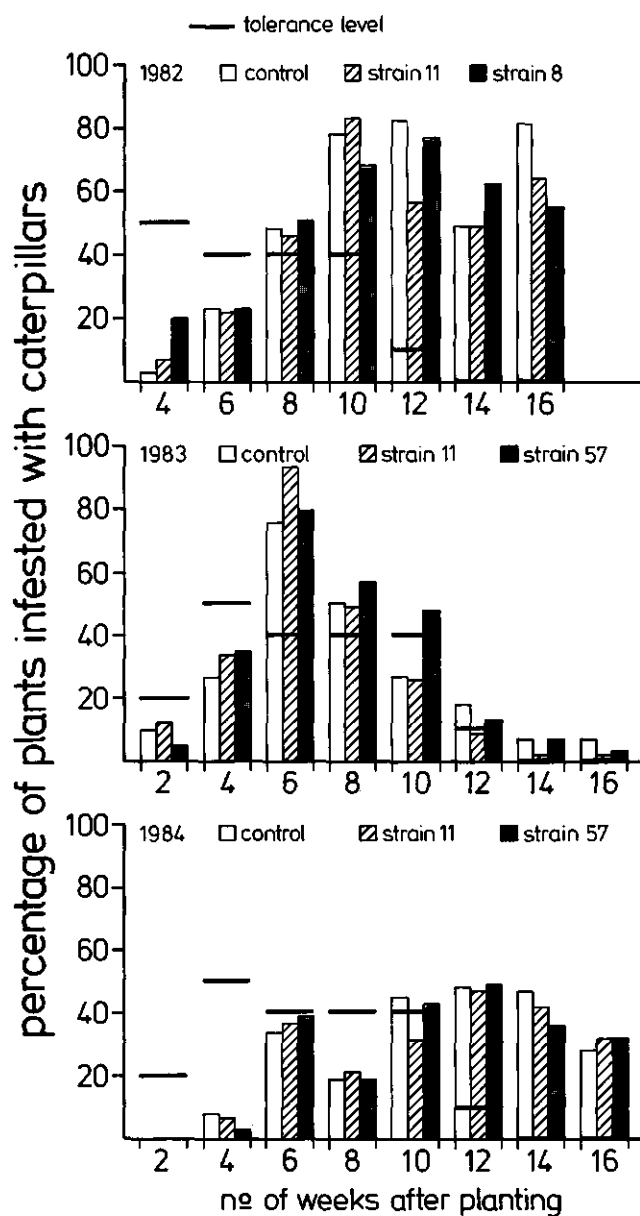
Figure 9.8. Percentage of Brussels sprout plants infested with caterpillars for different plant-growth stages in 1982, 1983 and 1984. Horizontal lines indicate tolerance levels.

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damage for treatments and control: 45% for strain 57, 47.5% for strain 11 and 57.2% for the control. This high figure for sprout damage, compared to leaf damage, shows that direct damage plays a more important role than indirect damage in Brussels sprouts crops.

DISCUSSION

The present study was carried out to compare the performance of different Trichogramma strains in parasitism of several lepidopterous host species in Brussels sprouts. Behavioural differences between these strains have been investigated in laboratory experiments, in particular the parasitization activity at low temperature (chapter 4) and the host-selection behaviour (chapters 5 and 6). Do the present field observations correspond to inferences made from the laboratory studies?

For mean temperatures below 15°C in 1984, parasitism occurred in plots inundated with strain 57, whereas it was not apparent for strain 11. This corresponds to the respective parasitization activities of the two strains at 12°C in the laboratory (97.5 and 17.5% of the females parasitizing, respectively, resulting in an average number of 19.1 and 3.2 parasitized hosts per female). The overall seasonal performance of strain 57 was better than that of strain 11 in 1984, but not in 1983. The latter year was the warmest of all seasons, and temperature possibly was not so much a limiting factor for parasite activity in this year as it was in other years. In 1984, however, parasitsm of M. brassicae eggs by strain 57 was higher within 1.5 m from the release site (22.4%) than between 1.5 and 3 m from this site (4.5%), which may have been due to temperature limited (searching) activity. Lower rates of parasitism by Trichogramma in cabbage crops during periods of cool weather have been reported for other countries by Hassan & Rost (1985) and Parker (1970).

Parasitism for strain 57 was twice as high in 1985 as in 1984. Weather conditions and host-egg densities were similar for the two years, but two times as many parasites were released in 1985 as in 1984. Strain 57 did not perform better than strain 82 (in 1985), which may suggest that the two strains have a similar activity response to temperature.

Host-species selection experiments in the laboratory indicated that Trichogramma generally prefer Mamestra eggs over Pieris eggs as a host

(chapter 6). However, females of strains 11 and 82 have the exceptional trait of accepting Pieris eggs nearly as readily as Mamestra eggs. In 1982 parasitism of Pieris eggs was higher for strain 11 than for strain 8. The two strains do not differ in parasitization activity at low temperature (chapter 4), so that the difference in field performance might be attributed to the difference in host preference. However, some unknown factors probably have also played a role in determining the effectiveness of the parasites, because strain 11 was also more effective than strain 8 in parasitizing M. brassicae eggs. Strain 8 is effective in controlling the corn borer, O. nubilalis, in West-Germany (Hassan 1982), whereas strain 11 originates from M. brassicae eggs on cabbage. Thus, it is possible that the two strains differ in habitat adaptability.

