Foraging behaviour of the egg parasitoid *Uscana lariophaga*

towards biological control of bruchid pests in stored cowpea in West Africa

Promotor:	dr. J.C. van Lenteren Hoogleraar in de Entomologie
Co-promotor:	dr. A. van Huis Universitair Hoofddocent Tropische Entomologie

NN08201, 2177

Frans van Alebeek

Foraging behaviour of the egg parasitoid *Uscana lariophaga*

towards biological control of bruchid pests in stored cowpea in West Africa

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van de Landbouwuniversiteit Wageningen,
dr. C.M. Karssen,
in het openbaar te verdedigen
op dinsdag 10 december 1996
des namiddags te half twee in de Aula.

In herinnering aan
Silent Spring *(1963)*
van Rachel Carson

BIBLIOTHEEK
LANDBOUWUNIVERSITEIT
WAGENINGEN

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Alebeek, Franciscus Adrianus Nicolaas van

Foraging behaviour of the egg parasitoid *Uscana lariophaga*: towards biological control of bruchid pests in stored cowpea in West Africa / Franciscus Adrianus Nicolaas van Alebeek. - [S.l. : s.n.]. - ill.
Thesis Landbouwuniversiteit Wageningen. - With ref. - With summary in Dutch.

ISBN 90-5485-613-0
Subject headings: biological control / parasitoids / foraging behaviour

Copyright 1996 F.A.N. van Alebeek

The research described in this thesis was carried out between 1990 and 1996 at the Department of Entomology of the Wageningen Agricultural University, in the last two years within the framework of the C.T. de Wit Graduate School Production Ecology. The project was financially supported by the European Union (project TS2 029 F ESF and TS3 CT 930201) and was coordinated in 1990-1993 by the Foundation for Biological Research (BION) (code 436.992) under supervision of the Netherlands Organization for Scientific Research (NWO).

The cover shows in the background a sample of cowpea seeds collected in February 1993 from a cowpea granary in the village of Gonou, Niger. Inset front cover: *Uscana lariophaga* parasitizing a *Callosobruchus maculatus* egg. Insets back cover: a traditional straw granary in Niger (top), a *Callosobruchus maculatus* female on cowpea seeds (middle), and an experimental cowpea field near Niamey, Niger (bottom). Photographs F.A.N. van Alebeek and Duotone Fotolocatie Binnenhaven. Cover design: Piet Kostense.

Printed by Grafisch Service Centrum Van Gilst B.V., Wageningen.

NN08201, 2107

Stellingen

1. Het idee dat het mogelijk is te spreken over DE functionele respons van een bepaalde sluipwesp of predator, berust op een illusie.
   Houck, M.A.; Strauss, R.E., 1985. Canadian Entomologist 117: 617-629.
   Dit proefschrift

2. Bij kleine, kortlevende eiparasieten is een aangeboren respons op geuren van hun gastheer voorspelbaar.
   Vet, L.E.M. *et al.*, 1995. In: R.T. Cardé & W.J. Bell (eds), *Chemical Ecology of Insects 2*, Chapman & Hall, New York, pp. 65-101.

3. Sluipwespen die volkomen lukraak naar gastheren zoeken, bestaan niet. Soorten waarbij dit het geval lijkt, zijn soorten wier gedrag we onvoldoende begrijpen.

4. De soortaanduiding *Callosobruchus maculatus* Fab. (Coleoptera; Bruchidae) zegt zonder nadere specificaties weinig tot niets over de biologie en het gedrag van een (willekeurige) populatie van deze soort.
   Mitchell, R., 1990. In: Fujii, K., *et al.* (eds), *Bruchids and legumes: economics, ecology and coevolution*. Proc. 2nd Int. Symp. Bruchids Legumes, Okayama (Japan), pp. 317-330.
   Tran, B.M.D.; Credland, P.F., 1995. Biological Journal of the Linnean Society 56: 483-503.

5. Het in het laboratorium bepalen van de intrinsieke groeisnelheid van natuurlijke vijanden, waarbij een overmaat aan gastheren wordt aangeboden in een qua omvang en structuur beperkte omgeving, negeert enkele voor de biologische bestrijding essentiële elementen van het zoekgedrag van die natuurlijke vijanden en leidt tot overschatting van de parasiterings- of predatiecapaciteit onder veldomstandigheden.
   Dit proefschrift.

6. De stelling van Barfield en Swisher: "Our institutions are not structured to tackle holistic ecological problems [*such as Integrated Pest Management, IPM*]. We do not have the technical knowledge, analytical tools nor institutional incentives to do so." is helaas maar al te juist.
   Barfield, C.S.; Swisher, M., 1994. In: D. Walmsley (ed), *New Stategies for the Caribbean Farmer*. Wageningen, CTA Seminar Proceedings, pp. 109-124.

7. Het schrijven van een goed wetenschappelijk artikel als het belangrijkste communicatiemedium in de wetenschap dient in de opleiding van promovendi veel meer aandacht te krijgen.

8. Veel wetenschappelijk onderzoek in de tropen dat wordt uitgevoerd met westers geld en westerse deskundigen, vertoont kenmerken die overeenkomen met die van de Nederlandse specerijenhandel in de Gouden Eeuw.

9. Het stedelijk groen, dat door vermesting, verdroging en versnippering van het Nederlandse landschap nu het ecosysteem met de grootste biodiversiteit vormt, wordt ten onrechte ondergewaardeerd in onderzoek en beleid.
   Owen, J.; Owen, D., 1975. Environmental conservation 2: 53-59.
   J. Owen, 1992. *The ecology of a garden: the first fifteen years.* Cambridge University Press.

10. Muziek maken, het bestuderen en genieten van vlinders en goed nadenken zijn relatief eco-vriendelijke handelingen.
    J. Lyklema, *1995.* Voordracht gehouden bij zijn afscheid als hoogleraar in de Fysische en Kolloidchemie aan de Landbouwuniversiteit te Wageningen

11. Vaste verzorg(st)ers van kinderen met werkzaamheden buiten het huishouden gedurende meer dan vijftien uur per week zouden na vijf jaar recht moeten hebben op het certificaat "Management in kleine non-profit organisaties" en "Conflicthantering en onderhandelen".

12. Voor mensen die leven onder een door welvaart en welzijn gekenmerkte rechtsorde, worden universele mensenrechten tot universele mensenplichten: wij hebben de plicht om mensenrechten te effectueren en toe te kennen aan mensen wier rechten worden geschonden.
    Graaf, M.H.K. van der, 1996. *Mensenrechten in spiegelbeeld.* Best, Uitgeverij DAMON bv.

13. Het plan voor de herintroductie van de zeearend (*Haliaëtus albicilla* L.) in Nederland zegt meer over de toestand van enkele Nederlandse natuurbeschermingsorganisaties dan over de toestand van de Nederlandse natuur.

Stellingen behorend bij het proefschrift: **Foraging behaviour of the egg parasitoid *Uscana lariophaga*: towards biological control of bruchid pests in stored cowpea in West Africa.** Frans van Alebeek. Wageningen, 10 december 1996.

Contents

Abstract

Foraging behaviour of the egg parasitoid *Uscana lariophaga:* towards biological control of bruchid pests in stored cowpea in West Africa. Frans van Alebeek, 1996. Thesis, Wageningen Agricultural University, Wageningen, The Netherlands. 176 pp.

Seed beetles cause considerable losses in traditionally stored cowpea (*Vigna unguiculata* Walp.) under subsistence farming conditions in West Africa. The indigenous egg parasitoid *Uscana lariophaga* Steffan (Hymenoptera; Trichogrammatidae) is evaluated as a candidate for a conservation strategy of biological control against the major pest *Callosobruchus maculatus* Fab. (Coleoptera; Bruchidae). Different aspects of *U. lariophaga*'s searching efficiency, such as host habitat location, host cluster location, the functional response, egg laying capacity and egg load, host handling time, arrestment response, photo- and geotaxis, dispersal and walking behaviour, have been investigated and results are reported in this thesis. *Uscana lariophaga* uses odour of uninfested cowpea seeds and of *C. maculatus* eggs to locate host habitats and host clusters therein. Host clusters with many eggs are more frequently located than clusters with few eggs, probably through olfaction. The parasitoid demonstrates a Holling Type II functional response, and the maximum number of hosts parasitized is determined by her ovarial egg load. Due to a strong arrestment response within host clusters, and area restricted searching behaviour after an oviposition, *U. lariophaga* parasitizes more eggs in host clusters with an even or clumped distribution than in clusters with a low density random distribution. *Uscana lariophaga* is positive phototactic and negative geotactic, and can move through cowpea stock over several meters a day. Under optimal conditions, *U. lariophaga* can significantly reduce losses in stored cowpea by *C. maculatus*. Further progress towards biological control of cowpea storage pests is discussed.

Dankwoord

Dankzij de steun en stimulansen van mijn ouders heb ik mijn plezier in insecten al heel jong kunnen ontwikkelen. Mede daardoor heb ik in mijn studie Planteziektenkunde de specialisatie Entomologie gekozen en heb ik nu al weer tien jaar interessant werk kunnen doen. Mijn gehele familie bedank ik voor hun belangstelling in het wel en wee van mijn onderzoek en de vorderingen van dit proefschrift. Franca, vanuit onze gedeelde geschiedenis en verwantschap in denken en werkkring, voel ik mij zeer gesteund door jouw aanwezigheid als paranimf bij de verdediging van dit proefschrift.

De komst naar Wageningen van mijn promotor Joop van Lenteren zorgde er mede voor dat ik mijn studie op een zinvolle manier kon richten op biologische bestrijding. Joop, in al die jaren heeft jouw enthousiasme steeds weer velen geïnspireerd. Bovendien bezorgde je mij een aantal afwisselende en leuke banen. Bedankt voor je snelle reacties op de eerste versies van mijn hoofdstukken, je opbouwende kritieken en je vertrouwen. Arnold van Huis was als dagelijkse begeleider betrokken bij al de ups en downs van mijn onderzoek. Vooral jouw brainstorm sessies, liefst in een buitenlands hotel met een sigaar en een borrel, hebben veel indruk gemaakt en mee richting gegeven aan dit proefschrift. Arnold, jouw tomeloze werkdrift en duizendpoterij was een bijzondere aansporing voor mij om door te gaan. Je hebt me volop de vrije hand gelaten om de lijnen van mijn onderzoek te kiezen. Bedankt voor je vaak nuchtere relativeringen waarmee je mijn neiging tot perfectionisme tot werkbare proporties wist terug te brengen.

Het in dit proefschrift beschreven onderzoek heb ik, in samenwerking met vele anderen, met veel plezier en voldoening uitgevoerd. Het schrijven aan dit proefschrift was soms echter een frustrerend gevecht met mijn perfectionisme, blinde vlekken en onzekerheden. Maar dit boek is er toch gekomen, mede door de hulp en steun van veel verschillende mensen.

In de afgelopen jaren hebben Gerard Pesch, Nanda Kaashoek en Mariët Cools met eindeloos geduld geholpen bij het kweken van de kevers en wespen en bij allerlei andere klussen voor ons onderzoek. Gerard voerde ook de vele honderden artikelen voor ons literatuursysteem in. Daarmee was ik verlost van diverse tijdverslindende klussen. Dank voor jullie hulp en kameraadschap.

Veel van het in dit proefschrift beschreven onderzoek is mede uitgevoerd door een aantal studenten. Dat waren Karin Koning, Edwin de Korte, Martijn Bezemer, Marcus van Es en Norbert Groot. Helaas heeft niet alles van wat jullie deden een plaats kunnen krijgen in dit proefschrift. Ik dank jullie oprecht voor jullie inzet, enthousiasme en volharding bij het vele saaie telwerk. Bovendien mocht ik delen in de resultaten van enkele studenten die hun onderzoek bij Arnold van Huis deden; Lidy Rouwkema, Marieke Bonhof en Sandor van Voorst, bedankt. Gert Ormel, jouw studieverlof bij onze onderzoeksgroep en jouw kritische, nauwgezette wijze van werken was leerzaam en stimulerend. Conny Schütte heeft als student en later als collega en vriendin belangrijke ideeën en inzichten bijgedragen aan mijn onderzoek. Conny, ik ben heel blij dat je deze steun tot het einde toe volhoudt door mij als paranimf terzijde te staan.

Simon Slumpa, Emana Getu Degaga and Antwi Kwaku, participated as MSc. students in my research. Thanks for your substantial contributions, directly or indirectly, to the ideas and work presented in this thesis. Raquel Perez Maluf, merci pour la traduction du Résumé. Jeffrey Harvey and Kate Flanagan, thanks for correcting the English in parts of this Thesis.

Al mijn collega's binnen de vakgroep Entomologie, met name de promovendi, wil ik bedanken voor de goede sfeer waarin we elkaar weten te relativeren, te stimuleren en te motiveren. Daarnaast hebben met name Marcel Dicke, Peter Mols, en Herman van Roermund met hun kritische kommentaren en discussies bijgedragen aan een betere doordenking en presentatie van mijn onderzoek. Herman Dijkman was steeds weer bereid om onze wensen voor klimaatkasten in te passen in zijn volle schema's en om technische problemen razendsnel opgelost te krijgen. Ook wil ik hier de steeds beschikbare ondersteuning vanuit het secretariaat noemen. Truus de Vries, Irene van Nes, Riet Tilanus en met nadruk Ans Klunder en Ineke Buunk: mijn oprechte dank.

De heer M.A.J. van Montfort en Gerrit Gort van de vakgroep Wiskunde (LUW) hebben hun wenkbrauwen gefronst over mijn experimentele opzet en vragen bij de analyses. Desondanks waren zij steeds bereid mee te denken en bruikbare hulpmiddelen aan te reiken. Gerrit, jouw hulp bij hoofdstuk 4 was onmisbaar.

De medewerk(st)ers van de bibliotheek van het Centrum voor Gewasbescherming hebben er voor gezorgd dat ik mijn eigen gedachtenspinsels steeds kon spiegelen aan wat anderen hebben geschreven. De collega's van de Technische Dienst en van Fotolocatie Binnenhaven van de Gecombineerde Diensten Binnenhaven verzorgden de materialen van veel proefopstellingen en de dia's van opstellingen en grafieken om mijn onderzoek aan anderen te kunnen presenteren en uitleggen. Piet Kostense realiseerde de omslag van dit boek. De medewerksters van de kantine hebben met hun goede zorgen mijn inwendige mens versterkt en wakker gehouden.

Je tiens particulièrement à remercier mes collègues de l'Institut de Biocénotique Expérimentale des Agrosystèmes de l'Université de Tours, pour leur hospitalité pendant mon séjour en 1988-1989, et pour leurs collaborations dans le projèt de CE: Professeur Jacques Huignard, Pascal Dupont, Mme. Daniëlle Rousse, Jean-Paul Monge, Laurent Leveque, et Mme. Anne Marie Cortesero. Je remercie également mes collègues africains pour leurs contributions, les traveaux indispensables sur le terrain, et leur hospitalité énorme pendant mes visites en Afrique de l'Ouest: Mme. Isabelle Glitho (Université de Benin, Lomé), Albert Ouedraogo (Université de Ouagadougou, Burkina Faso), Inezdane Alzouma (Université de Niamey, Niger), et Sagnia Sankung et ses collègues (Département de Formation en Protection des Végétaux, Centre Agrhymet, Niamey, Niger). Pascal et Sankung, grâce à vôtre amitié j'ai appris à connaître un petit peu plus d'Afrique de l'Ouest, et la vie et les problèmes des paysans. Ça, je n'oublierai jamais.

Thanks also to my colleagues within the project of the European Union, for the many stimulating discussions of plans and ideas. Peter Credland, Bruno Tran and Mike Profit of the University of London, Royal Holloway College, thanks for your contributions. Bruno, I greatly enjoyed our collaborations and chit-chat.

Een steun van een heel andere orde was voor mij de Puddingbroodjes-club, een plek waar ik ongegeneerd mijn benen op tafel kon leggen en van me af mocht mopperen. Birgit, Hanneke, Jan, Karen, Conny, Nanda en Ineke, de heerlijke maaltijden, de vriendschap en slappe-lachbuien hebben veel werkstress doen verdampen: ga zo door!

Johanna Baar zette mij met deskundige massage en hardhandige oefeningen weer recht overeind en hielp mij om het gebruik van mijn rechterarm weer terug te winnen na een ongelukkige breuk. Sylvia Blankestijn hielp mij met haar verhelderende inzichten om mijn innerlijke stem en stilte beter te verstaan.

Last but not least was er het gewone leven naast het werk. In tijden dat die twee heftig in conflict waren, is het mede aan de dierbare vriend(inn)en om mij heen te danken dat ik heb volgehouden. Namen noemen doet altijd anderen te kort. Toch wil ik expliciet Marcel, Alida, Conny, David, Janneke, Heleen, Kees, Piet, Lies, Machdy, Clare, Wouter Leen en mijn dierbare huisgenoten Tineke, Jaap, Klaske, Marieke, Liesbeth, Judith en Cora noemen, als redders van mijn geestelijke gezondheid. Tineke, bedankt voor de vele uren vlinderplezier die we samen deelden. En ik dank mijn Schepper voor de vlinders die elk jaar zo vrolijk door mijn blikveld en geest fladderen en mij zin in het leven geven.

Een jong gezin en een promotie-onderzoek vormen bepaald geen ideale combinatie. Ondanks hun soms vermoeiende vraag om zorg en aandacht, zijn onze dochters Liesbeth en Judith vooral enorme bronnen van vreugde en energie om door te gaan! Meiden, dat jullie mij nog lang mogen opvoeden.

Cora, toen ik aan dit promotie-onderzoek begon, hadden wij er geen besef van welke invloed dat werk ook zou hebben op jouw en ons leven. Jij hebt als geen ander in alle vreugde en frustraties rond dit proefschrift meegeleefd en -gedragen. Ik heb veel van jouw geduld en begrip gevraagd. Meer dan deze simpele woorden kunnen uitdrukken ben ik je daar dankbaar voor. Het besef dat we de afgelopen jaren dichter naar elkaar toe zijn gegroeid, doet veel van de moeilijke momenten vergeten en geeft aan de afronding van dit boek een gouden glans.

Wageningen
21 september 1996 Frans

Cowpea production and storage problems in West Africa

1

Abstract
More than half of the world's cowpea crop (estimated at 3.7 metric ton) is produced in West Africa. Losses caused by insect pests in the field and in storage are the major constraints for higher yields. Initial field infestations by the bruchid pest *Callosobruchus maculatus* as low as 1-2% of the seeds infested, may result in total loss of the harvest after 6 months of storage. Traditional and modern crop protection techniques and methods are being reviewed. Biological control of *C. maculatus* in a conservation strategy is an option compatible with subsistence farming conditions in West Africa. Conservation is a strategy in which environmental manipulations aim at increasing the impact of native natural enemies on indigenous pest populations. The larval and egg parasitoids that occure in stored cowpea in West Africa are being discussed.

Chapter 1

1.1 Cowpea production

Per capita food production in sub-Saharan Africa has declined over the last two decades (Yudelman, 1986; FAO, 1991; Weber, 1994). As a result, about 140 million people (35% of the total population) in sub-Saharan Africa were under-nourished in the early eighties (FAO, 1991). If Africa is to feed its increasing population, the production of pulses needs to increase. Pulses, i.e. legumes grown for their edible seeds, are a cheap and valuable source of protein, calcium, iron, thiamine and riboflavin (USDA, 1986; Walker, 1982), and have therefore been described as "poor man's meat" (Aykroyd & Doughty, 1982).

Africa's total annual production of cowpea (*Vigna unguiculata* Walp.) (Leguminosa; Papilionaceae) for 1990 was estimated at 3.36 metric tons (MT), of which 1.5 MT was produced in Nigeria and 368 000 MT in Niger (Nwokolo & Ilechukwu, 1996). Cowpea is grown extensively in about 15 African countries, of which Nigeria and Niger together produce half the world crop (Pandey & Westphal, 1989). Recent figures from FAO suggest that this proportion increased to about 80% in the early 90's (FAO, 1992).

Under subsistence farming conditions in Africa, average yields of cowpea, when intercropped, are 100 - 300 kilogram dried seeds per hectare (Pandey & Westphal, 1989). In large-scale, intensively managed monocropping systems, e.g. in California (USA) or at experimental fields at IITA (Nigeria), cowpea may yield up to 3000 kg/ha (Summerfield *et al.*, 1974; Kay, 1979).

In West Africa, cowpea is cultivated for seeds, pods and the leaves. Pods and leaves are consumed as green vegetables. The remaining leaves and haulms are used as green manure, as fodder or dried as hay for livestock. The production of cowpea as fodder is becoming more important, and prices of cowpea haulms are increasing (Speirs & Olsen, 1992). In subsistence farming in West Africa, cowpea also functions as a cash crop and stored cowpea is analogous to a savings account. At the time of harvest, the price of cowpea usually is low. After several months of storage, at the approach of the next rainy season, the price may have doubled. Farmers sell stored cowpeas whenever they need cash, but they may want to wait until the next growing season, when prices for sowing seed are high (Sagnia & Schütte, 1991; Speirs & Olsen, 1992; see also Chapter 2).