Parasitism of P. rapae eggs was not different between strain 57 and strain 82 in 1985, despite the difference in host preference between the two strains. Compared to 1982, parasitism during the peak density remained relatively low in this year, which suggests that one or more unknown factors were limiting parasitism of P. rapae eggs in 1985.

Although the present results are not fully understood, they demonstrate that the determination of behavioural differences between strains in the laboratory may help to explain their relative performance in the field. Hence this shows the feasibility of using behavioural variations among strains as criteria in a pre-introductory selection program for candidate natural enemies. Nevertheless, it is obvious that the candidate strains selected until now are not effective as a control agent. Even for the best performing strain (no. 57), the observed rates of parasitism of up to 50% appeared insufficient to suppress larval densities and to reduce sprout damage.

Knipling & McGuire (1968) estimated that ca. 80% egg parasitism would be sufficient for control of lepidopterous pests by Trichogramma. In experiments to control O. nubilalis, egg parasitism of about 90% resulted in larval reductions between 83 and 90% (Hassan 1982). Similar experiments conducted in France showed that only 10 to 20% of the corn plants in treated fields were infested with caterpillars, wheras 50 to 90% of the plants in control plots were infested (Voegelé et al. 1979). Shcheptilnikova (1974) reported larval reductions varying from 80 to 88% in cabbage fields in which 87 to 98% of M. brassicae eggs had been parasitized

by _Trichogramma_.

Several researchers (e.g. Gross 1981, Kot 1964, Parker 1970, Shcheptilnikova 1974) have stressed the poor performance of _Trichogramma_ when host densities are low. Gross (1981) suggested that this is due to a lack of host-associated, chemical searching stimulants (kairomones) and to a lack of encounters with hosts, which also may stimulate searching. Pena & Waddill (1983), however, found that parasitism of the tomato pinworm _Keiferia lycopersicella_ by _T. pretiosum_ varied independently of host density. Hirose et al. (1976) and Morrison et al. (1980) found a negative correlation between host density and overall percentage parasitism by _Trichogramma_, resulting from a negative correlation between egg parasitism for clusters and the number of eggs per cluster (Chesson 1982). In the present study parasitism of _M. brassicae_ did not appear related to egg density. By contrast, parasitism of _Pieris_ eggs appeared to be limited to density peaks. Parasitism was only observed if the density rose above its usual level of about 0.1 egg/plant. Peaks occurred when the weather was favourable (high temperatures), which probably favoured activity of the parasites.

Have low host densities been a cause of low parasitism in the present experiments? Hassan et al. (1978) found 90% parasitism of _O. nubilalis_ eggs at a (lowest) density of 0.23 clusters/plant. Shcheptilnikova (1974) reported over 85% parasitism of _M. brassicae_ eggs at a density of about 5 eggs per plant (1 cluster/m^2). According to Parker et al. (1971), the critical density for effective parasitism of _P. rapae_ eggs was about 0.3 eggs per plant. The reported densities are above those generally found in the present experiments. Comparable densities occurred only during peaks in the abundance of host eggs. It remains possible, therefore, that low host densities presently had an adverse effect on the rate of parasitism by _Trichogramma_.

Can parasitism of host eggs be increased by releasing more parasites? About 200,000 pupal parasites per ha (females and males) were introduced every two weeks in the first three years. This figure is similar to, or higher than the numbers used to control _O. nubilalis_ in Europe (Bigler 1986, Hassan 1982). In 1985 the number of introduced parasites was doubled. This might explain the two-fold increase, compared to the previous year, in parasitism of _M. brassicae_ eggs by strain 57. Weather conditions and host

densities appeared to be similar for the two years.

Knipling & McGuire (1968) predicted a deaccelerating increase of host-egg parasitism with increasing numbers of released Trichogramma. A positive validation of their model was obtained by Kot (1979) in a laboratory experiment. Ambiguous results, however, have been reported from the field. For instance, Houseweart et al. (1984) did not find a correlation between the release rate of T. minutum and parasitism of eggs of spruce budworm, Choristoneura fumiferana, whereas Smith et al. (1986) found an increase of 16 to 87% parasitsm when the release rate was increased 25 times (from 480,000 to 12 million females/ha).

This chapter shows that certain behavioural characteristics of Trichogramma females influence the rates of host finding and parasitization. However, strains selected for this study on the basis of results from behavioural studies in the laboratory were not sufficiently effective in the field. In order to determine which additional qualities are required for an effective strain, further research is probably necessary, especially on aspects of the searching behaviour. In principle, certain behavioural "inadequacies" might be remedied by releasing more parasites. Other behavioural traits, however, might turn out to be conditional, and therefore demand a more appropriate Trichogramma strain for effective control of Lepidoptera.

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Chapter 10. GENERAL DISCUSSION

SUCCESSFUL BIOLOGICAL CONTROL

During 100 years of inoculative biological control an estimated 3000-5000
introductions of control agents have been attempted worldwide, involving
some 1000 species of natural enemies against 200 pest species (Hokkanen
1985, Van Lenteren 1986a). One third of these introductions have resulted
in the establishment of natural enemies. Fifty eight percent of these have
achieved partial control and sixteen percent provides complete control
(Hall & Ehler 1979, Hall et al. 1980). Hall and colleagues found that the
success rate tends to decline with time (the "skimming off the cream"
effect). In contrast, the proportion of complete successes of all
successful cases (partial and complete) initially decreased, but has
steadily increased since 1930-40 (Hokkanen 1985).

The success ratio of inoculative introductions is considered poor by
some (e.g. Hokkanen 1985), but good or at least profitable by others (e.g.
DeBach 1974, Van Lenteren 1986a), especially when compared with chemical
control. Introduced species have largely been selected by trial and error
(chapter 2) which, in view of the limited funding for such projects, may be
considered as having provided good value for a small investment. Keeping in
mind that knowledge about ecological processes and empirical expertise with
biological control were more limited in the past than at present, the
trial-and-error method may have been a wise approach to the selection of
candidate natural enemies.

For inundative and seasonal inoculative biological control programs the
cost/benefit ratio of the method directly determines its continuous
feasibility (chapter 2). If an effective candidate agent has been found,
its production and release must be cost effective in order to establish it
as a method of pest control. Ramakers (1982) showed that biological control
in greenhouses may be cheaper than chemical control. Thus for biological
control methods which utilize mass produced control agents, the
availability of a suitable candidate agent seems imperative, because there
will be no commercial interest in mass producing an ineffective natural
enemy.

The effectiveness of a control agent is relative to the performance of other agents. For inundative biological control the effectiveness of a parasite may be defined as the ratio of the number of wasps released to the number of hosts killed. The present research has not yet yielded a sufficiently effective *Trichogramma* strain for releases against Lepidoptera on cabbage (chapter 9). However, laboratory pre-introductory selection did result in the identification of strains that appeared more effective than initially tested strains which were selected by trial and error (chapters 2 and 9). This result supports the use of a predictive approach to inundative biological control, which has also been demonstrated for seasonal inoculative biological control by Van Lenteren (1986a) and by Minkenberg & Van Lenteren (1986, 1987). The usefulness of different criteria for the selection of *Trichogramma* for inundative releases is discussed below. Results of the present research are used to evaluate predictive measures for certain criteria and to suggest important areas for further study.

SELECTION CRITERIA

Consideration of current knowledge and ideas on factors determining the effectiveness of natural enemies in suppressing pest populations has led to a list of nine possible criteria for pre-introductory selection of natural enemies (Table 2.1). These selection criteria are discussed below. Definitions of most of these criteria are reviewed and, as a consequence, renamed to clarify their meaning. Conclusions are summarized in Table 10.1, in which each criterion is given a coarse rating (low-medium-high) for three aspects: (1) usefulness to select candidate strains, (2) the amenability for manipulation by, for example, producers and users and (3) the current level of biological understanding on the way certain traits function and may be utilized. In addition, important research areas are indicated for each criterion.

Environmental risks

Serious negative aspects of natural enemies, such as (facultative) hyperparasitic habits of parasitic wasps or predation upon other beneficial species, may impede their use for any method of biological control

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Table 10.1. Evaluation of criteria for the selection of candidate *Trichogramma* strains to be used in inundative biological control programs (see Table 2.1) and major research areas identified to enhance the evaluation of selection criteria.

Criterion	Usefulness for pre-introd. selection	Amenability for manipulation by applier	Level of knowledge or utilization	Research areas
1. Environmental risks	high[1]	medium	low	Impact of releases on natural host communities
2. Tolerance of climatic extremes	high	medium	medium	Heritability of locomotory activity
3. Host-plant adaptation	medium	low	low	Dispersal to different habitats or plant types
4. Host selection	high	low	medium	Host selection in natural patches; host perception
5. Host suitability	medium	low	medium	Cytolytic process of killing hosts
6. Seasonal synchronization	low	high	high	Longevity of wasps in the field
7. Reproductive capacity	medium	high	high	Heritability of fecundity
8. Host-finding capacity	high	medium	low	Travel speed, dispersal by flight, role of kairomones
9. Culture method	high[1]	high[2]	medium	In vitro culture, quality of produced wasps

1) Conditional necessity rather than selection criterion for research program. 2) Manipulation by producer.