Cowpea is an annual herb, (sub)erect up to 80 cm tall, with trifoliolate alternating leaves (see inset on cover). Depending on the variety or cultivar, pods are curved or straight and contain between two and 18 seeds. Seeds are globular to reniform with a smooth or wrinkled pericarp, and white, buff, brown, red or black in colour (see cover). Seeds are between two and 12 mm long and weight five to 30 gram per 100 seeds (Duke, 1981; 1990). Cowpea is thought to originate from Africa, with a second centre of domestication in south Asia. Many wild and domesticated cowpea relatives can be found in different areas of Africa

(Summerfield *et al.*, 1974; Ng & Marechal, 1985; Padulosi & Ng, 1990; Smartt, 1990; Pienaar & van Wyk, 1992).

The crop can tolerate annual rainfall of 280-4100 mm, mean annual temperatures of 12.5-27.8°C, and a soil pH of 4.3-7.9 (Duke, 1981). Due to its resistance to drought, tolerance to acid soils, and ability to fix nitrogen, cowpea performs satisfactorily even on the low fertility soils situated in the northern savannas bordering the Sahara desert (Nwokolo & Ilechukwu, 1996). Low temperatures and a photoperiod shorter than 12 h 13 min strongly reduce yield. Early maturing cultivars produce pods in 50 days, while late cultivars may take 240 days to mature (Duke, 1990).

For low income families, cowpea is a much cheaper protein source than meat. The price of one kilogram of meat is about one tenth of a monthly salary of a janitor in Nigeria, and one fifth of a monthly salary in Sierra Leone (Nwokolo, 1996). Prices of cowpea seeds may be 50% to 70% lower. In Niger for example, the prices of meat are about 2.0 US $ per kilogram, which is between 3% and 10% of the monthly salary of a house guard (S.B. Sagnia, pers. comm.). In comparison, the cowpea price is only 0.8 US $/kg, or 2% of the salary (S.B. Sagnia, pers. comm.); this price is 50% lower just after harvest (Sagnia & Schütte, 1992).

Two popular cowpea dishes are prepared from decorticated and ground seeds (Dovlo *et al.*, 1976; Uzogara & Ofuya, 1992). Cowpea flour is mixed with onions and spices into a paste that is either deep-fried (akara balls) or steamed (moin-moin). Average families in northern Nigeria are reported to consume two to four kilogram grains per week (Ashraf, 1985). The use of cowpea, its nutritional qualities, and its importance in the diet of West African families is reviewed by Doughty & Orraca-Tetteh (1966), King *et al.* (1985), Phillips & McWatters (1991), Singh & Singh (1992), and Nwokolo & Ilechukwu (1996).

1.2 Field pests of cowpea and their control

Yields of cowpea in subsistence farming in Africa are only a fraction of those obtained in intensively managed monocropping systems (Summerfield *et al.*, 1974; Kay, 1979). In the drier savannas of Africa, insect pests are beyond doubt the major constraint in cowpea production (Singh & Allen, 1980; Youdeowei, 1989). This can be illustrated by yields increasing ten to twenty fold, commonly encountered when cowpea is protected by weekly insecticide applications during the flowering and pod-formation period (e.g. Booker, 1965a; Raheja, 1976, 1978; Matteson, 1982; Afun *et al.*, 1991; Alghali, 1993; Amatobi, 1995).

Singh *et al.* (1990) list 110 pest species of cowpea worldwide, and Booker (1965b) lists 85 insect species attacking cowpea plants in Nigeria, but only a few are considered of major importance. Insect field pests of cowpea and their impact on yield have been reviewed extensively by Singh & Van Emden (1979), Singh & Allen (1980), Singh & Jackai (1985), Jackai & Daoust (1986), Bal (1990), and Singh *et al.* (1990).

The major fields pests are (Singh *et al.*, 1990):

+ legume bud thrips (*Megalurothrips sjostedti* Trybom) (Thysanoptera, Thripidae),
+ coreid pod sucking bugs (*Clavigralla* spp.) (Hemiptera, Coreidae),
+ cowpea aphids (*Aphis craccivora* Koch) (Homoptera, Aphididae), and
+ legume pod borers (*Maruca testulalis* Geyer) (Lepidoptera, Pyralidae).

Thrips, aphids and coreid bugs influence pod formation, and coreid bugs and the pod borer directly attack the pods. Thrips and aphids are also involved in virus transmission, which may contribute to substantial losses. In unprotected monocrops, yield losses due to the major field pests may be 20-100% (Youdeowei, 1989).

Chemical control

Experiments to minimize the number of insecticide applications, often in monocrop cowpea systems with partly resistant varieties, have been conducted in several places. In general, two or three insecticide applications gave optimal yields (Nangju *et al.*, 1979; Matteson, 1982; Jackai *et al.*, 1985; Fisher *et al.*, 1987; Alghali, 1991a, 1993; Amatobi, 1995). In Nigeria, action thresholds (i.e. the infestation level at which insecticides are applied to prevent the infestation to reach the economic injury level) were tested to reduce the number of insecticide applications (Afun *et al.*, 1991).

In cowpea as a cash crop, insecticide applications have proven economically profitable. However, wide scale use of pesticides could result in the occurrence of insecticide-resistant strains of pests. Resurgence of pests, due to elimination of their natural enemies by pesticides applications, is another danger and has been observed in the cowpea aphid (Matteson, 1982).

Under subsistence farming conditions, pesticide applications in cowpea generally have remained beyond the reach of most farmers, mainly due to the lack of cash and because farm chemicals are poorly availability (Alghali, 1991a; Amatobi, 1995; Bottenberg, 1995; de Groot, 1995). Besides, the use of pesticides constitutes not only a health risk for poor farmers with few resources, but also a high economic risk given the possibility of crop failure (e.g. due to droughts) (Brader, 1986; van Huis, 1991).

For these reasons, crop protection methods not demanding financial inputs should be combined into Integrated Pest Management (IPM) systems. Low-input techniques, such as resistant varieties, biological and cultural control, should also include methods against diseases, nematodes and parasitic weeds (e.g. *Striga* sp.).

Integrated Pest Management (IPM)

IPM should be viewed as a strategy and an approach to developing technologies, based on four principles (Kiss & Meerman, 1991):

(i) integration of the management of any given pest (or diseases) into the overall farming system, and the integration of all appropriate measures to control any one pest;

(ii) use of biological measures to create an environment which discourages the build-up of pests and diseases, and encourages the incidence of natural enemies;

(iii) pest and disease levels should be kept below economically damaging levels (not to eradicate them); and

(iv) control measures are selected and implemented to minimize hazards to human health and the environment.

For Africa's subsistence level agriculture, the primary objectives of introducing IPM should be the need to increase food production through improved crop management, including pest control. Proposed pest management measures must recognize the likelihood of poor extension services, low levels of farming technology, and a general lack of capital and access to inputs. They should be based primarily on resistant crop varieties, improved cultural practices (including intercropping and crop rotations), and biological control (Kiss & Meerman, 1991).

Unfortunately, IPM " ..has already evolved from challenge to token to mainstream to buzzword to cliché, all in the space of two decades." (Dover, 1988, cited in Kiss & Meerman, 1991). In spite of all the work done so far, practical IPM schemes for cowpea, applicable at subsistence level agriculture, still remain to be developed and implemented (Jackai *et al.*, 1985; Kiss & Meerman, 1991). Most studies on cowpea discuss IPM in theory only, or concentrate on individual crop management components. At best, programmes achieve integration of chemical control with resistant cowpea varieties only. Several constraints are responsible for the slow development and implementation of IPM systems in Africa. For example, many national agricultural research and extension systems suffer from various financial, educational, organisational and administrative constraints. Extension services all over Africa are sparsely manned (and even more sparsely wo-manned); staff are poorly trained in IPM, poorly equipped and have insufficient funds to serve more than a few farmers (Zethner, 1995). With respect to insect pests of cowpea, the control methods currently available will be briefly discussed below.

Cultural control and intercropping

Cultural practices, such as crop rotation, manipulation of planting and harvesting dates, trap cropping and weed control are discussed by Singh *et al.* (1978), Ezueh (1982), Muleba & Ezumah (1985), Ofuya (1989) and Ezueh (1991). In Africa, cowpea is traditionally grown as an intercrop with maize, sorghum, or millet, and sometimes with cassava or cotton (Fisher *et al.*, 1987; Alghali, 1991a,b, 1992, 1993). From the farmers' point of view, reasons for this may be tradition, land shortage, more crops at harvest, improvement in cereal yields, increased soil fertility, and insurance against total crop failure (Alghali, 1991a). Cowpea grown as a monocrop in many cases produces less total grain and fodder yields than traditional mixtures of cowpea, sorghum and millet (Carr, 1989).

Plant protection specialists have evaluated the effects of intercropping on pests of cowpea. Intercropping cowpea with cereals reduces densities of foliage beetles and flower thrips, and increases the number of *Maruca* pod borers and pod sucking bugs (Singh *et al.*, 1990). However, many conflicting reports exist on the responses of a given pest to the same cropping system (Jackai *et al.*, 1985; Jackai & Daoust, 1986). When intercropped with cowpea, pest attack in sorghum was not significantly influenced (Dissemond & Weltzien, 1986). Cowpea yields depend on cowpea variety and planting dates, relative to the first rains and to the companion crop (Ezueh, 1982; Ezueh & Taylor, 1984; Muleba & Ezumah, 1985; Fisher *et al.*, 1987; Alghali, 1993). Planting in alternating single or double rows, instead of mixed intercropping, allows for more efficient weed control and insecticide applications (Jackai *et al.*, 1985; Fisher *et al.*, 1987).

Resistant varieties

From 1970 onwards, the International Institute for Tropical Agriculture (IITA, Ibadan, Nigeria) tries to develop insect-resistant cowpea lines. The world cowpea germplasm collection at IITA now holds over 15,000 cowpea lines (IITA, 1992). Resistance against several diseases and insect pests has been identified in wild relatives of cowpea. Varieties with resistance against leafhoppers (*Empoasca sp.*) and cowpea aphids (*Aphis craccivora*) do not require insecticide applications. Lines with some levels of resistance against the cowpea weevil (*Callosobruchus maculatus*), thrips (*Megalurothrips sjostedti*), pod borer (*Maruca testulalis*) and pod sucking bugs (*Clavigralla sp.*) have also been identified (Singh *et al.*, 1978; Singh & Allen, 1980; Redden *et al.*, 1984; Singh & Singh, 1990; IITA, 1992). Many lines with partial and/or moderate levels of resistance still have generally unacceptable seed characteristics and agronomic traits (Singh *et al.*, 1992). Most of the (partly) resistant cowpea lines released so far, require additional input of pesticides (against other pests or diseases) to attain their potential yields (Nangju *et al.*, 1979; Singh *et al.*, 1990).

In Nigeria, cowpea monocrops were introduced with the support of extension services. The packages included new high-yielding cowpea varieties, subsidized spray equipment and pesticides (Ashraf, 1985). However, a major devaluation of the national currency led to an increase in prices of pesticides and other inputs. This and the unreliable supply of pesticides, resulted in farmers gradually abandoning the packages in the drier northern states of Nigeria (IITA, 1992). Under heavy insect attack, improved varieties as monocrops yielded less dried seeds, produced less fodder, and received lower market prices than traditional intercropped cowpea varieties (IITA, 1992). At the same time, cowpea as a monocrop in many cases produced lower total grain and fodder yields than traditional mixtures of cowpea, sorghum and millet (Carr, 1989). This illustrates that new technological packages often fail to take into account the realities in the field and the needs of subsistence farmers to minimize their risks rather than to maximize their profits (Mensah, 1989; Cleaver, 1993).

Biological control

Biological control (i.e. the use of predators and parasitoids to control insect pests) in cowpea has received very little attention so far. It is mainly restricted to lists of predators or parasitoids compiled from the literature (Singh *et al.*, 1990). Such lists of natural enemies of certain cowpea pests in West Africa are given by Agyen-Sampong (1978), Usua & Singh (1978), Matteson (1981), Otieno *et al.* (1981), Don-Pedro (1983), and Ezueh (1991). Most reports on biological control of cowpea pests fail to quantify the impact of these agents. Pesticide applications may yield quantitative assessments of the effects of natural enemies (e.g. Matteson, 1981, 1982). Since the major cowpea pests are indigenous to Africa, conservation of the indigenous parasitoids, predators and pathogens offers the greatest scope for control (Yaninek & Cock, 1989).

1.3 Storage pests of cowpea and their control

In the Sudano-Sahelian zone, the cropping season for cowpea is four months (van Huis *et al.*, 1990). As a consequence, dried cowpea seeds and pods have to be stored over periods of up to one year. In West Africa, many different forms of traditional and modern storage structures are used (e.g. Hall, 1963; Appert, 1985; Sagnia & Schütte, 1992; Hama, 1994; Proctor, 1994). Most common are traditional granaries either made of a mixture of mud, straw and manure or made of the stems of wild grasses or millet. The variability in structures and materials is enormous (see also Chapter 2). Storage can be either in pods or as threshed seeds. Smaller containers in or near the house are often used to store cowpea seeds for home consumption and sowing seed for the next season.

Seed beetles (Coleoptera; Bruchidae) are the only serious post-harvest pests of cowpea (Hill, 1990; Singh *et al.*, 1990). In West Africa, *Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* Fab. (Coleoptera; Bruchidae) are the major species. In Central and Eastern Africa, *C. rhodesianus* and *C. subinnotatus* may also be important pests (Prevett, 1967; Southgate, 1978, 1979; Williams, 1980; Decelle, 1981; Taylor, 1981; Hill, 1990).

Bruchidius atrolineatus

The biology of *B. atrolineatus* has been investigated by teams of the University of Niamey (Niger) and the University of Tours (France). Females of *B. atrolineatus* are 2-3 mm long, greyish with black markings on the elytrae. They have simple serrate antennae, whereas males have profoundly pectinate antennae (Delobel & Tran, 1993). *Bruchidius atrolineatus* is mainly a field pest, arriving in cowpea fields as soon as the first flowers appear. The species is characterized by a reproductive diapause during the dry season and aestivates in so far unknown localities. The reproductive diapause is terminated by contact with cowpea pollen. Oviposition takes place on mature green and ripening pods. In Niger, *B. atrolineatus* usually is abundant in the field with 80%-90% of the pods infested. Average egg densities in the field are approximately ten eggs per pod (Huignard *et al.*, 1984, 1985). Cowpea pods infested with *B. atrolineatus* eggs and larvae are taken into storage after the harvest. Due to low temperatures and a short photoperiod, the second generation adults go into reproductive diapause. From February onwards, the diapausing beetles leave the storage structures (Alzouma, 1981; Huignard *et al.*, 1984, 1985; Alzouma *et al.*, 1985; Germain *et al.*, 1985, 1987).

Callosobruchus maculatus

The biology of *C. maculatus* has been extensively studied (e.g. Paddock & Reinhard, 1919; El-Sawaf, 1956; Howe & Currie, 1964; Tun, 1979; Credland & Dick, 1987; Giga & Smith, 1987; Mitchell, 1990; Sagnia, 1993, 1994). *Callosobruchus maculatus* is easy to rear and to manipulate in the laboratory. Therefore, this species has often been used as a model in studies on population dynamics (e.g. Utida, 1953, 1967; Bellows & Hassell, 1984, 1988; Toquenaga, 1993; Colegrave, 1994), physiology (e.g. Gatehouse *et al.*, 1985), and genetics (e.g. Møller *et al.*, 1990; Messina, 1989, 1993; Fox, 1993, 1994; Tran & Credland, 1995).

Females of *C. maculatus* (see inset on back cover) are 2-4 mm, brownish with black markings. They have slightly serrate antennae, brown legs and two apical spines on the hind femur. The elytra do not quite cover the abdomen, and in non-flying females, the pygidium is black with a median line of white hairs (Hill, 1990; Delobel & Tran, 1993). Two forms are being distinguished: the active or flying form, and the normal or flightless form (Sano-Fujii, 1986; Ouedraogo *et al.*, 1991). The flying form is the dispersing form that colonizes cowpea fields in the rainy season. It is characterised by a low fecundity and high longevity. *Callosobruchus maculatus* occurs in many local populations (or "strains"), each with distinctly different biological or behavioural characteristics adapted to local selection pressures (e.g. Credland, 1990; Mitchell, 1990; Tran & Credland, 1995).

Ripening pods are infested in the field. Eggs are glued to the pod or seed surface. When given a choice between pods and seeds, *C. maculatus* females preferably lay their eggs on the seeds (Alzouma, 1981). After five or six days, the hatching larva tunnels directly through the egg shell and through the pod wall into the seed below. The entire development of the larva and pupa take place inside the seed. Under optimal conditions (32°C), development from egg to adult may take 21-23 days (Hill, 1990). In Niger, *C. maculatus* eggs are usually scarce in cowpea fields, with only 10%-15% of the pods carrying one or two eggs (Huignard *et al.*, 1985; Germain *et al.*, 1987). Under field conditions, egg mortality may be as high as 45% to 90%, mainly due to egg parasitism and disappearance of eggs after rainfall (Sagnia, 1993, 1994). Within the storage structures, the normal form of *C. maculatus* continues to reproduce during six to seven generations per year. It has a lifetime fecundity of about 100 eggs per female. At the end of the storage season, due to crowding conditions, increased humidity levels and deterioration of the seeds, the flying form appears again and leaves the granaries.

Callosobruchus maculatus causes the major losses in stored cowpea in West Africa. Initial field infestations as low as one or two percent of the pods infested with bruchid eggs may lead to 80% of the pods attacked after six to eight months of storage (Caswell, 1961; Youdeowei, 1989). Surveys of cowpea on markets and in village stores in West Africa indicate that 20-90% of the beans are attacked (Caswell, 1970, 1974; Sharma, 1984; Seck *et al.*, 1991; see also Chapter 2).

Control methods

Many traditional protective measures for stored cowpea have been identified (e.g. Golob & Webley, 1980; van Huis, 1991; Dabire, 1992; Rees *et al.*, 1993; Hama, 1994; Lienard & Seck, 1994), but their effectiveness under local conditions has often not been shown. Traditional methods comprise:

+ storage of cowpea in the pod instead of threshed seeds, the pod wall acting as a physical and possibly toxic barrier (e.g. Akingbohungbe, 1976; Caswell, 1973, 1984; Kitch *et al.*, 1991);
+ the use of smoke; cowpea in Africa is often stored above the kitchen fire (Zehrer, 1980); the heat and smoke may have physical, repellent and insecticidal actions;
+ the use of plant parts with attributed insecticidal or repellent activity; several lists of plants with potentially effective ingredients have been published (e.g. Lambert *et al.*, 1985; Sagnia & Schütte, 1991; Belko, 1994); and
+ the use of inert substances, such as sand or ashes, between the seeds, to hamper dispersal of the bruchids, and to cause damage to the insect integument, resulting in death through desiccation (Wegmann, 1983; Ofuya, 1986; Wolfson *et al.*, 1991).

More modern methods of protection of stored cowpea include:

+ chemical control using insecticides (e.g. Lienard & Seck, 1994), liable to the same problems and constraints as formulated under field pest control above (see also: Taylor & Webley, 1979; Egwautu, 1987). In cowpea storage, pesticide applications may even cause increased losses, due to elimination of bruchid natural enemies (Caswell, 1973; 1980; see also Chapter 2);
+ hermetic storage, e.g in plastic bags with an internal cotton lining, causing suffocation of the bruchids (Caswell, 1974);
+ the use of plant oils which protect seeds from oviposition and which kill freshly laid bruchid eggs. Numerous reports describe tests with different locally available oils (e.g. Rees *et al.*, 1993); however, due to socio-economic constraints, practical application is very limited;

- the use of resistant cowpea varieties with thicker pod walls and seed testae, or which contain secondary plant chemicals that slow bruchid development and increase bruchid mortality (Redden *et al.*, 1984; Dick & Credland, 1986; Singh & Singh, 1990); and
- biological control through the inoculation or conservation of indigenous predators or parasitoids in storage (see below).

Other methods, e.g. modified atmospheres, temperature regulation, light or pheromone traps, insect growth regulators and gamma radiation are beyond the technical and financial means of most farmers in Africa (van Huis, 1991).

Biological control

In stored products, biological control has long been neglected because it was considered to be of little value (e.g. Haines, 1984). However, recently, attention focused on this strategy, due to increased consumers concern with pesticide residues in food, cases of insecticide resistant pests, and more restrictive pesticide legislation. The potentials and limitations for biological control of stored product pests have been reviewed by Brower *et al.* (1996; and references therein).

Three major strategies of biological control can be distinguished (Ehler, 1990):

i. classical biological control, i.e. the importation of natural enemies from another region, in order to suppress an introduced (exotic) pest;
ii. augmentation and inundation, i.e. the mass rearing of (exotic or native) natural enemies which are released as a kind of biological pesticide against the pest; and
iii. conservation, i.e. manipulation of the environment aimed at increasing the impact of native natural enemies on indigenous pest populations.

Conservation

Since *C. maculatus* and *B. atrolineatus* are indigenous to Africa, and because the local infrastructure and technical facilities do not allow for large-scale mass rearing and distribution of natural enemies, conservation of the indigenous parasitoids predators and pathogens offers the greatest scope for control (Yaninek & Cock, 1989; van Huis, 1991; van Huis *et al.*, 1991a). In subsistence farming systems, the presence of insects and mites in the stored product is often inevitable and therefore tolerated (van Huis, 1991; van Huis *et al.*, 1991a). Thus, the reduction in bruchid incidence by introducing natural enemies in storage, as a conservation strategy of biological control, should be an acceptable practice. For example, in Senegal, traditional practices of conserving a larval parasitoid against borer pests in a village millet storage system are employed (Bhatnagar, 1987). Such a system could also easily be improved into a simple and inexpensive parasitoid mass rearing facility at the village level for inoculations of granaries with parasitoids (Bhatnagar, 1987).