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(chapter 2). However, the primary concern is not with the negative trait itself, but rather with the degree of risk it may pose to the environment. Environmental risk therefore seems to be a more suitable term for this criterion. Practitioners of biological control generally consider it a safe method of pest management (e.g. Harris 1973, Caltagirone & Huffaker 1980). Pimentel (1980) reviewed adverse effects of introductions of exotic organisms for biological control purposes. The only example he cites for natural enemies of insects is the introduction of a predatory mite which upset the natural control of the coconut beetle in Fiji. In contrast, Howard (1983) contended that the threats of exotic introductions to endemic species are grossly underestimated, as indicated by population reductions and extinctions for various insects and their vertebrate predators in Hawaii, due to the indiscriminate introduction of numerous hymenopterous parasites.

Inundative biological control involves repeated releases of large numbers of natural enemies into crops. The possible impact of releases on alternative hosts in surrounding (natural) habitats should therefore be investigated as part of the development of the control program. Registration of biological control agents, comparable to that of pesticides, will probably become a regulation, which will require criteria to test their environmental safety. Trichogramma inundations are considered safe by appliers, perhaps because they usually release local strains collected from the pest species (chapter 2). However, if an exotic strain is released, or one that has been artificially selected, the possibility of increased egg parasitism of Lepidoptera other than the pest species itself is not imaginary, especially since most Trichogramma species are polyphagous (chapter 6). Permanent establishment of exotic species might be avoided by selecting strains which, for example, cannot overwinter in the new environment.

Tolerance of climatic extremes

Climatic adaptation involves the ability of natural enemies to effectively control a pest under ambient climatic conditions. However, in a broader sense, climatic adaptation concerns the ability of natural enemies to tolerate the extreme abiotic conditions of their environment. Climatic

tolerance is a determining factor for the survival and/or the reproduction of a natural enemy. For inoculative introductions survival under extreme conditions is an essential prerequisite for colonization, even if reproduction is limited to periods of more favorable conditions. For seasonal inoculative and inundative introductions the ability to reproduce is of immediate importance and should not be restricted by abiotic conditions.

In Trichogramma, tolerance to extreme environmental conditions has been primarily studied for high temperatures (chapter 4). For application in the temperate coastal climate of the Netherlands, low temperatures seem to present a major limiting factor to the parasitization activity of Trichogramma females (see host-finding capacity). Significant variation in proportional activity (i.e. the number of females engaged in locomotory and parasitization activity) occurred between strains (chapter 4) and appeared to correspond to the performance of strains of different activity released in the field (chapter 9). Temperature dependence of parasite activity, therefore, appears to be a useful criterion to select candidate strains for the present evaluation program.

Host-plant adaptation

The ability to attack a given species of host on all economically important host plants is considered important for inoculative biological control, but not for seasonal inoculative and inundative biological control (chapter 2). For the latter methods it has been suggested that a variable effectiveness of a natural enemy for different host plants of its (polyphagous) insect host is amenable for manipulative correction, e.g. by releasing more enemies. However, this may not be generally true, as shown for the greenhouse whitefly parasite Encarsia formosa, which is less effective on cucumber than on tomato (Van Lenteren et al. 1977, Hulspas-Jordaan & Van Lenteren 1978).

Different rates of egg parasitism are reported for the same host species on different plant types, usually by different Trichogramma spp. (e.g. Martin et al. 1971, Young & Price 1975, Lopez et al. 1982). Host-plant adaptation may be involved in host-habitat location, which has been demonstrated by Altieri et al. (1981) and Nordlund et al. (1985).

Furthermore, _Trichogramma_ species appear to be adapted to different plant structures (e.g. Thorpe 1985). Host-plant adaptations probably interact with host-species adaptations, and their combined effect results in strains of _Trichogramma_ being specialized and released in different crops, for example _T. maidis_ against the corn borer, _O. nubilalis_, and _T. pretiosum_ against the cotton bollworm, _H. zea_.

Plant adaptations have not been studied in the present selection program, but might be appropriate for further research. The propensity of parasite dispersal from the crop habitat to a different, habitual habitat should be investigated. Noldus & Van Lenteren (1983) did not find a response to cabbage volatiles in an airflow olfactometer for the present strain 11 (_T. maidis_). The species which were tested in the field, _T. evanescens_ and the related _T. maidis_ (chapter 9), appear to be adapted to field or shrub habitats, rather than to arboreal habitats as found for _T. minutum_ and _T. embryophagum_ (Kot 1964, Thorpe 1985).

Host specificity

Host specificity concerns the range of different host ages and host species accepted by a parasite. As a selection criterion host specificity is related to other criteria, such as environmental risks (narrow host range and low propensity of hyperparasitism), host suitability and density responsiveness (high host-finding capacity). If the latter criteria are evaluated for candidate parasites, host specificity might be investigated separately. Alternatively, a study of host specificity might be sufficient to permit evaluation of the other criteria also.

For inundative and seasonal inoculative releases host specificity is not considered an important selection criterion, apparently due to the assumption that releases are directed against a single pest species (Van Lenteren 1980). However, if two or more related species are to be controlled, the use of a single effective parasite is probably more economical (e.g. in costs of development of the program) than the use of different parasites for each host species. Host specificity, therefore, should be considered as an important selection criterion, because the occurrence of related host species in the same crop is not exceptional (e.g. Ehler & Miller 1978, Minkenberg & Van Lenteren 1987).