The feasibility of biological control of bruchids by conservation of indigenous parasitoids is currently under investigation (van Huis *et al.*, 1990, 1991a). Attention for biological control of bruchids has for a long time been limited to records of parasitoid species associated with different bruchid species (Steffan, 1981). Several lists of parasitoids for different bruchid species have been compiled (e.g. Parker, 1957; de Luca, 1961, 1962, 1965, 1970, 1977, 1980; Whitehead, 1975; Hetz & Johnson, 1988; Rasplus, 1988, 1990; Bapfubusa *et al.*, 1990; van Huis, 1991; and references therein).

In West Africa, a number of parasitoids of *B. atrolineatus* and *C. maculatus* and some predators have been reported from cowpea granaries. Generalist predators, such as the bug *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae), ant species (Hymenoptera: Formicoidea) and parasitic mites (Acari: e.g. *Pyemotes spp.*) can be encountered occasionally in cowpea stores. Generally, their impact is limited (Haines, 1984). In Niger, the egg parasitoid *Uscana lariophaga* Steffan (Hymenoptera; Trichogrammatidae) is frequently found (Lammers & van Huis, 1989; Monge *et al.*, 1991; see also Chapter 2). The solitary larval parasitoids *Eupelmus vuilleti* (Craw.), *E. orientalis* (Hymenoptera: Eupelmidae) and *Dinarmus basalis* (Rond) (Hymenoptera: Pteromalidae) develop on bruchid larvae inside the beans (Monge & Huignard, 1991; van Alebeek, 1991). Parasitoids attack bruchids in the field, and, upon harvest of the cowpea, they are transported, together with the bruchid pests, into the storage structures.

Larval parasitoids

Dinarmus basalis has a worldwide distribution, probably as a result of accidental transport together with its host (van Alebeek, 1991). This species is a potential candidate for biological control of bruchids in food legumes in Asia (e.g. Verma, 1990, 1991; Islam & Kabir, 1992, 1995). *Eupelmus vuilleti* and *E. orientalis* occur in Africa only (Rasplus, 1988). Their biology is very similar to that of *D. basalis* (van Alebeek, 1991). Levels of parasitism vary from 7% to 60% (Sowunmi, 1982; Monge & Huignard, 1991).

In recent years, the biology and behaviour of the larval parasitoids *E. vuilleti, E. orientalis* and *D. basalis* have been studied in detail, in order to evaluate their potential for biological control of cowpea bruchids in storage (Terrasse & Rojas-Rousse, 1986; Cortesero *et al.*, 1993, 1995; Cortesero & Monge, 1994, Doury & Rojas-Rousse, 1994; Thomas-Odjo & Gaspar, 1994; Doury *et al.*, 1995; Gauthier *et al.*, 1996; Terrasse *et al.*, 1996).

Eupelmus vuilleti and *D. basalis* concurrently attack the same host in the same storage structures. This has evoked several studies into the interspecific competition between the two parasitoid species (van Alebeek, 1991; Monge & Huignard, 1991; Monge *et al.*, 1991; van Es, 1993; van Alebeek *et al.*, 1993; Leveque *et al.*, 1993; Glitho & Amevoin, 1995; Glitho *et al.*, 1995; Monge *et al.*, 1995). In Niger and Togo, controlled inoculation of cowpea stores with *E. vuilleti* resulted in some reduction of the bruchid population, but still substantial losses occurred. However, inoculation with *D. basalis* significantly reduced bruchid populations, and losses were minimal. When both parasitoids were introduced, *E. vuilleti* exerted a strong negative effect on the *D. basalis* population. *Eupelmus vuilleti* selectively kills eggs and young larvae of *D. basalis* with its ovipositor, and may act as a facultative hyperparasitoid on older *D. basalis* larvae (van Alebeek *et al.*, 1993; Leveque *et al.*, 1993). The resulting control of bruchid populations by both parasitoids was much poorer than that by *D. basalis* alone (van Es, 1993; Glitho & Amevoin, 1995; Glitho *et al.*, 1985; Monge *et al.*, 1995).

Egg parasitoids

Uscana lariophaga Steffan (Hymenoptera; Trichogrammatidae) is another potential candidate for the biological control of bruchid pests in stored cowpea. Van Huis *et al.* (1991a) reviewed earlier attempts of biocontrol against bruchids elsewhere in the world. They list the species of *Uscana* known so far, and discuss the possibilities to use *U. lariophaga* in West Africa against *C. maculatus* and *B. atrolineatus* (van Huis *et al.*, 1991a). The taxonomy of the genus *Uscana* is difficult and needs revision (A. Polaszek, pers. comm.). Many samples of *Uscana spp.* from West Africa await identification. In addition to the *Uscana* species listed by van Huis *et al.* (1991a), some other species of *Uscana* have been described (Viggiani, 1979; 1992; Fursov, 1987; Yousuf & Shafee, 1987), and their potential use in biological control was discussed (Zerova & Fursov, 1989; Baker, 1990; Pintureau, 1991; Pinto & Stouthamer, 1994). In India and Bangladesh, the use of *Uscana mukerjii* Mani for the control of *Callosobruchus spp.* is under investigation (Islam *et al.*, 1991; Pajni, 1991, 1992; Kapila & Agarwal, 1991, 1995). In Chile, *Uscana senex* Grese has been tested in experimental mass releases against the pea weevil (*Bruchus pisorum*) (Gerding & Hormazabal, 1996).

Uscana lariophaga was first described from *C. maculatus* eggs in Mali (Steffan, 1954). In Niger, between four and 21% of *B. atrolineatus* eggs and between 25% and 60% of *C. maculatus* eggs were parasitized in cowpea fields (Alzouma, 1987). In experimental cowpea granaries, between 33% and 60% of *C. maculatus* eggs were parasitized (Lammers & van Huis, 1989; Monge *et al.*, 1991; see also Chapter 2). Very little is known about its life history, habitat-, host plant- and host preference in natural habitats.

Adults are 0.4 mm long; the thorax and abdomen are black in colour, the head, antennae and legs are yellowish-brown, and the eyes are red (see inset on front cover). Males and females can be differentiated by the shape of their antennae: in the male, the antennae are short with a blunt apical shape, whereas in the female the antennae are longer with a slender, spearlike tip (Steffan, 1954). At 30°C and in the absence of food and hosts, adult longevity is 2.5 days and lifetime fecundity is approximately 25 eggs (van Huis *et al.*, 1991a). The parasitization behaviour on host eggs is described in van Huis *et al.* (1991b). In Chapter 3 of this thesis, the research done so far on the biology and behaviour of *U. lariophaga* will be reviewed.

We cannot fully assess the potential of parasitoids for biological control in storage, until the biology and ecology of these natural enemies is thoroughly understood (Southgate, 1978). This thesis aims at contributing to this knowledge about *U. lariophaga*. The next Chapter gives the report of a survey to assess the relative impact of *U. lariophaga* in cowpea stores in West Africa. Chapter 3 deals with the objectives and research questions for the study of *U. lariophaga* biology and behaviour in a conservation strategy, and provides an outline of the structure of this thesis.

Acknowledgements

The draft of this paragraph was improved substantially by comments from Joop van Lenteren, Arnold van Huis, Sankung Sagnia, Frans Meerman, Conny Schütte, and Cora van der Sar.

References

Afun, J.V.K.; Jackai, L.E.N.; Hodgson, C.J., 1991. Calendar and monitored insecticide application for the control of cowpea pests. Crop Prot. 10: 363-370.

Agyen-Sampong, M.M., 1978. Pests of cowpea and their control in Ghana. In: S.R. Singh, H.F. van Emden & T.A. Taylor (eds), Pests of Grain Legumes: Ecology and Control. Academic Press, London, pp. 85-92.

Akingbohungbe, A.E., 1976. A note on the relative susceptibility of unshelled cowpeas to the cowpea weevil (*Callosobruchus maculatus* Fabricius) (Coleoptera: Bruchidae). Trop. Grain Legume Bull. 5: 11-13.

Alebeek, F.A.N. van, 1991. Interspecific host discrimination by two solitary ectoparasitoids of immature stages of Bruchidae. Meded. Fac. Landbouww. Rijksuniv. Gent 56/3b: 1011-1020.

Alebeek, F.A.N. van; Rojas-Rousse, D.; Leveque, L., 1993. Interspecific competition between *Eupelmus vuilleti* and *Dinarmus basalis*, two solitary ectoparasitoids of Bruchidae larvae and pupae. Entomol. exp. appl. 69: 21-31.

Alghali, A.M., 1991a. Studies on cowpea farming practices in Nigeria, with emphasis on insect pest control. Trop. Pest Manage. 37(1): 71-74.

Alghali, A.M., 1991b. The effect of plant spacings on cowpea, *Vigna unguiculata* Walp., insect pests and yields in two sites in Nigeria. Insect Sci. Applic. 12(5/6): 707-711.

Alghali, A.M., 1992. Development of insect management practices for sustainable production of cowpea in the semi-arid zones of West Africa. Proc. 3rd Int. Conf. Pl. Prot. in Tropics, Genting Highlands, 1990, Vol. V, pp. 85-92.

Alghali, A.M., 1993. Intercropping as a component in insect pest management for grain cowpea, *Vigna unguiculata* Walp production in Nigeria. Insect Sci. Applic. 14(1): 49-54.

Alzouma, I., 1981. Observations on the ecology of *Bruchidius atrolineatus* Pic. and *Callosobruchus maculatus* F (Coleoptera, Bruchidae) in Niger. Series Entomologica 19: 205-213.

Alzouma, I., 1987. Reproduction et développement de *Bruchidius atrolineatus* Pic. (Coléoptère: Bruchidae) aux dépens des cultures de *Vigna unguiculata* L. Walp (Leguminosae Papilionacea) dans un agrosystème sahélien au Niger. Thèse Université François-Rabelais, Tours (France).

Alzouma, I.; Huignard, J.; Leroi, B., 1985. La ponte de *Bruchidius atrolineatus* (Pic) (Col. Bruchidae) au cours de la maturation des gousses de *Vigna unguiculata* (Walp.) et ses conséquences sur le développement. Annls Soc. ent. Fr. (N.S.) 21(2): 207-217.

Amatobi, C.I., 1995. Insecticide application for economic production of cowpea grains in the northern Sudan savanna of Nigeria. Int. J. Pest Manage. 41(1): 14-18.

Appert, J., 1985. Le stockage des produits vivrièrs et sémencièrs. Technicien d'agriculture tropicale no. 4. Vol. 1: Dégats, pertes, et moyens de stockage, Vol. 2: Lutte contre les ravageurs, hygiène du stockage. Maisonneuve et Larose, Paris, 98 + 102 pp.

Ashraf, M., 1985. Farming-systems approach: research on cowpeas and extension. In: S.R. Singh & K.O. Rachie (eds.), Cowpea Research, Production and Utilization. John Wiley & Sons Ltd., London, pp. 341-349.

Aykroyd, W.R.; Doughty, J., 1982. Legumes in human nutrition. FAO Nutrition Studies no. 19, United Nations Food and Agriculture Organisation (FAO), Rome.

Baker, G.H., 1990. Natural enemies of pea weevil, *Bruchus pisorum* (Bruchidae), worthy of consideration as biological control agents. In: A.M. Smith (ed.), Proc. Nat. Pea Weevil Workshop, Dept. Agric. Rural Affairs, Melbourne, Victoria. Conf. Proc. Series 38: 80-83.

Bal, A.B., 1990. Les principaux insectes du niébé dans le Sahel. Bulletin d'Information en Protection des Végétaux de l'CTR/PV (Mali), Sahel PV Info 29: 8-14.

Bapfubusa, B.; Rasplus, J.Y.; Fabres, G., 1990. l'Entomofaune associée aux gousses des légumineuses du genre *Vigna* dans différents habitats de la zone forestière du Centre-Cameroun. Annls Soc. ent. Fr. (N.S.) 26(2): 203-210.

Belko, H., 1994. Plantes utilisées pour la conservation du niébé au Niger. Sahel PV Info 67: 2-18

Bellows, T.S.; Hassell, M.P., 1984. Models for interspecific competition in laboratory population of *Callosobruchus spp.* J. Animal Ecol. 53: 831-848.

Bellows, T.S.; Hassell, M.P., 1988. The dynamics of age-structured host-parasitoid interactions. J. Animal Ecol. 57(1): 259-268.

Bhatnagar, V.S., 1987. Conservation and encouragement of natural enemies of insect pests in dryland subsistence farming: problems, progress and prospects in the Sahelian zone. Insect Sci. Applic. 8: 791-795.

Booker, R.H., 1965a. Pests of cowpea and their control in northern Nigeria. Bull. ent. Res. 55: 663-672.

Booker, R.H., 1965b. List of insect species found in association with cowpeas at Samaru. Samaru Miscell. Paper 9, Inst. Agric. Res., Samaru, Ahmadu Bello University, Zaria, 4 pp.

Bottenberg, H., 1995. Farmers' perceptions of crop pests and pest control practices in rainfed cowpea cropping systems in Kano, Nigeria. Int. J. Pest Manage. 41(4): 195-200.

Brader, L., 1986. Problems of pesticide use in the tropics. J. Plant Prot. Tropics 3: 1-11.

Brower, J.H.; Smith, L.; Vail, P.V.; Flinn, P.W., 1996. Biological control. In: B. Subramanyam & D.W. Hagstrum (eds), Integrated Management of Insects in Stored Products. Marcel Dekker, Inc., New York, pp. 223-286.

Carr, S.J., 1989. Technology for small-scale farmers in sub-Saharan Africa. Experiences with food crop production in five major ecological zones. World Bank Technical Paper no. 109, World Bank, Washington, D.C., 106 pp.

Caswell, G.H., 1961. The infestation of cowpeas in the western regions of Nigeria. Trop. Sci. 3: 154-159.

Caswell, G.H., 1970. The storage of cowpeas in the northern states of Nigeria. Proc. Agric. Soc. Nigeria 5: 4-6.

Caswell, G.H., 1973. The storage of cowpea. Samaru Agric. Newsl. 15(2): 73-75.

Caswell, G.H., 1974. The development and extension of nonchemical control techniques for stored cowpeas in Nigeria. Proc. 1st Int. Working Conf. Stored Product Entomol., Savannah, Georgia, pp. 63-67.

Caswell; G.H., 1976. The storage of grain legumes.. In: Youdeowei, A. (Ed.): Entomology and the Nigerian economy. Entom. Soc. Nigeria, Occas. Publ. 18: 131-142.

Caswell, G.H., 1980. A review of the work done in the Entomology Section of the Institute for Agricultural Research on the pests of stored grain and their control. Samura Miscell. Paper N99, 12 pp.

Caswell, G.H., 1984. The value of the pod in protecting cowpea seed from attack by bruchid beetles. Samaru J. Agric. Res. 2(1/2): 49-55.

Cleaver, K.M., 1993. A strategy to develop agriculture in sub-Saharan Africa and a focus for the World Bank. World Bank Technical Paper no. 203, African Technical Department Series, World Bank, Washington, D.C., 140 pp.

Colegrave, N., 1994. Game theory models of competition in closed systems: asymmetries in fighting and competitive ability. OIKOS 71: 499-505.

Cortesero, A.M.; Monge, J.P., 1994. Influence of pre-emergence experience on response to host and host plant odours in the larval parasitoid *Eupelmus vuilleti*. Entomol. exp. appl. 72: 281-288.

Cortesero, A.M.; Monge, J.P.; Huignard, J., 1993. Response of the parasitoid *Eupelmus vuilleti* to the odours of the phytophagous host and its host plant in an olfactometer. Entomol. exp. appl. 69: 109-116.

Cortesero, A.M.; Monge, J.P.; Huignard, J., 1995. Influence of two successive learning processes on the response of *Eupelmus vuilleti* Crw (Hymenoptera: Eupelmidae) to volatile stimuli from hosts and host plants. J. Insect Beh. 8(6): 751-762.

Credland, P.F., 1990. Biotype variation and host change in bruchids: causes and effects in the evolution of bruchid pests. In: K. Fujii, A.M.R. Gatehouse, C.D. Johnson, R.M. Mitchell & T. Yoshida (eds), Bruchids and Legumes: economics, ecology and coevolution. Kluwer Academic Publishers, The Netherlands, pp. 271-287.

Credland, P.F.; Dick, K.M., 1987. Food consumption by larvae of three strains of *Callosobruchus maculatus* (Coleoptera: Bruchidae). J. Stored Prod. Res. 23(1): 31-40.

Dabire, C., 1992. Les méthodes traditionnelles de protection des stocks de niébé au Burkina Faso. Sahel PV Info 49: 7-13.

Decelle, J., 1981. Bruchidae related to grain legumes in the afro-tropical area. Series Entomologica 19: 193-197.

Delobel, A.; Tran, M., 1993. Les coléoptères des denrées alimentaires entreposées dans les régions chaudes. Faune tropicale XXXII, ORSTOM Editions, Paris, CTA, Wageningen, 425 pp.

Dick, K.M.; Credland, P.F., 1986. Changes in the response of *Callosobruchus maculatus* (Coleoptera: Bruchidae) to a resistant variety of cowpea. J. Stored Prod. Res. 22(4): 227-233.

Dissemond, A.; Weltzien, H.C., 1986. Influence of sorghum and cowpea intercropping on plant pests in a semi-arid area of Kenya. Med. Fac. Landbouww. Rijksuniv. Gent 51/3a: 1147-1155.

Don-Pedro, K.N., 1983. Level of parasitization of *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae) larvae in early and late cowpea (*Vigna unguiculata*) in Nigeria. Rèvue de Zoologie Africaine 97: 678-683.

Doughty, J.; Orraca-Tetteh, R., 1966. The contribution of legumes to African diets. In: W.R. Stanton (ed), Grain Legumes in Africa, United Nations Food and Agriculture Organisation (FAO), Rome, pp. 9-32.

Doury, G.; Rojas-Rousse, D., 1994. Reproductive potential in the parasitoid *Eupelmus orientalis* (Hymenoptera: Eupelmidae). Bull. ent. Res. 84: 199-206.

Doury, G.; Rojas-Rousse, D.; Periquet, G., 1995. Ability of *Eupelmus orientalis* ectoparasitoid larvae to develop on an unparalysed host in the absence of female stinging behaviour. J. Insect Physiol. 41(4): 287-296.

Dovlo, F.E.; Williams, C.E.; Zoaka, L., 1976. Cowpeas; home preparation and use in West Africa.. International Development Research Centre, Ottawa. 96 pp.

Duke, J.A., 1981. Handbook of Legumes of World Economic Importance. Plenum Press, London.

Duke, J.A., 1990. Introduction to food legumes. In: S.R. Singh (ed.), Insect Pests of Tropical Food Legumes. John Wiley & Sons, Chichester, pp. 1-42.

Egwautu, R.I., 1987. Current status of conventional insecticides in the management of stored product insect pests in the tropics. Insect Sci. Applic. 8: 695-701.

Ehler, L.E., 1990. Introduction strategies in biological control of insects. In: M. Mackauer, L.E. Ehler & J. Roland (eds.), Critical Issues in Biological Control. Intercept, Andover, pp. 111-134.

El-Sawaf, S.K., 1956. Some factors affecting the longevity, oviposition and rate of development in the southern cowpea weevil F., *Callosobruchus maculatus* F. (Coleoptera: Bruchidae).. Bull. Soc. Entomol. d'Egypte, 40: 29-95.

Es, M. van, 1993. l'Impact de *Dinarmus basalis* (Rond.) et *Uscana lariophaga* Steffan sur les populations de leur hôte *Callosobruchus maculatus* (Fab.). Rapport d'un stage, D.F.P.V., Niamey, 44 p.

Ezueh, M.I., 1982. Effects of planting dates on pest infestation, yield and harvest quality of cowpea (*Vigna unguiculata*). Expl. Agric. 18: 311-318.

Ezueh, M.I., 1991. Prospects for cultural and biological control of cowpea pests. Insect Sci. Applic. 12(5/6): 585-592.

Ezueh, M.I.; Taylor, A.T., 1984. Effects of time of intercropping with maize on cowpea susceptibility to three major pests. Trop. Agric. (Trinidad) 61: 82-86.

FAO, 1991. Sustainable agriculture and rural development in sub-Saharan Africa: regional document no. 1. FAO/Netherlands Conference on Agriculture and the Environment, 's-Hertogenbosch, the Netherlands, 15-19 april 1991. United Nations Food and Agriculture Organisation (FAO), Rome, 31 pp.

FAO, 1992. Agrostat Database, Commodity Cowpeas, dry. Unpublished report, 3 June 1992, United Nations Food and Agriculture Organisation (FAO), Rome.

Fisher N.M.; Raheja A.K.; Elemo K.A., 1987. Insect pest control for cowpea in crop mixtures. Expl. Agric. 23, 9-20.

Fox, C.W., 1993. A quantitative genetic analysis of oviposition preference and larval performance on two hosts in the bruchid beetle, *Callosobruchus maculatus*. Evolution 47(1): 166-175.

Fox, C.W., 1994. Maternal and genetic influences on egg size and larval performance in a seed beetle (*Callosobruchus maculatus*): multigenerational transmission of a maternal effect? Heredity 73: 509-517.

Fursov, V.N., 1987. New species of *Uscana* Girault (Hymenoptera, Trichogrammatidae) from Gruzia and the Ukraine. Entom. Rev. Washington 67(1): 17-26.

Gatehouse, A.M.R.; Butler, K.J.; Fenton, K.A.; Gatehouse, J.A., 1985. Presence and partial characterisation of a major proteolytic enzyme in the larval gut of *Callosobruchus maculatus*. Entomol. exp. appl. 39: 279-286.

Gauthier, N.; Monge, J.P.; Huignard, J., 1996. Superparasitism and host discrimination in the solitary ectoparasitoid *Dinarmus basalis*. Entomol. exp. appl. 79: 91-99.

Gerding, M.; Hormazabal, L., 1996. Effect of density release of *Uscana senex* Grese (Hymenoptera; Trichogrammatidae) on the pea weevil eggs parasitization. (Abstract) Proc. 20th Int. Congr. Entomol. Firenze, August 25-31, 1996, pp. 620.

Germain, J.F.; Huignard, J.; Monge, J.P., 1985. Influence des inflorescences de la plante hôte (*Vigna unguiculata*) sur la levée de la diapause reproductrice de *Bruchidius atrolineatus*. Entomologia exp. appl. 39(1): 35-42.

Germain, J.F.; Monge, J.P.; Huignard, J., 1987. Development of two bruchid populations (*Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (Fab.) infesting stored cowpea (*Vigna unguiculata* Walp) pods in Niger. J. Stored Prod. Res. 23(3): 157-162.

Giga, D.P.; Smith, R.H., 1987. Egg production and development of *Callosobruchus rhodesianus* (Pic) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) on several commodities at two different temperatures. J. stored Prod. Res. 23(1): 9-15.

Glitho, I.A; Amevoin, K., 1995. Compétition interspécifique entre deux parasitoides sympatriques, *Dinarmus basalis* Rond. (Pteromalidae) et *Eupelmus vuilleti* Craw. (Eupelmidae) et leur rôle dans le contrôle des populations de *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae) en zone Guinéenne. Paper presented at 15th WASA Conference, 18-22 September 1995. University of Cape Coast, Ghana.

Glitho, I.A; Tchamoou, K.S.; Tchokossi, E., 1995. Essai de lutte biologique contre *Callosobruchus maculatus* Fab., coléoptère Bruchidae déprédateur du niébé (*Vigna unguiculata* Walp.). Paper presented at 15th WASA Conference, 18-22 September 1995. University of Cape Coast, Ghana.

Golob, P.; Webley, D.J., 1980. The use of plants and minerals as traditional protectants of stored products. Report G 138, Tropical Products Institute, ODA, London, 38 pp.

Groot, A.A. de, 1995. The functioning and sustainability of village crop protection brigades in Niger. Int. J. Pest Manage. 41(4): 243-248.

Haines, C.P., 1984. Biological methods for integrated control of insects and mites in tropical stored products. III: The use of predators and parasites. Trop. stored Prod. Inf. 48: 17-25.

Hall, D.W., 1963. Some essential considerations on the storage of food grains (cereals, legumes and oilseeds) in tropical Africa. Farm Products Processing, Information Working Bulletin no. 24, Agricultural Engineering Branch, Land and Water Development Division, FAO, Rome, 176 pp.

Hama, B., 1994. Conservation du niébé en milieu paysan au Niger. Sahel PV Info 65: 2-14.

Hetz, M.; Johnson, C.D., 1988. Hymenopterous parasites of some bruchid beetles of North and Central America. J. Stored Products 24(3): 131-143.

Hill, D.S., 1990. Pests of stored products and their control. Belhaven Press, London, 280 pp.

Howe, R.W.; Currie, J.E., 1964. Some laboratory observations on the rates of development, mortality and oviposition of several species of Bruchidae breeding in stored pulses. Bull. ent. Res. 55(3): 437-477.

Huignard, J.; Leroi, B.; Alzouma, I.; Germain, J.F., 1985. Oviposition and development of *Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in *Vigna unguiculata* (Walp) cultures in Niger. Insect Sci. Applic., 6: 691-699.

Huignard, J.; Rojas-Rousse, D.; Alzouma, I., 1984. L'activité réproductrice et le développement de *Bruchidius atrolineatus* (Pic) sur les gousses sèches de *Vigna unguiculata* (Walp) en zone sahélienne, mise en évidence d'une diapause imaginale. Insect Sci. Applic. 5(1): 41-49.

Huis, A. van, 1991. Biological methods of bruchid control in the tropics: a review. Insect Sci. Applic. 12(1/2/3): 87-102.

Huis, A. van; Kaashoek, N.K.; Lammers, P.M., 1990. *Uscana lariophaga* (Hym.: Trichogrammatidae), egg parasitoid of two bruchid species of cowpea in West Africa. Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam, 1: 101-106.

Huis, A. van; Kaashoek, N.K.; Maes, H.M., 1991a. Biological control of bruchids (Col.: Bruchidae) in stored pulses by using egg parasitoids of the genus *Uscana* (Hym.: Trichogrammatidae): a review. In: F. Fleurat-Lessard & P. Ducom (eds.), Proceedings of the Fifth International Working Conference on Stored-product Protection, Bordeaux, France, September 9-14, 1990, pp. 99-108.

Huis, A. van; Wijkamp, M.G.; Lammers, P.M.; Klein Goldewijk, C.G.M.; Seeters, J.H. van; Kaashoek, N.K., 1991b. *Uscana lariophaga* (Hymenoptera: Trichogrammatidae), an egg parasitoid of bruchid beetle (Coleoptera: Bruchidae) storage pests in West Africa: host-age and host-species selection. Bull. Entomol. Res. 81: 65-75.

IITA, 1992. Crop improvement. Cowpea. In: Sustainable Food Production in Sub-Saharan Africa: IITA's Contributions, International institute of Tropical Africulture (IITA), Ibadan, Nigeria, pp. 112-137.

Islam, W.; Kabir, S.M.H., 1992. Mass culture and production of *Dinarmus basalis* (Rond.) (Hymenoptera: Pteromalidae) a larval pupal parasitoid of the pulse beetle, *Callosobruchus chinensis* (L.). Pakistan J. Zool. 24(2): 172-174.

Islam, W.; Kabir, S.M.H., 1995. Biological control potential of *Dinarmus basalis* (Rond.) (Hymenoptera: Pteromalidae), a larval-pupal ectoparasitoid of the pulse beetle, *Callosobruchus chinensis* (L.). Crop Prot. 14(6): 439-443.

Islam, W.; Kabir, S.M.H.; Kabir, A., 1991. Influence of host age on progeny production by *Uscana mukerjii* (Mani) (Hymenoptera: Trichogrammatidae). Bangladesh J. Zool. 19(2): 179-182.

Jackai, L.E.N.; Daoust, R.A., 1986. Insect pests of cowpea. Ann. Rev. Ent. 31: 95-119.

Jackai, L.E.N.; Singh, S.R.; Raheja, A.K.; Wiedijk, F., 1985. Recent trends in the control of cowpea pests in Africa. In: S.R. Singh & K.O. Rachie (eds.), Cowpea Research, Production and Utilization, John Wiley & Sons Ltd., London, pp. 233-243.

Kapila, R.; Agarwal, H.C., 1991. Biology of an egg parasite of *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae). In: F. Fleurat-Lessard & P. Ducom (eds.), Proc. 5th Int. Working Conf. Stored-Product Prot. Bordeaux, Sept. 9-14, 1990, Vol. 2, pp. 1265-1273.

Kapila, R.; Agarwal, H.C., 1995. Biology of an egg parasite of *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae). J. stored Prod. Res. 31(4): 335-341.

Kay, D.E., 1979. Food Legumes. Crop and product digest no. 3. Tropical Products Institute, London, 451 pp.

King, J.; Nnanyelugo, D.O.; Ene-Obong, H.; Ngoddy, P.O., 1985. Household consumption profile of cowpea (*Vigna unguiculata*) among low-income families in Nigeria. Ecol. Food Nutr. 16(3): 209-221.

Kiss, A.; Meerman, F., 1991. Integrated Pest Management and African Agriculture. World Bank Technical Paper Number 142, African Technical Department Series, World Bank, Washington, D.C., 122 pp.

Kitch, L.W.; Shade, R.E.; Murdock, L.L., 1991. Resistance to the cowpea weevil (*Callosobruchus maculatus*) larva in pods of cowpea (*Vigna unguiculata*). Entomol. exp. appl. 60: 183-192.

Lambert, J.D.H.; Gale, J.; Arnason, J.T.; Philogene, B.J.R., 1985. Bruchid control with traditionally used insecticidal plants *Hyptis spicigera* and *Cassia Nigricans*. Insect Sci. Applic. 6(2): 167-185.

Lammers, P.M.; Huis, A. van, 1989. *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae), egg parasitoid of the stored insect pests *Callosobruchus maculatus* (F.) and *Bruchidius atrolineatus* Pic. (Col.: Bruchidae): population studies in the field and in storage in Niger. Proc. Int. Conf. Int. Pest Manage. 3: 1013-1022.

Leveque, L.; Monge, J.P.; Rojas-Rousse, D.; Alebeek, F. van; Huignard, J., 1993. Analysis of multiparasitism by *Eupelmus vuilleti* (Craw) (Eupelmidae) and *Dinarmus basalis* (Rond) (Pteromalidae) in the presence of one of their common hosts, *Bruchidius atrolineatus* (Pic) (Coleoptera Bruchidae). Oecologia 94: 272-277.

Lienard, V.; Seck, D., 1994. Révue des méthodes de lutte contre *Callosobruchus maculatus* Fab. (Coleoptera; Bruchidae), ravageur des graines de niébé (*Vigna unguiculata* (L.) Walp) en Afrique tropicale. Insect Sci. Applic. 15: 301-311.

de Luca, Y., 1961. Contribution aux Bruchides (Coléoptères) d'Algerie. Leurs hôtes - leurs parasites - leurs stations. Mémoires Soc. Hist. nat. de l'Afrique du Nord, 7: 155 pp.

de Luca, Y., 1962. Contributions aux Bruchides (Col.) d'Algerie: leurs hôtes - leurs parasites - leurs stations. Ann. l'école nat. d'agric. d'Alger, III(6): 1-15.

de Luca, Y., 1965. Catalogue des métazoaires parasites et prédateurs de Bruchides (Coleoptera). J. Stored Prod. Res. 1: 51-98.

de Luca, Y., 1970. Catalogue des métazoaires parasites et prédateurs de Bruchides (Coléoptères). Ann. Soc. Hort. Hist. nat. Herault, 110(1,2,3): 81-88; 123-127; 177-184.

de Luca, Y., 1977. Catalogue des métazoaires parasites et prédateurs des Bruchides (Col.) (Troisieme Note). Bull. Soc. Etu. Sci. Nat. de Nimes, 55: 5-22.

de Luca, Y., 1980. Catalogue des métazoaires parasites et prédateurs des Bruchides (Col.) (4e Note). Bull. Soc. Etu. Sci. Nat. de Nimes, 86: 37-55.

Matteson, P.C., 1981. Egg parasitoids of hemipteran pests of cowpea in Nigeria and Tanzania, with special reference to *Ooencyrtus particiae* Subba Rao (Hymenoptera: Encyrtidae) attacking *Clavigralla tomentosicollis* Stal. (Hemiptera: Coreidae). Bull. ent. Res. 71: 547-554.

Matteson, P.C., 1982. The effects of intercropping with cereals and minimal permethrin applications on insect pests of cowpea and their natural enemies in Nigeria. Trop. Pest Manage. 28(4): 372-380.

Mensah, M.C., 1989. The challenge of sustainable agriculture in Africa. In: J.S. Yaninek & H.R. Herren (eds.), Biological Control: A Sustainable Solution to Crop Pest Problems in Africa, International Institute of Tropical Agriculture (IITA), Ibadan, pp. 8-17.

Messina F.J., 1989. Genetic basis of variable oviposition behavior in *Callosobruchus maculatus* (Coleoptera: Bruchidae). Ann. Entomol. Soc. Am., 82(6), 792-796.

Messina, F.J., 1993. Heritability and 'evolvability' of fitness components in *Callosobruchus maculatus*. Heredity 71(6): 623-629.

Mitchell, R., 1990. Behavioral ecology of *Callosobruchus maculatus*. In: K. Fujii, A.M.R. Gatehouse, C.D. Johnson, R.M. Mitchell & T. Yoshida (eds), Bruchids and Legumes: economics, ecology and coevolution. Kluwer Academic Publishers, The Netherlands, pp. 317-330.

Møller, H.; Smith, R.H.; Sibly, R.M., 1990. Evolutionary demography of a bruchid beetle. III. Correlated responses to selection and phenotypic plasticity. Func. Ecol. 4: 489-493.

Monge, J.P.; Dupont, P.; Idi, A.; Huignard, J., 1995. The consequences of interspecific competition between *Dinarmus basalis* (Rond) (Hymenoptera: Pteromalidae) and *Eupelmus vuilleti* (CRW) (Hymenoptera: Eupelmidae) on the development of their host populations. Acta Oecol. 16(1): 19-30.

Monge, J.P.; Huignard, J., 1991. Population fluctuations of two bruchid species *Callosobruchus maculatus* (F.) and *Bruchidius atrolineatus* (Pic) (Coleoptera, Bruchidae) and their parasitoids *Dinarmus basalis* (Rondani) and *Eupelmus vuilleti* (Crawford) (Hymenoptera, Pteromalidae, Eupelmidae) in a storage situation in Niger. J. African Zool. 105: 187-196.

Monge, J.P.; Ouedraogo, A.P.; Huignard, J., 1991. Development of two Bruchid species *Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (F.) and their larval parasitoids during storage of cowpea seeds *Vigna unguiculata* (Walp) in West Africa. In: F. Fleurat-Lessard & P. Ducom (eds.), Proc. 5th Int. Working Conf. Stored-Product Prot. Bordeaux, Sept. 9-14, 1990, Vol. 1, pp. 151-160.

Muleba, N.; Ezumah, H.C., 1985. Optimizing cultural practices for cowpea in Africa. In: S.R. Singh & K.O. Rachie (eds.), Cowpea Research, Production and Utilization, John Wiley & Sons Ltd., Chichester, pp. 289-295.

Nangju, D.; Flinn, J.C.; Singh, S.R., 1979. Control of cowpea pests by utilization of insect-resistant cultivars and minimum insecticide application. Field Crops Res. 2: 373-385.

Ng, N.Q.; Marechal, R., 1985. Cowpea taxonomy, origin and germ plasm. In: S.R. Singh & K.O. Rachie (eds.), Cowpea Research, Production and Utilization, John Wiley & Sons Ltd., Chichester, pp. 11-21.

Nwokolo, E., 1996. The need to increase consumption of pulses in the developing world. In: E. Nwokolo & J. Smart (eds.), Food and feed from legumes and oilseeds. Chapman & Hall, London, pp. 3-11.

Nwokolo, E.; Ilechukwu, S.N., 1996. Cowpea (*Vigna unguiculata* (L.) Walp.). In: E. Nwokolo & J. Smart (eds.), Food and feed from legumes and oilseeds. Chapman & Hall, London, pp. 229-242.

Ofuya, T.I., 1986. Use of wood ash, dry chilli pepper fruits and onion scale leaves for reducing *Callosobruchus maculatus* (Fabricius) damage in cow-pea seeds during storage. J. agric. Sci., Camb. 107: 467-468.

Ofuya, T.I., 1989. Effect of weeds on pre-flowering insect infestation and seed yield of cowpea (*Vigna unguiculata*) in a rainforest area of Nigeria. Experimental Agriculture 25: 343-347.

Otieno, D.A.; Odindo, M.O.; Okeyo-Owuor, J.B.; Sabwa, D.M., 1981. Studies on the legume pod-borer, *Maruca testulalis* (Geyer). 6: Field survey on pathogenic organisms. Insect Sci. Applic. 4: 211-215.

Ouedraogo, P.A.; Monge, J.P.; Huignard, J., 1991. Importance of temperature and seed water content on the induction of imaginal polymorphism in *Callosobruchus maculatus*. Entomol. exp. appl. 59: 59-66.

Paddock, F.B.; Reinhard, H.J., 1919. The cowpea weevil. Tex. Agric. Exp. Stn. Bull. 256: 92 pp.

Padulosi, S.; Ng, N.Q., 1990. Wild *Vigna* species in Africa: their collection and potential utilization. In: N.Q. Ng & L.M. Monti (eds), Cowpea Genetic Resoorces. Contributions in cowpea exploration, evaluation and research from Italy and the International Institute of Tropical Agriculture, IITA, Ibadan, pp. 58-77.

Pajni, H.R., 1991. Status of *Uscana mukerjii* (Mani) in the biocontrol of Bruchids (Trichogrammatidae; Hymenoptera). In: F. Fleurat-Lessard & P. Ducom (eds.), Proc. 5th Int. Working Conf. Stored-Product Prot. Bordeaux, Sept. 9-14, 1990, Vol. 2, pp. 1279-1280.

Pajni, H.R., 1992. Biological control of store bruchids (Bruchidae: Coleoptera). (Abstract) Proc. 19th Int. Congr. Entomol. Beijing, June 28 - July 4, 1992, pp. 586.

Pandey, R.K.; Westphal, E., 1989. *Vigna unguiculata* (L.) Walp.. In: L.J.G. v.d. Maesen & Sadikin Someatmadja (eds.), PROSEA No. 1, Pulses. Pudoc, Wageningen, pp. 77-81.

Parker, H.L., 1957. Notes sur quelques Bruches et leurs parasites éléves des graines de Légumineuses (Col.). Bull. Soc. Ent. Fr. 62: 168-179.

Phillips, R.D.; McWatters, K.H., 1991. Contribution of cowpeas to nutrition and health. Food Technology 45: 127-130.

Pienaar, B.J. & Wyk, A.E. van, 1992. The *Vigna unguiculata* complex (Fabaceae) in southern Africa. S. Afr. J. Bot. 58(6): 414-429.

Pinto, J.D.; Stouthamer, R., 1994. Systematics of the Trichogrammatidae with emphasis on *Trichogramma*. In: E. Wajnberg & S.A. Hassan (eds.), Biological Control with Egg Parasitoids, IOBC, CAB International, Wallingford, pp. 1-36.

Pintureau, B., 1991. We know *Trichogramma*, but what else in Trichogrammatidae? Insect Parasitoids, 4th European Workshop, Perugia 3-5 April, 1991, REDIA, 74(3) (Appendice): 375-377.

Prevett, P.F., 1967. Notes on the biology, food plants and distribution of nigerian Bruchidae (Coleoptera), with particular reference to the northern region. Bull. Entomol. Soc. Nigeria 1: 3-6.

Proctor, D.L., 1994. Grain storage techniques. Evolution and trends in developing countries. FAO Agricultural Services Bulletin No. 109, FAO, Rome, 277 pp.

Raheja, A.K., 1976. Assessment of losses caused by insect pests to cowpeas in Northern Nigeria. PANS 22(2): 229-233.

Raheja, A.K., 1978. Yield losses from pests and the economics of chemical pest control on cowpea in Northern Nigeria. In: S.R. Singh, H.F. van Emden & T. Ajibola Taylor (eds.), Pests of grain legumes, Academic Press, London, pp. 259-265.

Rasplus, J.Y., 1988. La communauté parasitaire des Coléoptères séminivores de légumineuses dans une mosaique forêt-savanne en Afrique de l'Ouest (Lampto - Côte d'Ivoire). Thesis, Univ. d'Orsay-Paris XI: 437 pp.

Rasplus, J.Y., 1990. Structure spatio-temporelle d'une communauté parasitaire inféodée aux Coléoptères séminivores de Légumineuses à Lamto (RCI). Bull. Soc. Ent. Fr., 95(1-2), 5-15.

Redden, R.J.; Singh, S.R.; Luckefahr, M.J., 1984. Breeding for cowpea resistance to bruchids at IITA. Prot. Ecol. 7(4): 291-303.

Rees, D.P.; Dales, M.J.; Golob, P., 1993. Alternative methods for the control of stored-product insect pests: A bibliographic database. Natural Resources Institute, Chatham.

Sagnia, S.B., 1993. Parasitism, disappearance and eclosion of eggs of *Callosobruchus maculatus* exposed in the field at three different periods of the rainy season. In: AAIS (African Association of Insect Scientists), Beneficial African Insects: a Renewable Natural Resource, Proc. 10th Meeting Scient. Conf. AAIS, 5-10 Sept. 1993, Mombasa, pp. 53-55.

Sagnia, S.B., 1994. Mortality factors affecting *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) under field conditions in Niger. J. stored Prod. Res. 30(1): 71-74.

Sagnia, S.B.; Schütte, C., 1991. Cowpea storage system at village level in the State of Kano, Nigeria. Département de Formation en Protection des Végétaux (DFPV), CILSS, Niamey, 26 p.

Sagnia, S.B.; Schütte, C., 1992. Le système de stockage du niébé en milieu villageois dans l'état de Kano, Nigeria. Sahel PV Info 46: 6-15.

Sano-Fujii, I., 1986. The genetic basis of the production of the active form of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). J. Stored Prod. Res. 22(3): 115-123.

Seck, D.; Sidibe, B.; Haubruge, E.; Hemptinne, J.-L.; Gaspar, C., 1991. La protection chimique des stocks de niébé et de mais contre les insectes au Senegal. Med. Fac. Landbouww. Rijksuniv. Gent 56/3b: 1225-1233.

Sharma, S.S., 1984. Review of literature on the losses caused by *Callosobruchus* species (Bruchidae: Coleoptera) during storage of pulses. Bulletin of Grain Technology 22: 62-71.