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The present studies of variability among Trichogramma strains included investigations of host-age and host-species selection. The preferred ages of different species of host eggs had to be determined before host-species selection experiments could be carried out. The effectiveness of a parasite may also be reduced if host acceptance is limited to certain ages of hosts. For the host species tested, host age selection did not occur in all six Trichogramma strains tested (chapter 5). However, females of nine out of twelve strains tested for host-species selection preferred M. brassicae eggs over P. brassicae and P. rapae eggs as a host (chapter 6). Host-species selection is a useful criterion for the selection of candidate strains, because parasitism of Pieris eggs in the field was predominantly observed for a strain (no. 11) that did not show a preference for either of the host species in the laboratory (chapter 9).

The host range for strains in the field might be narrower than observed in the laboratory. Parasitism of Pieris eggs in the field was not apparent for strains of wasps which accept Pieris eggs in a laboratory test if the preferred M. brassicae eggs are not offered simultaneously. This question requires further investigation in order to develop the right kind of laboratory test for host selection, i.e. a test that correctly predicts host-selection behaviour of parasites in the field. Present host-species selection experiments showed that wasps may select host eggs from a distance without physical contact. The distance from which hosts are perceived by the parasites determines the probability of host discovery and their host-finding capacity (see below). Evaluation of this trait might therefore be a useful measure for the present criterion.

Host suitability

Host suitability concerns the ability of the parasite to complete development in the host and seems a more direct term for this criterion than internal synchronization. It was not considered an important selection criterion for inundative releases (Table 2.1), because their aim may be fulfilled if the host is killed. However, present experiments on the suitability of different hosts for Trichogramma (chapter 8) showed that the rate of success of killing hosts is proportional to the duration of the oviposition period. If only one egg was laid in the eggs of M. brassicae

and P. brassicae, instead of the usual clutch size of two eggs per host, only half of the host eggs were killed. Thus, the supposition that host acceptance (= oviposition) in Trichogramma includes killing of the host egg (Strand 1986) may not be correct. The parasitization process and the properties of the substances involved in lysis of the host contents need further elucidation. The present findings suggest that, if host eggs are not always killed when attacked, host suitability should be considered as a criterion in the selection of candidate strains.

Eggs of the present host species were usually effectively killed by females of various strains (chapter 8), because most females laid two or more eggs per host egg. However, for P. brassicae eggs the clutch size of parasite eggs per unit of volume was about half that for M. brassicae eggs (0.05 mm^3 host per egg). This is probably due to an adaptation of the wasps to the inferior quality of the contents of Pieris eggs, because they laid the optimal number of eggs per Pieris egg for maximal survival of the progeny (chapter 8). In theory it seems possible that, in response to host quality parameters, Trichogramma females could lay too few eggs to kill the host. The requirement for wasps to lay a sufficient number of eggs in order to kill the host egg would be another reason, beside optimal clutch survival, for their having a mechanism of host-volume measurement (Schmidt & Smith 1987a).

Seasonal synchronization

Seasonal synchronization with the host refers to the temporal (external) correspondence between the proper stages of the life cycles of host and parasite. For inundative biological control, natural synchronization is not an important criterion. The applier can synchronize populations of the parasite and the host by introducing the parasites at the proper time. In fact, inundative releases are a way of artificially synchronizing the occurrence of hosts and sufficient numbers of parasites in places where they are actually poorly synchronized (Coppel & Mertins 1977). Host phenology, especially host-egg density, determines decisions regarding the timing and frequency of releases. Thus, the presence of host eggs must be regularly monitored or predicted by means of model computations (Ridgway et al. 1981).

The age of host eggs may significantly affect their susceptibility to parasitism by Trichogramma (chapter 5). Since young eggs are generally most susceptible, it may be of crucial importance that female wasps are searching for host eggs from the very beginning of the oviposition period of the host insect. Bigler & Brunetti (1986) demonstrated that pheromone-trapping of O. nubilalis in corn facilitates appropriate planning of T. maidis releases, because it offers a lead-time of a few days to estimate egg numbers and to program parasite releases (Witz et al. 1985).

Trichogramma are usually introduced into the field as immatures in the pupal or pharate adult stage, mechanically or by hand (chapter 2). Wasp emergence can be manipulated and synchronized by exposure of pupae to low temperature on the day before release (Morrison et al. 1978, Bouse & Morrison 1985). In the laboratory, wasps may live for a few weeks at moderate temperatures, provided they can feed on honey or another food substance (e.g. Ashley & Gonzalez 1974, Walter 1983b), but little is known about the life span of females in the field. Mark-and-recapture studies by Stern et al. (1965) suggested that wasps may live for several days. Keller et al. (1985) stressed the potential hazard of dew and rain drops on plant leaves for survival of Trichogramma, due to their minute size. Under variable, unpredictable weather conditions the introduction of parasite pupae of mixed ages into the field may provide for a continuous supply of sufficient foraging wasps for several weeks (Hassan 1982, Bigler 1986).