Singh, B.B.; Singh, S.R., 1990. Breeding for bruchid resistance in cowpea. In: Fujii, K. et all. (Eds.): Bruchids and legumes: economics, ecology and coevolution; Proc. 2nd Int. Symp. Bruchids Legumes, Okayama, pp. 219-228.

Singh, S.R.; Allen, D.J., 1980. Pests, diseases, resistance and protection in cowpeas. In: R.J. Summerfield & A.H. Bunting, Advances in Legume Science, pp. 419-443.

Singh, S.R.; Emden, H.F. van, 1979. Insect pests of grain legumes. Ann. Rev. Entomol. 24: 255-278.

Singh, S.R.; Emden, H.F. van; Taylor, T.A., 1978. The potential for the development of integrated pest management systems in cowpeas. In: S.R. Singh, H.F. van Emden & T.A. Taylor (eds), Pests of Grain Legumes, Academic Press, London, pp. 329-335.

Singh, S.R.; Jackai, L.E.N., 1985. Insect pests of cowpeas in Africa: their life cycle, economic importance and potential for control. In: S.R. Singh & K.O. Rachie (eds.), Cowpea Research, Production and Utilization, John Wiley & Sons Ltd., London, pp. 217-231.

Singh, S.R.; Jackai, L.E.N.; Dos Santos, J.H.R.; Adalla, C.B., 1990. Insect pests of cowpea. In: S.R. Singh (ed.), Insect Pests of Tropical Food Legums, John Wiley & Sons, Chichester, pp. 43-89.

Singh, S.R.; Jackai, L.E.N.; Thottappilly, G.; Cardwell, K.F.; Myers, G.O., 1992. Status of research on constraints to cowpea production. In: G. Thottappilly, L.M. Monti, D.R. Mohan Raj & A.W. Moore (eds.), Biotechnology: Enhancing Research on Tropical Crops in Africa, CTA/IITA, Ibadan, pp. 21-26.

Singh, U.; Singh, B., 1992. Tropical grain legumes as important human foods. Economy Botany 46(3): 310-321.

Smartt, J., 1990. Grain legumes: evolution and genetic resources. Cambridge University Press, Cambridge.

Southgate, B.J., 1978. The importance of the Bruchidae as pests of grain legumes, their distribution and control. In: S.R. Singh; H.F. van Emden; T.A. Taylor (Eds.), Pests of grain legumes: Ecology and control, Academic Press, London.

Southgate, B.J., 1979. Biology of the Bruchidae. Ann. Rev. Entomol. 24: 449-473.

Sowunmi, E.O., 1982. Effect of storage length and insecticide treatment on cowpea (*Vigna unguiculata* L. Walp). Trop. Grain Legume Bull. 25: 21-27.

Speirs, M. & O. Olsen, 1992. Indigenous integrated farming systems in the Sahel. World Bank Technical Paper no. 179, African Technical Department Series, World Bank, Washington, D.C..

Steffan, J.R., 1954. Note sur le genre *Uscana* Girlt. (Hym. Trichogrammatidae) et description d'espèces nouvelles parasites de bruches. Bull. Museum, 2e serie, 26(6): 667-673.

Steffan, J.R., 1981. The parasites of bruchids. In: V. Labeyrie (ed.), The Ecology of Bruchids Attacking Legumes (Pulses). Proc. Int. Symp. Tours, April 16-19, 1980. Dr. W. Junk Publishers, The Hague, pp. 223-229.

Summerfield, R.J.; Huxley, P.A.; Steele, W., 1974. Cowpea (*Vigna unguiculata* (L.) Walp.). Field Crop Abstr. 27(7): 301-312.

Taylor, A.T., 1981. Distribution, ecology and importance of bruchids attacking grain legumes and pulses in Africa. Series Entomol. 19: 199-203.

Taylor, R.W.D.; Webley, D.J., 1979. Constraints on the use of pesticides to protect stored grains in rural conditions. Trop. Stored Prod. Inf. 38: 5-9.

Terrasse, C.; Nowbahari, B.; Rojas-Rousse, D., 1996. Sex ratio regulation in the wasp *Eupelmus vuilleti* (Crwf.), and ectoparasitoid on bean weevil larvae (Hymenoptera; Pteromalidae). J. Insect Beh. 9: 251-263.

Terrasse, C.; Rojas-Rousse, D., 1986. Distribution de la ponte et évitement du super-parasitisme chez l'hyménoptère solitaire *Bruchocida vuilleti* Cwf. (Hym.: Eupelmidae), parasite des stades larvaires de son hôte, *Callosobruchus maculatus* F. (Col.: Bruchidae). J. Appl. Entomol. 101: 243-256.

Thomas-Odjo, A.A.; Gaspar, C., 1994. Utilisation de *Dinarmus basalis* (Rondani) (Hymenoptera; Pteromalidae) pour le contrôle de *Callosobruchus maculatus* (F) (Coleoptera Bruchidae). Meded. Fac. Landbouww. Univ. Gent 59/2a: 451-456.

Toquenaga, Y., 1993. Contest and scramble competitions in *Callosobruchus maculatus* (Coleoptera: Bruchidae) II. Larval competition and interference mechanisms. Res. Popul. Ecol. 35: 57-68.

Tran, B.M.D.; Credland, P.F., 1995. Consequences of inbreeding for the cowpea seed beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Biol. J. Linnean Soc. 56: 483-503.

Tun, S.B. 1979. A life history study of the cowpea weevil in northern Nigeria, *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae). Samura Miscellaneous Paper 83: 7-13.

USDA, 1986. Composition of foods: legumes and legume products. Human Nutrition Information Centre, Agricultural Handbook no. 8-16, United States Department of Agriculture, Washington.

Usua, E.J.; Singh, S.R., 1978. Parasites and predators of the cowpea pod-borer, *Maruca testulalis* (Lepidoptera: Pyralidae). Nigerian J. Entomol. 2: 100-102.

Utida, S., 1953. Interspecific competition between two species of bean weevil. Ecology, 34(2): 301-307.

Utida, S., 1967. Damped oscillation of population density at equilibrium. Res. Popul. Ecol. 9: 1-9.

Uzogara, S.G.; Ofuya, Z.M., 1992. Processing and utilization of cowpeas in developing countries: a review. J. Food Proc. Pres. 16: 105-147.

Verma, R., 1990. Host habitat location and host location by *Dinarmus basalis*, a parasite of bruchids of stored legumes. Indian J. Exper. Biol. 28: 179-184.

Verma, R., 1991. Life-history and some aspects of biology of *Dinarmus basalis* (Hymenoptera, Pteromalidae), a parasite of stored legume bruchids. Acta Entomol. Bohemoslov. 88: 359-366.

Viggiani, G., 1979. Ricerche sugli Hymenoptera Chalcidoidea 62. *Uscana spermophagi* n.sp. (Trichogrammatidae) parassita di *Spermophagus sericeus* Geoffr. (Col. Bruchidae). Boll. de Lab. di Ent. Agraria 'Filipo Silvestre' 36: 51-54.

Viggiani, G., 1992. New species of Trichogrammatidae (Hymenoptera: Chalcidoidae) from South America. "Redia" Giornale di Zoologica, 75(Nt): 253-265.

Walker, A.F., 1982. Physiological effects of legumes in the human diet: a review. Journal of Plant Foods 4: 5-14.

Weber, A. 1994. Population growth, agricultural production and food supplies. In: E-C. Oerke, H-W. Dehne, F. Schönbeck & A. Weber, Crop production and crop protection: estimated losses in major food and cash crops. Elsevier Science B.V., Amsterdam, pp. 1-44.

Wegmann, E., 1983. Holzaschen als wirksames Mittel zur Bekaempfung von *Callosobruchus maculatus* (F.) in traditionellen Bohnenlagern Westafrikas.. Gesunde Pflanzen 35(9): 229-234.

Whitehead, D.R., 1975. Parasitic Hymenoptera associated with bruchid-infested fruits in Costa Rica. J. Wash. Acad. Sci., 65(3): 108-116.

Williams, J.O., 1980. Note on Bruchidae associated with stored products in Nigeria. Trop. Grain Legume Bull. 21: 5-10.

Wolfson, J.L.; Shade, R.E.; Mentzer, P.E.; Murdock, L.L., 1991. Efficacy of ash for controlling infestations of *Callosobruchus maculatus* (F.) (Coleoptera; Bruchidae) in stored cowpeas. J. stored Prod. Res. 27: 239-243.

Yaninek, J.S.; Cock, M.J.W., 1989. Identifying pest problems in relation to implementing biological control in Africa. In: J.S. Yaninek & H.R. Herren (eds.), Biological Control: A Sustainable Solution to Crop Pest Problems in Africa, IITA, Ibadan, pp. 116-126.

Youdeowei, A., 1989. Major arthropod pests of food and industrial crops in Africa and their economic importance. In: J.S. Yaninek & H.R. Herren (eds.), Biological Control: A Sustainable Solution to Crop Pest Problems in Africa, IITA, Ibadan, pp. 31-50.

Yousuf, M.; Shafee, S.A., 1987. Taxonomy of Indian Trichogrammatidae (Hymenoptera: Chalcidoidae), p. 163-164. Indian Jl. Syst. Entomol. 4: 55-200.

Yudelman, M., 1986. Forty years of Agricultural development. ISP Guest Lecture Series no. 4, Nairobi, The International Centre of Insect Physiology and Ecology, ICIPE Science Press, 30 pp.

Zehrer, W., 1983. The effect of the traditional preservatives used in Northern Togo and of neem oil for control of storage pests. Proc. 2nd Int. Neem Conf.: 453-460.

Zerova, M.D.; Fursov, V.N., 1989. Chalcids - parasites of Bruchidae - are promising species for introduction. In: O.A. Skarlato (ed.), Introduction and Application of Beneficial Arthropods in Plant Protection, 1989, Trudy Simposiuma, Leningrad, pp. 76-80.

Zethner, O., 1995. Practice of Integrated Pest Management in tropical and sub-tropical Africa: an overview of two decades (1970-1990). In: A.N. Mengech, K.N. Saxena & H.N.B. Gopalan (eds.), Integrated Pest Management in the Tropics, Current Status and Future Prospects, John Wiley & Sons, New York, pp. 1-67.

Natural suppression of bruchid pests in stored cowpea in West Africa

2

Abstract
Stored cowpea was collected from village storage units and from a market in Niger, to study the occurrence of bruchid pests and their parasitoids. High variability in storage methods and structures and in the levels of bruchid and parasitoid attack was observed. The village of origin, the cowpea variety and the type of storage structure all had significant effects on the percentage of beans attacked by bruchids. Damage to beans was highest in the Northern Ouallam region, and parasitism was highest in the South near Say. Bruchid eggs parasitized by *Uscana spp.* were present in 69 % of all samples. Emergence holes and adults of larval parasitoids (*Eupelmus spp.* and *Dinarmus spp.*) were found in 92 % of all samples. Some samples with high levels of egg or larval parasitism showed the potential for natural control, given the right conditions. Storage units seem to function as isolated units with little dispersal between them. The use of insecticides seems to decrease the impact of parasitoids, leading to higher bruchid populations and losses. Comparing the results of this survey with the outcome of interviews with farmers in Nigeria and Niger, it appears that traditional storage methods and protection techniques disappear gradually.

A slightly different version of this chapter has been published as:
Alebeek, F.A.N. van, 1996. Natural suppression of bruchid pests in stored cowpea (*Vigna unguiculata* (L.) Walp.) in West Africa. International Journal of Pest Management 42: 55-60.

Chapter 2

Introduction

Surveys of cowpea on markets and in village stores in West Africa indicate that 20-90% of the beans are attacked by bruchid pests (*B. atrolineatus* and *C. maculatus*) (Caswell, 1970, 1974; Seck *et al.*, 1991). Several parasitoids of these bruchids have been reported for West Africa, i.e. the egg parasitoid *U. lariophaga* and the solitary larval parasitoids *Eupelmus vuilleti, E. orientalis* and *Dinarmus basalis* (Lammers & van Huis, 1989; Monge *et al.*, 1991; Monge & Huignard, 1991; van Alebeek, 1991).

For assessing the potential of natural control of storage bruchid pests, a better knowledge of the occurrence of the bruchids, their parasitoids, and factors influencing their abundance is necessary. Therefore, a sampling survey of traditional cowpea stores was held in Niger. The results from this sample survey were compared with the outcome of interviews held with farmers in Northern Nigeria (Sagnia and Schütte, 1992) and in several Departments of Niger in 1992 (P. Dupont *et al.*, unpublished data). In those interviews, a detailed questionnaire was used to collect basic information on current cowpea storage systems and protection methods at the village level. This chapter reports on the results of the sampling survey, assesses the effect of indigenous parasitoids on bruchids in cowpea stores in Niger, and aims at identifying factors that may enhance the impact of those parasitoids.

Material and methods

Sampling methodology

In February 1992, a number of villages and a regional market in Niger were visited to sample cowpea in storage units. As cowpea is harvested near the end of October (van Huis *et al.*, 1990), storage duration at the date of sampling was between three and four months. This corresponds to four or five overlapping generations of bruchids, five or six generations of larval parasitoids, and 10 to 12 generations of egg parasitoids (van Huis *et al.*, 1990; van Alebeek, 1991).

Near Ouallam (about 100 km North of Niamey), I found stored cowpea in two villages and took eight samples. Cowpea was stored as grains for sowing seed. Near Say (about 50 km South of Niamey), stored cowpea was found in three villages and 11 samples were taken. Here, cowpea was stored in the pod for home consumption. The average annual rainfall is lower in the Ouallam region (354 mm) than in the Niamey (526 mm) and Say (525 mm) region (Sivakumar *et al.*, 1993).

At the market of Baleyara (100 km East of Niamey), eight samples were bought from women farmers offering small quantities of cowpea, supposedly from the local region.

Additionally, 25 samples were taken from 50 kg bags, belonging to several merchants. These merchants may travel as far as Nigeria to obtain their goods.

For each village sample, I reviewed the type of storage structure, storage methods, and protective measures. I interviewed local producers on the origin and use of the stored cowpea sampled, and the use of protection methods (Table 2.1). Samples were collected in plastic bags, labelled, and stored at -20° C. Subsamples of 100 beans were taken, and beans were examined individually under the binocular. All traces of bruchids and parasitoids, i.e. adults, emergence holes and bruchid egg shells, were noted. Samples obtained as pods (Say region) were first inspected (40 pods per sample). Subsequently, those pods were threshed, and a subsample of 100 beans was again examined, for comparison with other samples taken directly as beans.

Impact assessments

Adults of the beetles and the larval parasitoids mentioned in the introduction can easily be identified. Dead and living adults were present in most samples. Eggs of *C. maculatus* and *B. atrolineatus* can be distinguished, because the first are ovoid in shape whereas the latter are elongated (see Figure 1 in: Huignard *et al.*, 1985). Unparasitized bruchid eggs become filled with whitish powder when the larva penetrates the bean. Eggs which have been parasitized by *Uscana spp.* turn black. Bruchid species cannot be indentified on the basis of their emergence holes in beans (2-3 mm diameter). Emergence holes of larval parasitoids are smaller (0.5-1.5 mm diameter) than those of the bruchids (Figure 1 in: Huignard *et al.*, 1985; see also cover), but do not allow for identification to the species either.

The impact of bruchids and their parasitoids was estimated by four sample measurements:

+ the number of beans attacked by bruchids (i.e. beans showing any trace of bruchid eggs and/or any emergence holes);
+ the percentage of *C. maculatus* eggs parasitized by *Uscana spp.* (the very few eggs of *B. atrolineatus* on beans were neglected);
+ the percentage of emergence holes belonging to larval parasitoids, and
+ the number of *C. maculatus* and *B. atrolineatus* eggs on pods compared with those on beans in samples from the Say region.

Analyses

Because of highly skewed distributions, non-parametric statistics were applied. The Mann-Whitney *U*-test ($\alpha = 0.05$) was used to test for differences between samples from the Say and the Ouallam region, and those from farmers and merchants in Baleyara. Statistical Analysis Systems programming (SAS Institute Inc., 1988) was used for an ANOVA of the data from the village stores in the Say and Ouallam region. For this, data were first transformed to ranks and subsequently through a Van der Waerden transformation into normal distributions (proc rank normal=vw, SAS Institute Inc., 1988). Observations on the store characteristics (Table

2.1) were used as possible explanatory variables for the bruchid and parasitoid levels encountered. In all samples of beans, eggs of *B. atrolineatus* were very rare and were therefore ignored in most analyses.

Results

The number of samples of known origin is limited, because it was rather difficult to find stored cowpeas at the village level. The main characteristics of the samples taken near Say and Ouallam are summarized in Table 2.1.

Table 2.1 Occurrence of some major characteristics of cowpea stores in two regions in Niger.

Region		Say	Ouallam
Number of villages		3	2
Total number of samples[1]		11	8
Storage in:	+ straw granaries	5	0
	+ cotton bags	4	3
	+ plastic bags	2	2
	+ otherwise	1	2
Cowpea stored as:	+ pods	11	0
	+ threshed seeds	0	8
Quantity of cowpea stored:	+ less than 50 l	1	2
	+ 50 l or more	10	5
Cowpea varieties used:	+ Dan Louma	0	6
	+ a mixtures of varieties	3	2
	+ unknown	8	0
Protective measures taken:	+ plant materials	5	0
	+ pesticides	0	5
	+ none	6	0
Position of storage unit:	+ inside the village	2	8
	+ outside the village	9	0
Exposure of storage unit:	+ in sunshine	8	3
	+ in shaded conditions	3	4

1: Subtotals of characteristics may be less when information is lacking, or may be more because some samples show more characteristics at the same time.

A huge variability was found between nearby storage units within villages, between villages and between regions. This variability refers to storage methods and techniques, but also to the infestation and levels of parasitism encountered. Eggs of *C. maculatus* were present in 96% of all samples, traces of *Uscana spp.* egg parasitoids in 69%, and traces of larval parasitoids (*Eupelmus spp.* and *Dinarmus spp.*) in 92% of all samples. Of the predators known to occur, a single specimen of the predator *Xylocoris flavipes* (Reuter) was encountered.

Ranges of the number of beans attacked by bruchids, the percentage of *C. maculatus* eggs parasitized by *Uscana spp.*, and the percentage of emergence holes belonging to larval parasitoids demonstrate the heterogeneity in the samples (Figure 2.1). No differences in these parameters were found between Baleyara samples from small producers and those from large merchant's stocks (Mann-Whitney *U*-test, α = 0.05). The number of beans attacked by bruchids was lower and the percentage of *C. maculatus* eggs parasitized was higher in the Say than in Ouallam region. The percentage of emergence holes from larval parasitoids was also higher in the Say region, but not significantly.

An ANOVA was applied to the transformed data from the village stores in the Say and Ouallam region. Only three variables could be used in this analysis: village, storage type, and cowpea variety. All other variables (Table 2.1) were too correlated to allow for statistical analysis. Between village, storage type or cowpea variety, there were no significant differences in the percentage of *C. maculatus* eggs parasitized by *Uscana spp.*, and the percentage of emergence holes belonging to larval parasitoids. The variables village (df=4, F=4.05, p=0.08), storage type (df=6, F=4.03, p=0.07) and cowpea variety (df=2, F=4.98, p=0.06) all had a weakly significant effect on the number of beans attacked by bruchids.

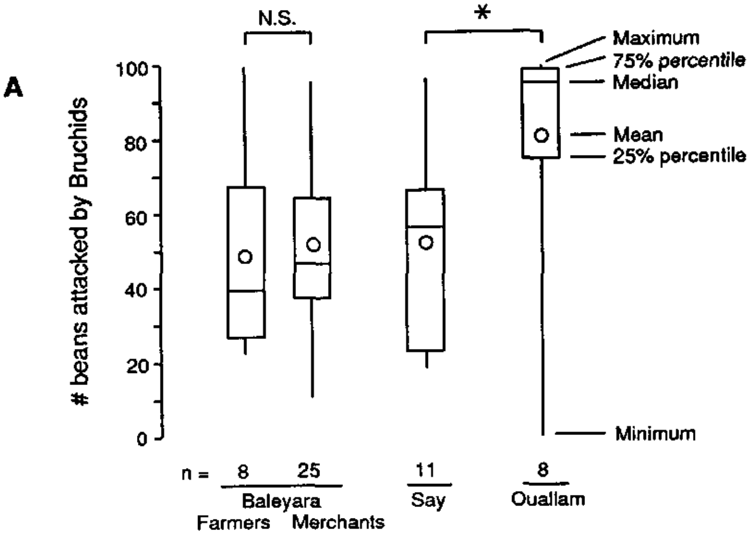

Figure 2.1.A Cowpea infestation rates of samples gathered on the market of Baleyara, and in villages near Say and Ouallam, Niger in 1992. **A.** Number of beans (per 100) attacked by bruchids. Symbols are explained in the top right. N.S. = difference not significant, * = $p < 0.05$ (Mann-Whitney U-test). (See also next page).

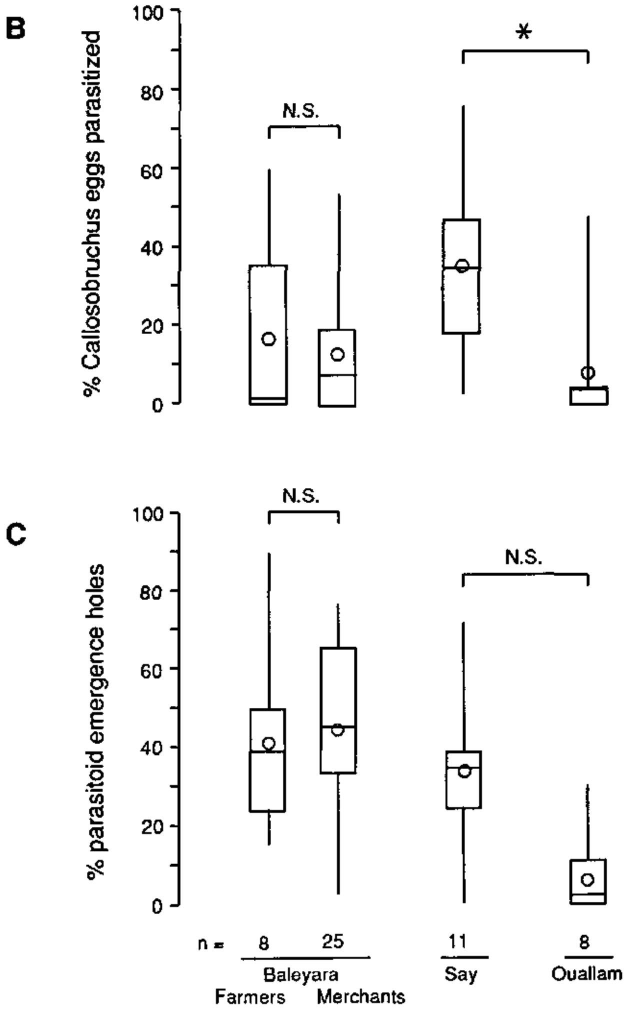

Figure 2.1 **Continued**. Rates of parasitism in cowpea samples gathered on the market of Baleyara, and in villages near Say and Ouallam, Niger in 1992. **B**. Percentage of *C. maculatus* eggs parasitized by *Uscana* spp. **C**. Percentage of emergence holes from larval parasitoid. Symbols are explained in Fig. 2.1.A (previous page).

Results from the pod counts in the Say region were compared with those from subsamples of 100 beans, after the pods were threshed (Figure 2.2). Remarkable differences were found in oviposition by *C. maculatus* and *B. atrolineatus* on pods and beans. Eggs of

C. maculatus were very rare on pods, but abundant on the threshed beans from the same samples. Eggs of *B. atrolineatus* were abundant on pods, but rare on beans. Differences between pods and beans are highly significant (Wilcoxon Signed-Ranks test, $p < 0.001$) for both bruchid species.

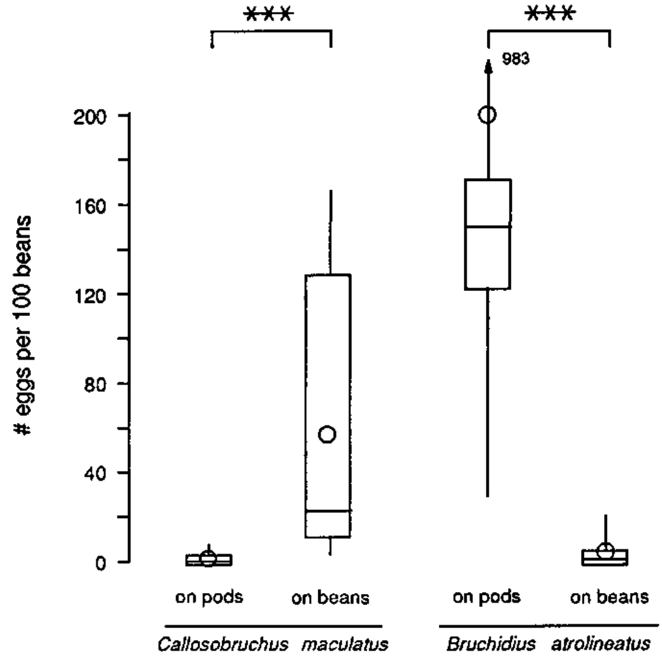

Figure 2.2. *C. maculatus* and *B. atrolineatus* eggs on pods and on beans in 11 samples from the Say region. For comparison, the pod results were transformed to means per 100 beans by dividing the counts on pods by the number of beans in those pods. Symbols as in Figure 2.1.A. *** = $p < 0.001$.

Discussion

The sometimes observed high levels of egg parasitism and larval parasitism (73% and 89% respectively, the maxima in Fig. 2.1B and 2.1C) were associated with the lowest levels of bruchid attack in the samples. Together with the high incidence of samples with egg and larval parasitism (69% and 92%), these findings clearly demonstrate the potential for successful natural suppression of bruchids by parasitoids from the genera *Uscana*, *Dinarmus* and *Eupelmus*. What remains is to identify the factors which may help to realize this potential, and to translate these factors into recommended storage practices.

The initial objective was to use the observations as presented in Table 2.1 to identify possible factors that determine the severity of bruchid attack and/or rates of parasitism. However, the number of samples is rather limited for this purpose, and, in addition, most sample characteristics are strongly correlated. Only the variability in village, storage type and cowpea variety could be tested, and all three significantly influenced the percentage of beans attacked by bruchids. Village, as a factor influencing bruchid attack, may be an aggregated variable. West Africa is populated by a number of ethnic groups, each with its own culture and tradition, and each with different agricultural systems and plant protection practices. In this survey, villages with different ethnic inhabitants probably comprise a number of correlated factors and variables that, directly or indirectly, influence the level of beans attacked by bruchids in the stores.

Bruchid attack is also influenced by the type of storage unit. Possibly, different storage units create different internal conditions and microclimates, affecting bruchid and parasitoid population dynamics. For example, differences in mean temperatures (caused e.g. by storage type and its exposure to sunshine) will have different effects on the intrinsic rate of increase of the egg parasitoid *U. lariophaga* and the cowpea weevil *C. maculatus* (van Huis *et al.*, 1994). Cowpea variety also affects bruchid attack levels. This confirms the large variability in bruchid resistance generally observed in different cowpea varieties (Kitch *et al.*, 1991). Identification of other factors and assessing their effects remains very difficult, and direct experimental approaches are necessary.

Three straw granaries in the village of Lountchia (Say region) are comparable in all variables described in Table 2.1. However, huge differences in bruchid attack and in rates of parasitism were found between the three granaries (Table 2.2).

Table 2.2 A comparison of samples from three nearby granaries with identical characteristics. Sample size is 100 seeds per storage unit.

storage unit[1]	% beans attacked by bruchids	% bruchid eggs parasitized by *Uscana spp.*	% emergence holes from larval parasitoids
1	86	2	71
2	19	33	0
3	24	73	16

1: Description of the storage units: Niger, Say region, village of Lountchia, 3 straw granaries filled with pods, cowpea variety unspecified, treated with local plant material (*Heliotropium indicum*), located in full sunshine outside the village. Unit 1 and 2 are 10 m apart, unit 3 is approx. 1 km away.

This suggests that storage units can be regarded as ecological islands, with relatively little migration between them. Lammers and van Huis (1989) for several months monitored nine experimental granaries standing together. Large differences in population dynamics and egg parasitism were found between those granaries (Lammers, personal communication). Matokot *et al.* (1987) also reported large differences in bruchid population dynamics in stored groundnuts in Congo between five granaries within the same village. If little dispersal between storage units occurs, then initial conditions within each storage unit will have a strong impact upon the subsequent development of pest and parasitoid populations.

Large differences were found between numbers of *C. maculatus* and *B. atrolineatus* eggs on pods and on beans (Figure 2.2) in samples from the Say region. The first infestation of young, mature pods occurs in the field. In Niger, *B. atrolineatus* usually is abundant in the field with 80%-90% of the pods infested and average densities of approx. 10 eggs per pod; *Callosobruchus maculatus* eggs usually are scarce, with only 10%-15% of the pods carrying 1-2 eggs (Huignard *et al.*, 1985; Germain *et al.*, 1987). After harvest and storage of those pods, *B. atrolineatus* has only one more generation, that will oviposit on the (predominantly intact) pods. Subsequent offspring of *B. atrolineatus* are in reproductive diapause and leave the granary (Germain *et al.*, 1987). This explains why most *B. atrolineatus* eggs are on the pod walls. However, *C. maculatus* continues to reproduce in the store over several generations, gradually building up its population density. In the course of time, pods will show more emergence holes, and due to handling by the farmer (e.g. taking out quantities for consumption or sale) more pods become cracked and split. Thus, beans in the interior of the pods become available for ovipositing *C. maculatus* females. The pod wall acts as an important mortality factor for the first instar bruchid larva drilling in from the egg (e.g. Caswell, 1973; Kitch *et al.*, 1991). If given a choice between pods and seeds, *C. maculatus* females predominantly oviposit on seeds (84%) (Alzouma, 1981). Thus, the increased availability of oviposition sites on beans when *C. maculatus* population densities are building up explains why, in the samples from the Say region, most *C. maculatus* eggs were found on the beans. In sampling cowpea, care should be taken to account for this difference in phenology and behaviour, to prevent gross errors in estimating egg densities.

These sample results stem from one limited period in time, reflecting the accumulated traces of bruchid and parasitoid attack over a 3 to 4 month period. Calculations of parasitism based on these results are probably subject to several biases (van Driesche, 1983; van Driesche *et al.*, 1991). For example, my calculations assume that all traces are preserved over time. The loss of egg shells on beans, and especially the question whether egg shells of parasitized and unparasitized eggs dislodge at the same rate, remains to be investigated.

For many farmers, stored cowpea is analogous to a savings account. At the time of harvest, the price of cowpea usually is low. However, after several months of storage, at the approach of the next rainy season, this price may have doubled. Farmers sell stored cowpeas whenever they need cash, but they may want to wait until the next sowing season, when prices are high (Sagnia & Schütte, 1991).

However, when selecting sampling sites for this survey in the main cowpea production areas around Niamey, cowpea storage appeared to occur on a very limited scale. Several villages did not store any cowpea (whereas sorghum and millet were abundantly stored). Interviews demonstrated that traditional on-farm storage is gradually disappearing. The reason for this is probably that losses due to bruchids make it very difficult to preserve cowpea over periods of several months. In Nigeria, 68% of the farmers had used granaries in the past, but only 23% did so in 1992. There, traditional granaries are being replaced by modern concrete or mud-built stores, covered by metal roofs, in which pesticide treated cowpea is stored in plastic bags (Sagnia & Schütte, 1992). In Niger, 90% of the farmers interviewed had stored cowpea in granaries in the past, but only 33% did so in 1990 (P. Dupont, personal communication). Those farmers probably sell their cowpea directly upon harvest to merchants or cooperatives with better storage and protection facilities. In doing so, farmers refrain from potential gains as described above. Apparently, those potential gains do not compensate for the losses expected from bruchid attack during storage.

Farmers continue to store limited quantities (less than five 50 l bags) for home consumption or as sowing seed. Cowpea destined for home consumption (e.g. in the Say region) is not protected by pesticides but by traditional methods (Table 2.1). This is also the practice in Nigeria (Sagnia & Schütte, 1992) and in Niger (P. Dupont, personal communication). It is not clear whether people refrain from pesticide applications on cowpea used for home-consumptions for health or for economic reasons.

In cowpea destined for the market, pesticide use is widespread and tends to replace traditional methods (Sagnia & Schütte, 1991). However, traditional methods still prevail in storage for home consumption. About 50% of the farmers interviewed mentioned the use of plant material (over 15 species mentioned) as protectants. The use of ashes or sand is mentioned by about 25% of the farmers. The effect of many traditional methods still requires further verification (Rees *et al.*, 1993). Side effects of traditional methods on naturally occurring parasitoids should be an integral part of the verification of traditional methods for stored cowpea protection.

According to the interviews in Niger, 71% of the farmers use pesticides on their cowpea stock produced for sale at the market (P. Dupont, personal communication). The effectiveness of these treatments was not investigated. In this sampling survey, the highest levels of bruchid attack appeared in the Ouallam region. These levels are associated with the lowest levels of both egg and larval parasitism (Fig. 2.1). Near Ouallam, 5 out of 8 farmers explicitly reported the use of Deltamethrine (K-Othrine, powder) on their stored cowpea (Table 2.1). Most of the bruchid's life cycle takes place inside the seeds. In contrast, egg parasitoids spend their entire life cycle and larval parasitoids about half of their life cycle on the seed surface, thus being exposed to toxic substances more than their hosts. In general, herbivores are found to be resistant to pesticides much more frequently than parasitoids. Possible explanations for this phenomenon, such as the pre-adaptation and food limitation hypothesis, are discussed by Tabashnik (1990) and Hoy (1990). Thus, the use of pesticides may have caused disruption to the natural suppression of bruchids. Eliminating the parasitoids without sufficient control of the bruchids might explain the results found in Ouallam. The same phenomenon was reported by Caswell (1973, 1980), who found 50% seed damage in unprotected cowpea stock, versus 90% damage in stock treated with BHC. He attributed the additional damage to the fact that the pesticide killed the hymenopteran parasitoids (*Dinarmus spp.*) which otherwise would have had an important impact on bruchid population growth (Caswell, 1973).

Natural control of stored cowpea bruchids clearly has potential. In this survey, cowpea variety, storage structure type and the use of pesticides were identified as variables that can be manipulated to realize this potential to a greater extent. Several other factors, e.g. storage in pods versus seeds, the quantity of cowpea stored, the use of protective plant materials, and the exposure of storage units to sun or shade, remain to be studied in an experimental approach.

Acknowledgements

I thank the staff of the "Département de Formation en Protection des Végétaux" (DFPV), Niamey, Niger, for their hospitality and provision of facilities in the survey. Special thanks go to Sankung Sagnia (DFPV) and Pascal Dupont (University of Niamey) for their friendship, support, and sharing of data, and to Sama Gagare (DFPV), as skilful guide and interpreter during field trips. The assistance of M.A.J. van Montfort (WAU Department of Mathematics) in the analysis is gratefully acknowledged. Valuable comments on an earlier draft were given by J.C. van Lenteren and A. van Huis.

References

Alebeek, F.A.N. van, 1991. Interspecific host discrimination by two solitary ectoparasitoids of immature stages of Bruchidae. Meded. Fac. Landbouww. Rijksuniv. Gent 56/3b: 1011-1020.

Alzouma, I., 1981. Observations on the ecology of *Bruchidius atrolineatus* Pic. and *Callosobruchus maculatus* F (Coleoptera, Bruchidae) in Niger. Series Entomol. 19: 205-213.

Caswell, G.H., 1970. The storage of cowpeas in the northern states of Nigeria. Proc. Agric. Soc. Nigeria 5: 4-6.

Caswell, G.H., 1973. The storage of cowpea. Samaru Agric. Newsl. 15(2): 73-75.

Caswell, G.H., 1974. The development and extension of nonchemical control techniques for stored cowpeas in Nigeria. Proc. 1st Int. Working Conf. Stored Product Entomol., Savannah, Georgia, pp. 63-67.

Caswell, G.H., 1980. A review of the work done in the Entomology Section of the Institute for Agricultural Research on the pests of stored grain and their control. Samura Miscellaneous Paper N99, 12 pp.

Driesche, R.G. van, 1983. Meaning of "percent parasitism" in studies of insect parasitoids. Environ. Entomol. 12: 1611-1622.

Driesche, R.G. van; Bellows, T.S.; Elkinton, J.S.; Gould, J.R.; Ferro, D.N., 1991. The meaning of percentage parasitism revisited: solutions to the problem of accurately estimating total losses from parasitism. Environ. Entomol. 20: 1-7.

Germain, J.F.; Monge, J.P.; Huignard, J., 1987. Development of two bruchid populations (*Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (Fab.) infesting stored cowpea (*Vigna unguiculata* Walp) pods in Niger. J. Stored Prod. Res. 23(3): 157-162.

Hoy, M.A., 1990. Pesticide resistance in arthropod natural enemies: variability and selection responses. In: R.T. Roush & B.E. Tabashnik (eds), Pesticide Resistance in Arthropods. Chapman and Hall, New York, pp. 203-236.

Huis, A. van; Kaashoek, N.K.; Lammers, P.M., 1990. *Uscana lariophaga* (Hym.: Trichogrammatidae), egg parasitoid of two bruchid species of cowpea in West Africa. Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam, 1: 101-106.

Huis, A. van; Arendse, P.W.; Schilthuizen, M.; Wiegers, P.P.; Heering, H.; Hulshof, M.; Kaashoek, N.K., 1994. *Uscana lariophaga*, egg parasitoid of bruchid beetle storage pests of cowpea in West Africa: the effect of temperature and humidity. Entomol. exp. appl. 70: 41-53.

Huignard, J.; Leroi, B.; Alzouma, I.; Germain, J.F., 1985. Oviposition and development of *Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in *Vigna unguiculata* (Walp) cultures in Niger. Insect Sci. Applic., 6: 691-699.

Kitch, L.W.; Shade, R.E.; Murdock, L.L., 1991. Resistance to the cowpea weevil (*Callosobruchus maculatus*) larva in pods of cowpea (*Vigna unguiculata*). Entomol. exp. appl. 60: 183-192.

Lammers, P.M.; Huis, A. van, 1989. *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae), egg parasitoid of the stored insect pests *Callosobruchus maculatus* (F.) and *Bruchidius atrolineatus* Pic. (Col.: Bruchidae): population studies in the field and in storage in Niger. Proc. Int. Conf. Int. Pest Manage. 3: 1013-1022.

Matokot, L.; Mapangou-Divassa, S.; Delobel, A., 1987. Evolution des populations de *Caryedon serratus* (Ol.) (Coleoptera: Bruchidae) dans les stocks d'arachide au Congo. L' Agronomie Tropicale, 42: 69-74.

Monge, J.P.; Huignard, J., 1991. Population fluctuations of two bruchid species *Callosobruchus maculatus* (F.) and *Bruchidius atrolineatus* (Pic) (Coleoptera, Bruchidae) and their parasitoids *Dinarmus basalis* (Rondani) and *Eupelmus vuilleti* (Crawford) (Hymenoptera, Pteromalidae, Eupelmidae) in a storage situation in Niger. J. African Zool. 105: 187-196.

Monge, J.P.; Ouedraogo, A.P.; Huignard, J., 1991. Development of two Bruchid species *Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (F.) and their larval parasitoids during storage of cowpea seeds *Vigna unguiculata* (Walp) in West Africa. In: F. Fleurat-Lessard & P. Ducom (eds), Proc. 5th Int. Working Conf. Stored-Product Prot., Bordeaux, Sept. 9-14, 1990, Vol. 1, pp. 151-160.

Rees, D.P.; Dales, M.J.; Golob, P., 1993. Alternative methods for the control of stored-product insect pests: A bibliographic database. United Kingdom, Natural Resources Institute, Chatham.

Sagnia, S.B.; Schütte, C., 1991. Cowpea storage system at village level in the State of Kano, Nigeria. Département de Formation en Protection des Végétaux (DFPV), CILSS, Niamey, 26 p.

Sagnia, S.B.& C. Schütte, 1992. Le système de stockage du niébé en milieu villageois dans l'état de Kano, Nigeria. Sahel PV Info 46: 6-15.

SAS Institute Inc., 1988. SAS / STAT^{TM} User's Guide, release 6.03 SAS Institute Inc., Cary, 1028 pp.

Seck, D.; Sidibe, B.; Haubruge, E.; Hemptinne, J.-L.; Gaspar, C., 1991. La protection chimique des stocks de niébé et de mais contre les insectes au Senegal. Meded. Fac. Landbouww. Rijksuniv. Gent 56/3b: 1225-1233.

Sivakumar, M.V.K.; Maidoukia, A.; Stern, R.D., 1993. Agroclimatology of West Africa: Niger (2nd edition). International Crops Research Institute for the Semi-Arid Tropics, Niamey, ICRISAT Inf. Bull. 5, 108 p.

Tabashnik, B.E., 1990. Modeling and evaluation of resistance management tactics. In: R.T. Roush & B.E. Tabashnik (eds), Pesticide Resistance in Arthropods. Chapman and Hall, New York, pp. 153-182.

Uscana lariophaga as a candidate for the biological control of bruchid pests in stored cowpea in West Africa

3

Abstract

In a conservation strategy of biological control, one has to choose which characteristics of a natural enemy should be studied, and which environmental factors should be manipulated, to improve the impact of the natural enemy on the pest population. In this chapter, the main characteristics of a parasitoid for a conservation strategy are reviewed. At the same time, the work done so far on the egg parasitoid *Uscana lariophaga* is summarised. Searching efficiency was the main characteristic that required further investigation. This searching efficiency involves several characteristics. Parasitoid foraging behaviour can be regarded as a hierarchical series of subsequent steps of decreasing scale. In each step, the parasitoid 'zooms in' her searching behaviour and concentrates on a smaller, specific part of the habitat (see Figure 3.1): host habitat location, location of host clusters within the habitat, and once the parasitoid arrives within a host cluster, the location of hosts within that cluster, and the response to different densities (i.e. functional response) and different distributions of the host. The presentation of research on searching efficiency of *U. lariophaga* in this thesis follows this hierarchical approach, as explained in this chapter.

Chapter 3

In the previous chapter, the considerable losses caused by bruchid pest populations in stored cowpea in West Africa have been mentioned. Strategies to control bruchids in storage should be compatible with the agro-ecological and socio-economic characteristics of the Sahelian farming systems (see § 1.3). Given the occurrence of several indigenous parasitoids in cowpea stores, and the successful natural control that sometimes occurs (see Chapter 2), a conservation strategy of biological control is an option to be explored.