Reproductive capacity

The potential reproductive capacity of a natural enemy is usually expressed by the intrinsic rate of natural increase (r_m value), which combines development time and mortality of immatures, adult survival and fecundity into the rate of population growth (Pak & Oatman 1982b). Parasites develop at the detriment of their hosts (solitary or gregariously), and may also kill them by host feeding. Thus, the host-death rate caused by activity of the parasites should offset the rate of increase of the host in the absence of parasites (Huffaker et al. 1977, Van Lenteren 1986a). The potential reproductive impact on the host population is usually determined in the laboratory under optimal conditions of host availability. In practice, however, parasites may not find the hosts as readily as in the laboratory

and, consequently, the field host-death rate per female parasite may be lower.

The usefulness of laboratory studies on interspecific differences in reproductive adaptation to ambient temperatures between candidate natural enemies has been demonstrated for inoculative and seasonal inoculative biological control programs (e.g. Force & Messenger 1968, Vet & Van Lenteren 1981). For inundative biological control the reproductive potential does not appear to be a useful selection criterion, because a limited parasite fecundity can, in theory, be adjusted by releasing more parasites. However, Smith & Hubbes (1986b) did find that differences in field performance between various indigenous geographical strains of T. minutum, in parasitism of spruce budworm eggs, C. fumiferana, corresponded to differences in reproductive capacity between strains determined in the laboratory.

If host eggs are laid in clusters rather than singly, the effectiveness of a parasite as a control agent may be reduced if a single female cannot parasitize all hosts in a patch (Hassell 1982a). Present field observations (chapter 9) showed that the fecundity of Trichogramma females may be too low to parasitize each egg in clusters of M. brassicae containing more than 20 eggs, which make up a considerable fraction of the egg population, especially toward the end of the season. Thus if the biological control effectiveness of wasps is limited by their reproductive capacity, fecundity might be a useful selection criterion for inundative biological control.

In the present study, the oviposition rates at a low extreme temperature (chapter 4) served as a measure for limitation of reproduction (parasitization activity), rather than for potential reproductive capacity. However, the heritability of fecundity should be investigated, because the parasitization rate did not appear related to proportional locomotory activity of females. Intraspecific genetic variation in fecundity and degree of egg-cluster parasitism between Trichogramma strains has been found, so that these traits might be increased in a strain by linear selection (Smith & Hubbes 1986a, Chassain & Boulétreau 1987).

Host-finding capacity

Density responsiveness is mentioned in chapter 2 as a selection criterion
related to the host-finding ability of natural enemies. The question of
density dependent host-parasite interactions, such as spatial heterogeneity,
parasite aggregation and mutual interference, is of great interest in the
theory of population regulation by effective natural enemies (Waage
& Hassell 1982, Lessels 1985, Hassell 1986). As a result, density dependent
behaviour is commonly seen as an essential feature of the searching
efficiency or capacity of candidate natural enemies, but it also seems to
be the most difficult selection criterion for experimental evaluation
(Huffaker et al. 1977, Van Lenteren 1986a).

   Coexistence of pest and natural enemy at a low density seems to be an
essential feature of inoculative and seasonal inoculative biological
control, but not of inundative biological control (chapter 2). In addition
to density dependent behavioural responses of parasites, any trait of their
searching behaviour that has a significant influence on the probability of
host finding might be a useful selection criterion, especially traits
related to the searching effort (e.g. travel speed, travel time and
responses to searching stimulants). Since the measure of success of a
natural enemy is in the actual finding of hosts rather than in the
searching for hosts, host-finding capacity seems the most appropriate term
for this criterion.

   The host-finding capacity of a parasite is the number of hosts found
per unit of time. However, this does not seem to be a useful measure for
evaluation, because it is only conveniently determined under controlled
conditions at a relatively high host density (Hassell 1986). At a high host
density, the host-finding capacity of a parasite is limited by her egg
complement and/or by her handling time. In the present field experiments
(chapter 9), host densities averaged between 1 and 2 eggs per plant for most
of the season. Since host eggs were mostly laid in clusters, the
percentages of plants bearing any eggs usually were less than 10%. Thus, if
parasites were searching for hosts, they were probably foraging on leaves
without hosts.

   The host-parasite interaction occurring in the field suggests that it
is practically unrealistic to express the host-finding capacity of

inundatively released Trichogramma in terms of an absolute deterministic measure, such as the area traversed or the area of discovery (Nicholson 1933, Hassell 1982b). These measures assume that parasites search randomly, whereas it is becoming increasingly apparent that host searching is directed or stimulated by physical or chemical cues from the habitat or host, e.g. substances functioning as synomones or kairomones (Nordlund 1981, Vinson 1984). A stochastic approach therefore seems to be more appropriate for making predictions about the host-finding capacity of natural enemies (Morrison 1986), which requires a better understanding on factors influencing the host-finding process in comparison with what is presently known (Keller et al. 1985).

In the present study, selection of candidate strains of Trichogramma on possible differences in one or more aspects of their host-finding capacity has not been attempted as yet. If further selection is necessary, however, good options seem to be available for experimental analysis of host-finding traits in the laboratory. For example, Bigler et al. (1988) found that differences in travel speed between a number of strains of T. maidis in the laboratory corresponded consistently, i.e. for several seasons, to differences in performance in the field. This suggests that travel speed might be a useful measure to select strains.

Another possibility to select Trichogramma strains might be based on differences in the response of females to kairomones. Close range host-finding behaviour is stimulated by contact kairomones eliciting a combination of klinokinetic and orthokinetic responses in the wasps (Beevers et al. 1981, Gardner & Van Lenteren 1986). These kairomones are present in the wing scales of adult hosts and are deposited by ovipositing females on and around the eggs (Gueldner et al. 1984). Intraspecific differences in response to wing scales of the present host species among candidate Trichogramma strains may influence their host-finding capacity and might be a useful selection criterion. Furthermore, the sex pheromone of the adult-female host has been found to function as a volatile kairomone for Trichogramma, probably playing a role in host-community location (Lewis et al. 1982, Noldus & Van Lenteren 1985a). Since sex pheromone is a more hostspecific kairomone source than host scales (Noldus et al. 1986), inter- and intraspecific differences in kairomonal responses among Trichogramma strains may be more common for volatile than for contact kairomones.

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Culture method

A good culture method is an essential prerequisite for the use of a natural
enemy in seasonal inoculative and inundative biological control programs
(chapter 2). The ability to culture a given natural enemy is a conditional
necessity of concern to the producer, rather than a useful selection
criterion for the researcher conducting an evaluation program. The
widespread use of Trichogramma as an inundatively released control agent is
a consequence of the feasibility of mass production on factitious host
species. However, host-specific species or strains, such as T. nubilale,
cannot be readily cultured on factitious host eggs, which may limit their
practical use (Burbutis & Goldstein 1983, Goldstein et al. 1983). The
present study (chapters 6-9) indicates that successful performance of
Trichogramma against Pieris spp. in the field may require a specialized
strain. In fact, the present collection of geographical strains (chapter 2)
was limited to strains that could be maintained on eggs of E. kuehniella.
     Host species commonly used to mass produce Trichogramma have relatively
small eggs, which produce small Trichogramma (chapter 3). Smaller
Trichogramma females generally have a lower fecundity (e.g. Stinner et al.
1974, Hassan et al. 1978) a shorter longevity (e.g. Marston & Ertle 1973,
Lewis et al. 1976) and a lower walking speed (e.g. Biever 1972, Marston &
Ertle 1973) than larger females from natural hosts. In the past, the
quality of mass produced Trichogramma has received little attention from
practitioners, although several accounts of reduced field effectiveness
have been reported for laboratory cultures of strains (e.g. Ashley et al.
1973, Nagarkatti & Nagaraja 1978, Bigler et al. 1982). Adverse genetic
changes that might take place in mass cultured strains should be monitored
in order to maintain the quality of Trichogramma as a control agent.
Population-genetic studies and the monitoring of the quality of mass
produced parasites require the quantification of biological traits (Bigler
et al. 1987), for which several of the presently discussed selection
criteria may be useful.

The present pre-introductory selection program did not yet yield an effective candidate strain of Trichogramma for application. Even moderate levels of host-egg parasitism (up to 50%) did not appear to result in significant reductions of larval host populations. A relevant question therefore is whether introduced Trichogramma, or egg parasites in general, have the potential of being effective biological control agents. An apparent advantage of a high level of egg parasitism, compared to parasitism in later developmental stages of the host, is a lower number of pest larvae feeding on the host plant, because no larvae emerge from parasitized eggs. Ever since Thompson (1928) stressed the distinction between apparent mortality (i.e. percentage egg parasitism) and real mortality (i.e. percentage overall mortality), however, there seems to exist a general confusion over the effectiveness of egg parasites (e.g. DeBach 1964).

If egg parasitism and early larval mortality of a pest host are comparable, increased egg parasitism by introduced parasites will contribute little to suppression of the population of older larvae, which are most damaging (e.g. Tucker 1935, Pickles 1936). In a model study on density dependent larval mortality of the sorghum stalk borer, Chilo partellus, Van Hamburg & Hassell (1984) showed that the success of egg-parasite releases is dependent on the level of egg parasitism, the subsequent level of early larval mortality and the degree to which these levels are density dependent. These factors will vary between hosts and, perhaps, parasite species and might explain some of the erratic results obtained with inundative releases of Trichogramma.