3.1 Evaluation criteria for a conservation strategy of biological control in stored cowpea

In a conservation strategy of biological control, environmental manipulations aim at increasing the impact of native natural enemies on indigenous pest populations. Conservation studies should start with detailed life-table studies to assess the impact of different predator and parasitoid species in the agro-ecological system under study. After assessing key mortality factors and key natural enemies, one can consider the kind of manipulations that enhance pest suppression. Environmental factors which can be manipulated in conservation strategies have been reviewed e.g. by Rabb *et al.* (1976), Van Lenteren (1987), Dent (1991) and Dutcher (1993). These include:

1. manipulation of the climate
2. changing the habitat structure, including a wide range of cultivation practices
3. manipulation of food and other resources of natural enemies
4. manipulation of natural enemies

Examples for bruchid control with indigenous parasitoids will be discussed under the different evaluation criteria mentioned below.

Conservation strategies usually aim at the total complex of indigenous natural enemies. To select the type and extend of environmental (abiotic and biotic) factors to be manipulated, we have to assess their impact on the natural enemy and bruchid pest populations. Thus, one needs criteria to choose which factors should be studied.

Manipulation of some factors may have the same (positive) effect on each natural enemy species present in the system. However, when certain factors affect each beneficial species differently, it causes conflicting objectives for different natural enemies. In such a case, it may be necessary to select key natural enemy species on which the conservation strategy should focus.

Therefore, criteria to evaluate the effectiveness of natural enemies can be used as guidelines for studies of factors and their impact on different beneficials. Lists of criteria have been reviewed extensively for classical biological control (e.g. Huffaker *et al.*, 1977; Van Lenteren, 1986), for inundative biological control (Hirose, 1986; Smith & Hubbes, 1987; Pak, 1988), and for seasonal inoculative biological control (Van Lenteren & Woets, 1988; Minkenberg, 1990).

Based on criteria proposed by Van Lenteren (1986) and Pak (1988), and using my own experiences with the stored cowpea environment, I propose a set of eight criteria (Table 3.1). These criteria need to be considered for the study of natural enemies of stored cowpea bruchids in a conservation strategy. Compared to Pak (1988), I propose the following changes:

+ criterion 3 (Host plant adaptation) is renamed as *Compatibility with storage environment*;
+ criterion 4 and 5 are combined into one new criterion: *Host selection and suitability*;
+ criterion 7 (Host finding capacity) is renamed as *Searching efficiency*;
+ criterion 9 (Culture method) is dropped, since in a conservation strategy, natural enemies are not mass reared in the lab;
+ a new criterion 8, *Compatibility with other control methods*, is added.

Also based on Van Lenteren (1986) and Pak (1988), with additions from Minkenberg (1990), the type of factors to be measured for each criterion are listed in Table 3.1.

Not all of the natural enemy's criteria listed in Table 3.1 can be manipulated to the same extend. For example, criterion 1 (Environmental risks) and 4 (Host selection and suitability) are mainly descriptive characteristics of a beneficial species, and cannot easily be changed. On the other hand, manipulating the climate, the provision of food or changing the habitat structure in a cowpea granary will almost certainly influence the natural enemy's mortality (criterion 2), dispersal (criterion 3), its intrinsic rate of increase (criterion 6) and several aspects of its searching efficiency (criterion 7). Without prior knowledge of the natural enemy's main characteristics, the effects of environmental manipulations are difficult to predict. Therefore, evaluation criteria can help to identify which environmental manipulations will most effectively suppress pest populations. The criteria listed in Table 3.1 will be discussed below, including a review of the work done so far on *U. lariophaga* and the larval parasitoids occurring in the stored cowpea system.

1. Environmental risks

Since the parasitoids under consideration all are indigenous to the region, any environmental risks or negative traits, if any, have to be taken for granted. If conservation would increase the beneficials' impact on non-target organisms, conflicts of interest may arise. However, one can also use negative traits in selecting which natural enemy or enemies should become the focus of manipulations. For example, the two larval parasitoids present in stored cowpea in Niger, *Dinarmus basalis* (Rond) (Hymenoptera; Pteromalidae) and *Eupelmus vuilleti* (Craw) (Hymenoptera; Eupelmidae), both seem promising candidates for biological control (Van Alebeek, 1991). They have a very similar biology and life history and reproduce well on the bruchid pests *C. maculatus* and *B. atrolineatus*. However, some indications have been found that *E. vuilleti*, in contrast to *D. basalis*, cannot fully exploit the host complement in lab experiments (Leveque *et al.*, 1993) and in storage conditions (P. Dupont & S. Sagnia,

Table 3.1 Criteria for the evaluation of target natural enemies of stored cowpea bruchids in a conservation strategy, the type of measurements to be made, the extend to which these criteria have been studied for the egg parasitoid *Uscana lariophaga* Steffan so far, and the factors studied and reported in this Thesis.

Criterion[1]	Type of measurements[1]	Studied so far	In this Thesis
1. Environmental risks	- Hyperparasitism	--	--
	- Effects on non-target hosts	--	--
2. Tolerance of climatic extremes	- Developmental time and mortality at different temperatures and relative humidities	Van Huis *et al.*, 1994a	--
	- Lower and upper thresholds for development and reproduction	Van Huis *et al.*, 1994a	--
3. Compatibility with storage environment	- Colonization of and migration from storage structures	--	Chapter 2
	- Dispersal within storage structures and geotaxis	--	Chapter 5 and 8
	- Effects of light conditions	Van Huis & Appiah, 1995	Chapter 8
4. Host selection and suitability	- Host age and host species preference	Van Huis *et al.*, 1991a	--
	- Development, survival, sex ratio and reproduction on different hosts	Van Huis *et al.*, 1991a	--
5. Seasonal synchronization	- Developmental time and longevity	Van Huis *et al.*, 1991a Van Huis *et al.*, 1994a	--
6. Reproductive capacity	- Intrinsic rate of increase (r_m)	Van Huis *et al.*, 1994a	--
	- The effect of food availability	Van Huis *et al.*, 1991b	--
	- The effect of temperature (see criterion 2)	Van Huis *et al.*, 1994a	--
7. Searching efficiency	- Host habitat location	Van Huis *et al.*, 1994b	Chapter 4
	- Host (community) location within the habitat	Van Huis *et al.*, 1994b	Chapter 5
	- Dispersal, travel speed (see criterion 3)	--	Chapter 5 and 8
	- Functional response	--	Chapter 6, 7 and 8
	- Aggregation and walking behaviour	--	Chapter 6, 7 and 8
	- Mutual interference	--	--
8. Compatibility with other control methods	- effects on development, survival and reproduction	--	Chapter 2

1: Adapted from Van Lenteren (1986), Pak (1988) and Minkenberg (1990).

Niamey, unpublished results). Even worse, when occurring simultaneously, *E. vuilleti* acts as a (facultative) hyperparasitoid on *D. basalis*, thereby upsetting the otherwise satisfactory control of bruchid populations by the latter (Van Alebeek *et al.*, 1993; Leveque *et al.*, 1993). Therefore, a conservation strategy should focus on *D. basalis*. However, selective exclusion of *E. vuilleti* from the storage environment may be difficult.

2. Tolerance to climatic extremes

In the Sahelian climatic conditions, maximum temperatures may drop to as low as 10-15° C in December - February or exceed 45° C in April - May (Sivakumar *et al.*, 1993). The lower and upper thresholds for development in *U. lariophaga* are 15 and 42.5° C respectively. Mortality during development was below 20% at temperatures from 17.5 to 35° C (Van Huis *et al.*, 1994a). A conservation strategy may aim at preventing the extreme maximum temperatures in granaries (e.g. through shading or increased aeration of structures), or aim at increasing temperatures inside the granary in the cold period (e.g. by increased exposure to sunshine during daytime or some form of insulation during cool nights). Activity at different temperatures may be a criterion to test for parasitoids. Cortesero *et al.* (1991) studied this activity for the larval parasitoid *E. vuilleti*.

3. Compatibility with storage environment

Host acceptance on different plants was named host plant adaptation by Pak (1988). I adjusted this criterion to compatibility with the storage environment. Natural enemies have evolved under certain field conditions, and are adapted to disperse in this environment and to forage for suitable hosts. In the (typically man-made) storage environment, a number of factors differ markedly from field conditions. In stores, host densities are much higher than in the field, and distributions may be more aggregated. The architecture of stored dried cowpea (either as pods or as threshed beans) is very much different from green cowpea plants in the field. In the field, wind may carry olfactory information guiding parasitoids to their hosts. In stores, cyclic convection air flows may cancel odour gradients and confuse orientation.

Habitat differences, such as light and temperature conditions, may also exist between closed clay structures and more open, straw granaries (Lammers & Van Huis, 1989). Stores are dark inside, which may cause problems for the activity of parasitoids adopted to field conditions. Van Huis & Appiah (1995) studied the effect of photoperiod on parasitism and the rhythm of eclosion in *U. lariophaga*. Cracks and crevices in the walls of granaries allow local light gradients within stores. This may attract positive phototactic insects to leave the store. Emigration of the two bruchid species from storage structures is a main characteristic of their seasonal life history (Germain *et al.*, 1987; Monge *et al.*, 1991). Emigration of parasitoids has not been quantified, but could influence the level of control inside stores. In Chapter 2, I concluded that storage structures seem to function as ecological islands, with relatively little exchange between them. This suggests that, even though emigration from stores may occur, immigration into neighbouring stores is neglectable.

Lammers & Van Huis (1989) found that *U. lariophaga* attains significant rates of parasitism (up to 60%) in storage structures. Monge & Huignard (1991) report similar rates for the larval parasitoids *E. vuilleti* and *D. basalis*. This demonstrates that these parasitoids are able to disperse within the storage environment, and to find and parasitize their hosts under the given conditions.

4. Host selection and suitability

Since two bruchid species, *B. atrolineatus* and *C. maculatus*, occur simultaneously in stored cowpea in Niger, target natural enemies should preferably accept both as a host and be able to reproduce on them successfully. At the onset of the storage period, *B. atrolineatus* is the dominant species. But after one or two generations, *B. atrolineatus* goes into reproductive diapause and leaves the store (Germain *et al.*, 1987; Monge *et al.*, 1991). *Callosobruchus maculatus* starts at low densities, but continues to reproduce and gradually builds up its densities. Successful parasitoids should easily switch from one host to the other. Van Huis *et al.* (1991a) demonstrated that *U. lariophaga* accepts both bruchid species readily. There are some differences between the host species in suitability and in preference, but in a no-choice situation *U. lariophaga* readily switches from one to the other bruchid species (Van Huis *et al.*, 1991a).

5. Seasonal synchronisation

For a good synchronisation, a parasitoid's developmental time should be comparable to that of its host. If the parasitoid develops faster than its host, the parasitoid's longevity should allow the parasitoid to "wait" until a new cohort of suitable host stages develops ("waiting time", *sensu* Minkenberg, 1990). This is less critical if the host occurs in overlapping generations.

For bruchids in cowpea stores, overlapping host generations occur later in the storage season. However, at the time of harvest, bruchid and parasitoid population densities are low and generations may not overlap. Suitable host stages for parasitization may then be virtually absent at certain moments.

Synchronisation may cause problems for *U. lariophaga*, which life cycle takes only about 9 days, with a longevity of 2-3 days, compared to the developmental time of *C. maculatus* of about 20 days (Van Huis *et al.*, 1994a). Increasing the longevity of *U. lariophaga* through honey as an additional food supply (Van Huis *et al.*, 1991b) could be a conservation strategy.

The two larval parasitoids, *D. basalis* and *E. vuilleti*, have a developmental time of 12 to 18 days, and a longevity of approx. 20 - 30 days, which allows them to bridge gaps until the next bruchid generation (Van Alebeek, 1991).

6. Reproductive capacity

A good natural enemy is supposed to have an intrinsic rate of increase (r_m) of at least the same value as that of its host, and preferably higher (Van Lenteren, 1986). However, this only applies to host and parasitoid populations with a stable age distribution (Minkenberg, 1990). Van Huis *et al.* (1994a) calculated the r_m for *U. lariophaga* between 17.5 and 40° C, which is always higher than that of its host *C. maculatus*. Relative humidity did not influence this result.

The provision of food, i.e. honey to *U. lariophaga* females increased their fecundity three-fold and their longevity five-fold (Van Huis *et al.*, 1991b), thereby increasing the r_m with 15%.

7. Searching efficiency

The definition of this criterion and the problems of how to assess density dependent parasitism and stability in field populations has been the centre of much debate (Minkenberg, 1990). Searching efficiency should comprise a diversity of measurements such as host (habitat) location, within patch behaviour including functional response, aggregation, walking speed and mutual interference (Table 2.1). Searching efficiency is considered a key criterion in determining the success of natural enemies in classical and inundative biological control. Recent studies to evaluate the characteristics of successful natural enemies suggest that the parasitoids' searching behaviour may even be more important than the parasitoids' intrinsic rate of increase (Van Roermund, 1995; van Steenis, 1995). This is largely due to the fact that the r_m is commonly estimated under laboratory conditions with limitless host supply at unrealistic high densities, thereby practically eliminating spatial aspects of host location behaviour.

In a conservation strategy, manipulation of the storage climate or the habitat structure, and provision of food will almost certainly influence the host location behaviour, functional response and walking speed of natural enemies. Therefore, this criterion also is of major interest in a conservation strategy.

Concerning host habitat and host location of *U. lariophaga*, olfaction studies indicated that females are attracted and arrested by odours of clean cowpea seeds, fresh *C. maculatus* eggs, and sex pheromones from virgin *C. maculatus* females (Van Huis *et al.*, 1994b). Host (habitat) location by *D. basalis* is guided mainly by vision and short range olfactory cues (Verma, 1990). Host location by *E. vuilleti* is influenced by odours of cowpea seeds, *B. atrolineatus* larvae, pupae, faeces and female sex-pheromones (Cortesero *et a*l., 1993). Most of the other measurements (e.g. within patch behaviour, functional response, mutual interference, aggregation and travel speed, Table 3.1) have not been investigated for *U. lariophaga* yet, nor for the two larval parasitoids mentioned.

8. Compatibility with other control methods

The integration of other (traditional) control methods with the conservation of parasitoids in stored cowpeas requires more attention. A number of traditional protective measures has been described (Sagnia & Schütte, 1992; Rees *et al.*, 1993): the use of sand, ashes, plant materials with repellent and/or insecticide properties, and vegetable oils (see § 1.3). Most of the bruchids' life cycle takes place inside the seeds, as the larvae develop. Thus, they are largely protected against most of the protective methods mentioned above. In contrast, egg parasitoids spend their entire life cycle and larval parasitoids about half of their life cycle on the seed surface, being exposed to protective measures more than their hosts. The use of oils may deter *U. lariophaga* from accepting host eggs, and may kill developing parasitoids inside the host egg (S. Sagnia, personal communication). The effect of most of the protective measures still awaits verification. In Chapter 2, I indicated that the use of chemical pesticides resulted in lower parasitization rates and higher bruchid attack rates, probably due to the death of parasitoids.

The naturally occurring combinations of beneficial insect species in cowpea stores may not always result in optimal pest suppression. Under criterion 1 (environmental risks) I already mentioned the combined effect of the two larval parasitoids *E. vuilleti* and *D. basalis*, which resulted in poorer bruchid suppression compared to *D. basalis* alone.

Evaluating Uscana lariophaga

As described before, a number of criteria have already been (partly) studied for *U. lariophaga* (Table 3.1). Some criteria, such as 1. *Environmental risks*, 3. *Adaptation to storage environment*, and 5. *Seasonal synchronisation* cannot easily be evaluated in the laboratory. Those evaluation criteria require measurements on non-target host parasitization, colonization of cowpea fields and storage structures, and population synchronisation during the storage season. Such measurements should be performed under field conditions in West Africa.

However, searching efficiency is a key evaluation criterion which still requires further study. Searching efficiency comprises a number of possible measurements (Table 3.1) open to investigations in the laboratory. These measurements have become the focus of the research described in this thesis.

3.2 *Uscana* foraging behaviour: structure of this thesis

The research described in this thesis includes an evaluation of some aspects of criterion 3: *compatibility with the storage environment*, but mainly concentrates on criterion 7: the *searching efficiency* of *U. lariophaga* (Table 3.1). Parasitoids that emerge as adults from the pupae, generally do not find hosts abundantly available on the same spot and at the same time. In order to be able to reproduce, they have to go and search for hosts in a huge and variable environment. This searching behaviour is not at random. Foraging parasitoid are guided towards their targets by a hierarchy of (internal and environmental) stimuli, often in the form of visual, acoustic or olfactory information (Van Alphen & Vet, 1986).

For the convenience of thinking and research, the searching behaviour of parasitoids has traditionally been divided into a series of hierarchical steps, e.g. host habitat preference, potential host community location, host location, host examination, ovipositor probing, etc. This hierarchical approach and the steps within this approach have been extensively reviewed (Arthur, 1981; Van Lenteren, 1981; Vinson, 1981, 1984, 1985; Weseloh, 1981; Wellings, 1991; Godfray, 1994; Vet *et al.*, 1995; and references therein). In each step, tactile, visual and chemical stimuli may play an important role in locating the parasitoid's target.

In reality, parasitoid behaviour is more continuous and variable than the hierarchical scheme suggest, and certain steps may be absent or added. Furthermore, in recent years, it has become clear that learning often plays a paramount role in parasitoids' foraging behaviour, and that parasitoids' experiences may change the role and importance of different stimuli considerably (Van Alphen & Vet, 1986; Vet, 1988; Lewis *et al.*, 1990; Vet *et al.*, 1990, 1991, 1995; Vet & Dicke, 1992; Turlings *et al.*, 1993).

However, theory predicts that in short-lived egg parasitoids, learning is of little importance, and that fixed responses to a limited set of stimuli determine the foraging behaviour of egg parasitoids (Vet *et al.*, 1995). For *U. lariophaga*, the hierarchical approach as used by Vinson (1984; 1985) is useful, and will be used as a framework for the presentation of research on the parasitoid's foraging behaviour in this thesis. This has been illustrated in Figure 3.1.

The first step in the foraging behaviour of a parasitoid usually is host habitat location (Figure 3.1.A). Parasitoids are assumed to use more general olfactory cues related to the host's food plant or habitat, in order to locate potential habitats in which the host might be found. Some Trichogrammatidae have been shown to use sex pheromones of their host as long distance cues (Lewis *et al.*, 1982; Noldus, 1989; Nordlund, 1994). In **Chapter 4**, the olfactory cues in host habitat location of *U. lariophaga* are being analyzed.

Within the habitat, olfactory cues related to the host itself (kairomones) or to the host's food (e.g. synomones) may guide the parasitoid towards host communities or host clusters (Figure 3.1.A, step 2). Therefore, in **Chapter 4**, I looked at the role of volatile host odours in host (community) location by *U. lariophaga*.

The finding of host clusters is further investigated and described in **Chapter 5**. Within a storage structure, apart from volatile host stimuli, other (a)biotic factors (e.g. gravity, light) may play a role in *U. lariophaga*'s foraging behaviour and dispersal within the cowpea seed mass. The investigation of these factors partly relates to criterion 3 in the evaluation of natural enemies for conservation (Table 3.1): *compatibility with storage environment.*

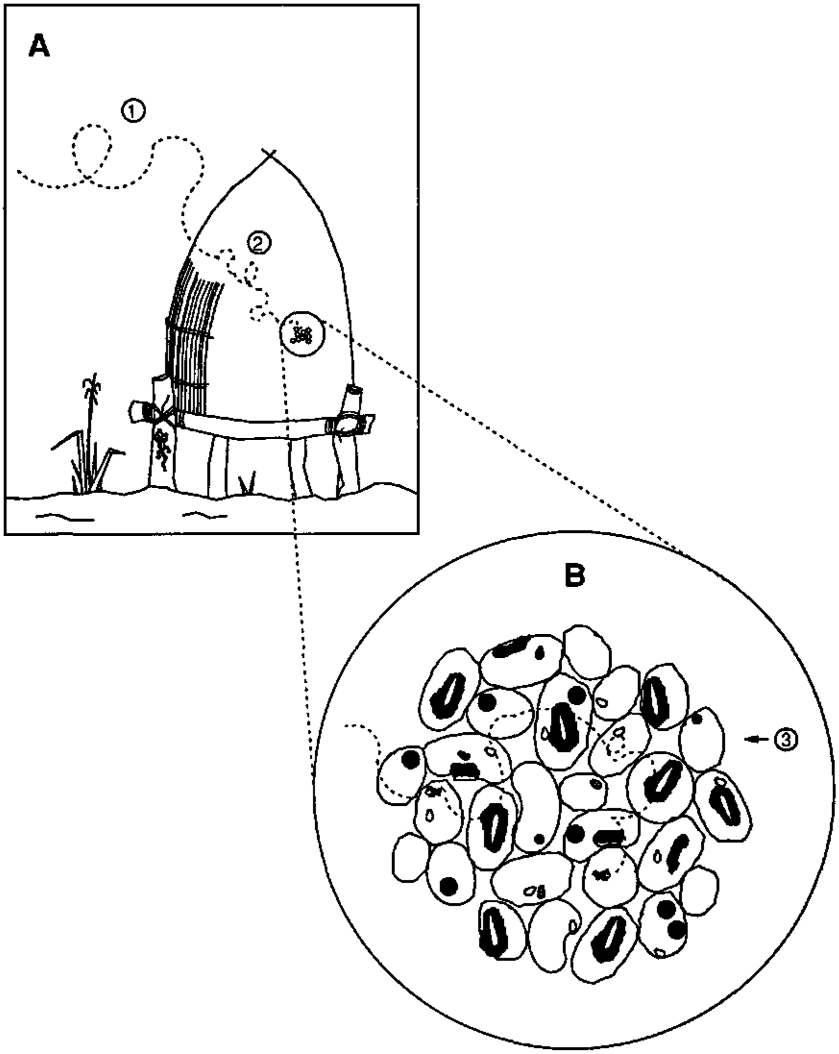

Figure 3.1 Schematic illustration of the hierarchical steps in *Uscana lariophaga*'s foraging behaviour, as discussed in this thesis. A. Step 1: Host habitat location. Step 2: Host cluster location within the habitat. B. Step 3: Within-cluster behaviour (e.g. functional response, arrestment, walking behaviour). For further details, see text.