Egg parasites account for 15% of the successful inoculative biological control agents, and their success ratio is similar to that of larval parasites and better than that of pupal parasites (Hokkanen 1985). Egg parasites occur in sixteen families of Hymenoptera, of which the Trichogrammatidae, Mymaridae and Scelionidae are composed exclusively of egg parasites (Clausen 1940). Examples of succesful inoculative control by egg parasites are known for the Scelionidae (e.g. the North American Telenomus alsophilae against the geometrid Oxydia trychiata in Columbia, Drooz et al. 1977) and for the Mymaridae (e.g. Patasson nitens imported

from Australia into Africa to control the eucalyptus weevil, Gonipterus scutellatus, Tooke 1955) but not for the Trichogrammatidae. Greathead (1986) therefore concluded that trichogrammatids are less succesful control agents than species from the other egg-parasite families. Strand (1986) suggested that, for unknown reasons, Trichogramma spp. are less specialized in parasitizing certain species within their major host group (Lepidoptera) than the scelionids (specialized on Lepidoptera, Hemiptera or Orthoptera) and the mymarids (specialized on Homoptera).

The preoccupation with Trichogramma, instead of other egg parasites, for inundative biological control seems to stem largely from its polyphagous nature and feasibility of mass production on factitious hosts, rather than from superior biological traits determining effectiveness as a control agent. For example, Hirose (1986) found that Telenomus dendrolimi was a more effective control agent of Papilio xuthus (Papilionidae) in Japan than Trichogramma dendrolimi, due to its longer life span and reproductive period. Apart from the success of inundative releases of T. maidis against the corn borer, O. nubilalis, in Europe, applied at an estimated 10.000 ha (Van Lenteren 1987) there is little good evidence of consistently successful application of Trichogramma in pest control. Investigation of the factors determining the effectiveness of Trichogramma in providing control of the European corn borer might result in identification of important criteria for the selection of effective strains.

IMPROVING TRICHOGRAMMA EFFECTIVENESS

If an effective "wild" strain of Trichogramma cannot be found in nature, it might be attempted to improve the effectiveness of existing laboratory strains. The major approaches suggested toward this aim are enhancement of the activity of released parasites in the field (Powell 1986), and genetic improvement of a strain by selective breeding (Roush 1979).

Trichogramma has been the subject of extensive research on manipulation of the searching behaviour by the application of contact or volatile kairomonal substances to the habitat, which primarily at low host densities might increase the effectiveness of host finding (Gross 1981, Lewis et al. 1981, 1982). Gross et al. (1981) demonstrated that pre-release oviposition

and kairomone experience may stimulate searching behaviour and increase retention time of wasps in a target area. This response might suppress dispersal of the wasps from a crop habitat. In order to reinforce the response to contact kairomones applied to the habitat, Gross et al. (1984) distributed supplemental (sterilized) host eggs in the field, which increased the rate of finding natural host eggs. These manipulative approaches to stimulate the searching behaviour of Trichogramma might be useful for the present application against Lepidoptera on cabbage, because present field studies (chapter 9) suggested that low host densities played a role in limiting the effectiveness of host finding.

The present research on selection criteria to evaluate the effectiveness of Trichogramma strains may be useful in directing which qualitative traits need genetic improvement of the parasites, because the lack of criteria to measure quality or to direct linear selection appears to be a major limiting factor for putting ideas into practice (Hoy 1976, Bigler et al. 1987, Roush 1979). If natural selection acts against the artificially selected trait, or if outbreeding with a natural population occurs, an artificially selected enemy will gradually revert to a "wild" genotype after inoculative introduction. The prospects of genetic improvement seem to be especially promising for inundatively introduced natural enemies, because this method offers the opportunity to maintain the quality of the selected trait of a strain by applying a continuous selection pressure in the mass production (Hoy 1976, Roush 1979).

Genetic variability in behavioural traits determing effectiveness of a natural enemy are the basic requirement for artificial selection (e.g. Hoy 1979) and several selection criteria have been suggested in the present discussion. Successful attempts of artificial selection of Trichogramma in the laboratory have been reported for fecundity (Brenière 1965c, Ram & Sharma 1977a,b) and host-finding capacity (Urquijo 1946, 1950). Application of a genetically improved strain has not been reported. However, incorporation of certain technical measures into a mass-production system to maintain the quality of the Trichogramma culture, for example long-distance host finding requiring dispersal by flight (Bigler et al. 1982), may be considered as applications of continuous artificial selection.

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ART OR SCIENCE

Inundative biological control is a technical enterprise, which offers opportunities to leave behind the primitive "hunting-and-gathering" phase of biological control (Hoy 1979). The trial-and-error approach to the selection of candidate control agents has been a useful element of this primitive phase. In the past, several ecologists and entomologists have expressed their concerns about the orthodox approach to the choice of effective natural enemies. Krebs (1972) commented that "until we can explain why an introduction program is a success or a failure, biological insect pest suppression will remain an art, not a science". This was confirmed by Harris (1973), who reviewed the selection of agents for the biological control of weeds. Van Lenteren (1980) discussed the question of whether the art does have to become a science, while Hokkanen (1985) suggested that the selection of effective natural enemies "should probably best regarded as an art, based on science".

I believe that if biological control is to gain wide adoption as a method of pest control, it needs to be based on research by scientists, who should enjoy art to give relaxation to their puzzled and weary minds. These scientists should study behavioural interactions between natural enemies and their hosts or prey in order to find useful criteria and methods to evaluate or monitor the effectiveness of candidate natural enemies. The present study helps to lead the way.

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