Once arrived within a host cluster (Figure 3.1.B, step 3), individual females respond to the local density of the host. This so-called functional response is an important characteristic in biological control. For *U. lariophaga*, this trait was investigated in **Chapter 6**.

In the course of the season, *C. maculatus* egg densities may vary widely in space and time. Furthermore, geographical strains of *C. maculatus* have been found which demonstrate different egg dispersion behaviour (e.g. Credland, 1990; Mitchell, 1990; Tran & Credland, 1995). Therefore, in **Chapter 7**, I looked at the effect of different host distributions on the functional response of *U. lariophaga.*

At the farmers level, cowpea is stored in the pod, or pods are being threshed and cowpea is stored as seeds. In **Chapter 8**, I tested whether this would have consequences for the dispersion and functional response of *U. lariophaga* in two different habitats: in cowpea pods and seeds.

Finally, in the discussion (**Chapter 9**), the relevance of our findings are being discussed in the light of questions like: Which environmental manipulations can improve the searching efficiency of *U. lariophaga*? Can *U. lariophaga* contribute to a significant reduction in losses of stored cowpea due to bruchids? How does the impact of *U. lariophaga* on bruchid populations compare to that of the larval parasitoids occurring in the cowpea storage system? Can *U. lariophaga* be combined with larval parasitoids, and do such combinations result in significantly lower seed losses?

References:

Alebeek, F.A.N. van, 1991. Interspecific host discrimination by two solitary ectoparasitoids of immature stages of Bruchidae. Meded. Fac. Landbouww. Rijksuniv. Gent 56/3b: 1011-1020.

Alebeek, F.A.N. van; Rojas-Rousse, D.; Leveque, L., 1993. Interspecific competition between *Eupelmus vuilleti* and *Dinarmus basalis*, two solitary ectoparasitoids of Bruchidae larvae and pupae. Entomol. exp. appl. 69: 21-31.

Alphen, J.J.M.; Vet, L.E.M., 1986. An Evolutionary Approach to Host Finding and Selection. In: J.R. Waage & D. Greathead (eds), Insect parasitoids. Proc. 13th Symp. Royal Entomol. Soc., Acad. Press, London, pp. 23-61.

Arthur, A.P., 1981. Host acceptance by parasitoids. In: D.A. Nordlund, R.L. Jones & W.J. Lewis (eds), Semiochemicals, their role in pest control. John Wiley & Sons, New York, pp. 97-120.

Cortesero, A.M.; Monge, J.P.; Huignard, J., 1991. Influence of climatic conditions and host physiology on egg-laying behaviour and development of *Eupelmus vuilleti* (Eupelmidae). Insect Parasitoids, 4th European Workshop, Perugia 3-5 April, 1991, REDIA 74(3) (Appendice): 301-302.

Cortesero, A.M.; Monge, J.P.; Huignard, J., 1993. Response of the parasitoid *Eupelmus vuilleti* to the odours of the phytophagous host and its host plant in an olfactometer. Entomol. exp. appl. 69: 109-116.

Credland, P.F., 1990. Biotype variation and host change in bruchids: causes and effects in the evolution of bruchid pests. In: K. Fujii, A.M.R. Gatehouse, C.D. Johnson, R.M. Mitchell & T. Yoshida (eds), Bruchids and Legumes: economics, ecology and coevolution. Kluwer Academic Publishers, The Netherlands, pp. 271-287.

Dent, D., 1991. Insect Pest Management. CAB International, Wallingford.

Dutcher, J.D., 1993. Recent examples of conservation of arthropod natural enemies in agriculture. In: R.D. Lumsden & J.L. Vaughn (eds.), Pest Management: Biologically Based Technologies, Proc. Beltsville Symp. 18 Agric. Res. Service, USDA, Beltsville, pp. 101-108.

Germain, J.F.; Monge, J.P.; Huignard, J., 1987. Development of two bruchid populations (*Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (Fab.) infesting stored cowpea (*Vigna unguiculata* Walp) pods in Niger. J. Stored Prod. Res. 23(3): 157-162.

Godfray, H.C.J., 1994. Parasitoids. Behavioral and Evolutionary Ecology. Princeton University Press, Princeton.

Hirose, Y., 1986. Biological and ecological comparison of *Trichogramma* and *Telenomus* as control agents of lepidopterous pests. J. appl. Ent. 101: 39-47.

Huffaker, C.B.; Luck, R.F.; Messenger, P.S., 1977. The ecological basis of biological control. In: Proc. 15th Int. Congr. Entomol. August 1976, Washington D.C., pp. 560-586.

Huis, A. van; Appiah, S.O., 1995. *Uscana lariophaga*, West-African egg parasitoid of the cowpea bruchid beetle *Callosobruchus maculatus*: photoperiod, parasitization and eclosion interactions. Entomol. exp. appl. 76: 1-6.

Huis, A. van; Arendse, P.W.; Schilthuizen, M.; Wiegers, P.P.; Heering, H.; Hulshof, M.; Kaashoek, N.K., 1994a. *Uscana lariophaga*, egg parasitoid of bruchid beetle storage pests of cowpea in West Africa: the effect of temperature and humidity. Entomol. exp. appl. 70: 41-53.

Huis, A. van; Kaashoek, N.K.; Maes, H.M., 1991b. Biological control of bruchids (Col.: Bruchidae) in stored pulses by using egg parasitoids of the genus *Uscana* (Hym.: Trichogrammatidae): a review. In: F. Fleurat-Lessard & P. Ducom (eds.), Proc. 5th Int. Working Conf. Stored-product Prot. Bordeaux, Sept. 9-14, 1990, pp. 99-108.

Huis, A. van; Schütte, C.; Cools, M.H.; Fanget, Ph.; Hoek, H. van der; Piquet, S.P., 1994b. The role of semiochemicals in host location by *Uscana lariophaga*, egg parasitoid of *Callosobruchus maculatus*. Proc. 6th Int. Working Conf. Stored-Product Prot., April 1994, Canberra, pp. 1158-1164.

Huis, A. van; Wijkamp, M.G.; Lammers, P.M.; Klein Goldewijk, C.G.M.; Seeters, J.H. van; Kaashoek, N.K., 1991a. *Uscana lariophaga* (Hymenoptera: Trichogrammatidae), an egg parasitoid of bruchid beetle (Coleoptera: Bruchidae) storage pests in West Africa: host-age and host-species selection. Bull. ent. Res. 81: 65-75.

Lammers P.M.; Huis, A. van, 1989. *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae), egg parasitoid of the stored insect pests *Callosobruchus maculatus* (F.) and *Bruchidius atrolineatus* Pic. (Col.: Bruchidae): population studies in the field and in storage in Niger. Proc. Int. Conf. Int. Pest Manage. 3: 1013-1022.

Lenteren, J.C. van, 1981. Host discrimination by parasitoids. In: D.A. Nordlund, R.L. Jones & W.J. Lewis (eds), Semiochemicals, their role in pest control. John Wiley & Sons, New York, pp. 155-179.

Lenteren, J.C. van, 1986. Evaluation, mass production, quality control and release of entomophagous insects. In: K. Franz (ed.), Biological Plant and Healt Protection, Fortschritte der Zoologie 32. G. Fischer Verlag, Stuttgart, pp. 31-56.

Lenteren, J.C. van, 1987. Environmental manipulation advantageous to natural enemies of pests. In: V. Delucchi (ed), Protection integree: quo vadis? PARASITIS 86, pp. 123-163.

Lenteren, J.C. van; Woets, J., 1988. Biological and integrated pest control in greenhouses. Ann. Rev. Entom. 33: 239-269.

Leveque, L.; Monge, J.P.; Rojas-Rousse, D.; Alebeek, F. van; Huignard, J., 1993. Analysis of multiparasitism by *Eupelmus vuilleti* (Craw) (Eupelmidae) and *Dinarmus basalis* (Rond) (Pteromalidae) in the presence of one of their common hosts, *Bruchidius atrolineatus* (Pic) (Coleoptera Bruchidae). Oecologia 94: 272-277.

Lewis, W.J.; Vet, L.E.M.; Tumlinson, J.H.; Lenteren, J.C. van; Papaj, D.R., 1990. Variations in parasitoid foraging behavior: essential element of a sound biological control theory. Environ. Entomol. 19: 1183-1193.

Lewis, W.J.; Nordlund, D.A.; Gueldner, R.C.; Teal, P.E.A.; Tumlinson, J.H., 1982. Kairomones and their use for management of entomophagous insects. 13. Kairomonal activity for *Trichogramma* spp. of abdominal tips, excretion, and a synthetic sex pheromone blend of *Heliothis zea* (Boddie) moths. J. Chem. Ecol. 8(10): 1323-1331.

Mitchell, R., 1990. Behavioral ecology of *Callosobruchus maculatus*. In: K. Fujii, A.M.R. Gatehouse, C.D. Johnson, R.M. Mitchell & T. Yoshida (eds), Bruchids and Legumes: economics, ecology and coevolution. Kluwer Academic Publishers, The Netherlands, pp. 317-330.

Minkenberg, O.P.J.M., 1990. On seasonal inoculative biological control. Doctoral Thesis. Wageningen Agricultural University, Wageningen, 230 pp.

Monge, J.P.; Huignard, J., 1991. Population fluctuations of two bruchid species *Callosobruchus maculatus* (F.) and *Bruchidius atrolineatus* (Pic) (Coleoptera, Bruchidae) and their parasitoids *Dinarmus basalis* (Rondani) and *Eupelmus vuilleti* (Crawford) (Hymenoptera, Pteromalidae, Eupelmidae) in a storage situation in Niger. J. African Zool. 105: 187-196.

Monge, J.P.; Ouedraogo, A.P.; Huignard, J., 1991. Development of two Bruchid species, *Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (F.), and their larval parasitoids during storage of cowpea seeds *Vigna unguiculata* (Walp) in West Africa. In: F. Fleurat-Lessard & P. Ducom (eds), Proc. 5th Int. Working Conf. Stored-Product Prot. Bordeaux, Sept. 9-14, 1990, Vol. 1, pp. 151-160.

Noldus, L.P.J.J., 1989. Semiochemicals, foraging behaviour and quality of entomophagous insects for biological control. J. appl. Entomol. 108: 425-451.

Nordlund, D.A., 1994. Habit location by *Trichogramma*. In: E. Wajnberg & S.A. Hassan (eds.), Biological Control with Egg Parasitoids, IOBC, CAB International, Wallingford, pp. 155-163.

Pak, G.A., 1988. Selection of *Trichogramma* for innundative biological control. A study on behavioural variations among strains and species of an egg-parasite genus. Doctoral Thesis. Wageningen Agricultural University, Wageningen, 224 pp.

Rabb, R.L.; Stinner, R.E.; Bosch, R. van den, 1976. Conservation and augmentation of natural enemies. In: C.B. Huffaker & P.S. Messenger (eds), Theory and Practice of Biological Control. Academic Press, New York. pp. 233-254.

Rees, D.P.; Dales, M.J.; Golob, P., 1993. Alternative methods for the control of stored-product insect pests: A bibliographic database. Natural Resources Institute, Chatham, 161 p.

Roermund, H.W.J. van, 1995. Understanding biological control of greenhouse whitefly with the parasitoid *Encarsia formosa*. From individual behaviour to population dynamics. Doctoral Thesis, Wageningen Agricultural University, Wageningen.

Sagnia, S.B.; Schütte, C., 1992. Le système de stockage du niébé en milieu villageois dans l'état de Kano, Nigeria. Sahel PV Info 46: 6-15.

Sivakumar, M.V.K.; Maidoukia, A.; Stern, R.D., 1993. Agroclimatology of West Africa: Niger (2nd edition). ICRISAT Info. Bull. 5, International Crops Research Institute for the Semi-Arid Tropics; Direction de la Meteorologie Nationale du Niger, Niamey, 108 p.

Smith, S.M.; Hubbes, M., 1987. Strains of the egg parasitoid *Trichogramma minutum* Riley II. Utilization for release against the spruce budworm. J. Appl. Entomol. 102: 81-93.

Steenis, M. van, 1995. Evaluation and application of parasitoids for biological control of *Aphis gossypii* in glasshouse cucumber crops. Doctoral Thesis, Wageningen Agricultural University, Wageningen.

Tran, B.M.D.; Credland, P.F., 1995. Consequences of inbreeding for the cowpea seed beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Biol. J. Linnean Soc. 56: 483-503.

Turlings, T.C.J.; Wäckers, F.L.; Vet, L.E.M.; Lewis, W.J.; Tumlinson, J.H., 1993. Learning of host-finding cues by Hymenopterous parasitoids. In: D.R. Papaj & A.C. Lewis (eds.), Insect Learning, Ecological and Evolutionary Perspectives, Chapman & Hall, London, pp. 51-78.

Verma, R., 1990. Host habitat location and host location by *Dinarmus basalis,* a parasite of bruchids of stored legumes. Indian J. Exper. Biol. 28: 179-184.

Vet L.E.M., 1988. The influence of learning on habitat location and acceptance by parasitoids. In: M. Bouletreau & G. Bonnot (eds), Parasitoid insects, European Workshop, Lyon, September 7-10, 1987, Les Colloques de l' INRA 48: 29-34.

Vet, L.E.M.; Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. Annu. Rev. Entomol. 37: 141-172.

Vet, L.E.M.; Lewis, W.J.; Cardé, R.T., 1995. Parasitoid foraging and learning. In: R.T Cardé & W.J. Bell (eds), Chemical Ecology of Insects 2, Chapman & Hall, London, pp. 65-101.

Vet, L.E.M.; Lewis, W.J.; Papaj, D.R.; Lenteren, J.C. van, 1990. A variable-response model for parasitoid foraging behavior. J. Insect Beh. 3(4): 471-490.

Vet, L.E.M.; Wäckers, F.L.; Dicke, M., 1991. How to hunt for hiding hosts: the reliability-detectability problem in foraging parasitoids. Neth. J. Zool. 41(2-3): 202-213.

Vinson, S.B., 1981. Habitat location. In: D.A. Nordlund, R.L. Jones & W.J. Lewis (eds), Semiochemicals, their role in pest control. John Wiley & Sons, New York, pp. 51-78.

Vinson, S.B., 1984. Parasitoid - host relationship. In: W.J. Bell and R.T. Cardé (eds), Chemical Ecology of Insects. Chapman and Hall Ltd., London, pp. 205-233.

Vinson, S.B., 1985. The behaviour of parasitoids. In: G.A. Kerkut & L.I. Gilbert (eds), Comprehensive Insect Physiology, Biochemistry and Pharmacology, Pergamon Press, Oxford, 9: 417-469.

Wellings, P.W., 1991. Host location and oviposition on animals. In: W.J. Bailey & J. Ridschill-Smith (eds.), Reproductive Behaviour of Insects; Individuals & Populations. Chapman & Hall, London, pp. 75-107.

Weseloh, R.M., 1981. Host location by parasitoids. In: D.A. Nordlund, R.L. Jones & W.J. Lewis (eds), Semiochemicals, their role in pest control. John Wiley & Sons, New York, pp. 79-96.

Analysing host-habitat and host location in *Uscana lariophaga*, using Cox's proportional hazards model

4

Abstract

Uscana lariophaga Steffan is an egg parasitoid of the bruchid species *Callosobruchus maculatus* (Fabricius) and *Bruchidius atrolineatus* Pic in West Africa, which cause damage in stored cowpea (*Vigna unguiculata,* Leguminosae). In a no-choice situation, the attractiveness of some odour sources to the parasitoid was studied in a simple diffusion olfactometer. Cox's proportional hazards model was used for data analysis. The results showed that cowpea beans were more attractive to naive *U. lariophaga* females than glass beads. Baits with host eggs were more attractive than baits without eggs. Experienced females did not perform any better than naive ones. The relationship between the inclination to fly of the parasitoids, and the baits used, was difficult to interpret. Changes in searching activity between the different weeks of experimentation were observed. Only a small part of this variation could be explained by (absolute) changes in barometric pressure.

This chapter has been published, in a slightly different form, as:
Ormel, G.J.; Gort, G.; Alebeek, F.A.N. van, 1995. Analysing host location in *Uscana lariophaga* (Hymenoptera: Trichogrammatidae), an egg parasitoid of bruchids (Coleoptera: Bruchidae), using Cox's proportional hazards model. Bulletin of Entomological Research 85: 113-123.

Chapter 4

Introduction

The foraging behaviour of parasitoids can be divided into a series of hierarchical steps, e.g. host habitat preference, potential host community location, host location, host examination, ovipositor probing, etc. (e.g. Vinson, 1984; 1985; Chapter 3). In the first stage, habitat location, volatiles produced by the host-plant are often used as orientation cues. The second and third stage comprise the host community location process and host location within the community, in which olfactory, contact or visual stimuli can play a role.

We studied the role of host-plant odours and host eggs in the host habitat, host community and host location behaviour of the egg parasitoid *Uscana lariophaga* Steffan (Hymenoptera: Trichogrammatidae), a parasitoid of the bruchid beetles *Callosobruchus maculatus* (Fabricius) and *Bruchidius atrolineatus* (Pic). Larvae of these beetles can cause considerable losses during storage of leguminous crops Jackai & Daoust, 1986). *Uscana* spp. appear to be host specific at the family level, making them particularly suitable as potential biological control agents (Van Huis *et al.*, 1991a).

There are various reports on the use of host-plant and host related substances in the foraging process of egg parasitoids. The use of volatile host-plant substances in habitat location has been demonstrated by Nordlund *et al.* (1985); host related substances have been reported by, for example, Teal *et al.* (1986) and Shu & Jones (1989). The involvement of substances associated with the host's eggs, the parasitoids' ultimate targets, has been described by e.g. Noldus & Van Lenteren (1985) and Renou *et al.* (1989). Ferreira *et al.* (1979) and Bourarach & Hawlitzky (1984), found that *Trichogramma* spp. were attracted to eggs of Noctuidae. Wysoki & de Jong (1989) examined the attraction of *Trichogramma platneri* Nagarkatti to eggs of some lepidopterous pests of avocado *(Persea americana* (Lauraceae)).

Data on host-finding cues *of Uscana* spp. are virtually non existent. Preliminary studies, using a four-armed airflow olfactometer, showed that *U. lariophaga* females responded to volatiles emanating from the host-plant and the host (Van Huis *et al.*, 1994).

In the present study, the attractiveness of various odour sources to *U. lariophaga* females was investigated by using petri dishes as diffusion olfactometer. Cox's proportional hazards model was used to analyze the results.

Materials and methods

Rearing of insects.
The insects, *Callosobruchus maculatus* and *Uscana lariophaga*, were collected in December 1990 in the Niamey region of Niger. Beetles were reared on cowpeas *(Vigna unguiculata,* var.

Blackeye) in plastic petri dishes at 60 ± 10% r.h. and fluctuating temperature: 35 ± 0.5°C, coinciding with a photoperiod of 12 h, and 25 ± 0.5°C with a scotophase of 12 h (Van Huis *et al.,* 1991b). *U. lariophaga* parasitoids were reared in glass tubes at 30 ± 0.5°C, 30 ± 10% r.h. and a photoperiod of L:D 12:12. Fresh *C. maculatus* eggs (0 - 48 h) on cowpea beans were offered for parasitization and removed after two days. Adults were provided with a drop of honey to increase fecundity and longevity (Van Huis *et al.,* 1991a).

Preparation of naive and experienced Uscana lariophaga *females.*

Every day at 17.00 h parasitized eggs about to eclose were placed in clean glass tubes with a drop of honey. The following morning, freshly emerged adults were isolated in gelatine capsules (gelcaps), their age varying from 0-24 hours. This range was probably much less, because most parasitoids emerged over a period of 4 hours around the start of the photophase (Van Huis & Appiah, 1995). Adults were sexed using distinct antennal characteristics and used in experiments on the same day. Isolated females were used in a random sequence to minimize possible behavioral variations due to different gelcap residence times.

Naive parasitoids were mated females without previous contact with host eggs. To obtain experienced adults, naive females were allowed to complete one entire oviposition sequence (described by Van Huis *et al.*, 1991b) on a cowpea bean with young (0 - 20 h) *C. maculatus* eggs. On finishing post-oviposition behaviour, the females were isolated in a gelcap for a period of 45 to 60 minutes at 25°C before being used (long gelcap stay). This resting period served to neutralize a possible arrestment reaction due to the oviposition experience, which might interfere with the response to the odour source (Gardner & Van Lenteren, 1986).

Olfactometer.

Glass petri dishes (diam. 14 cm) with lids served as diffusion olfactometer. This simple set-up has been used in several experiments to study attraction or orientation in various insect species (e.g., Strand *et al.,* 1989; Mendel *et al.,* 1990). We used the same set-up, since it imitated the dispersion of odours under storage conditions. In a petri dish, as under storage conditions, the air hardly moves, which means that odour molecules disperse by molecular diffusion. In order to insert a gelatine capsule holding a female, a hole was drilled in the centre of the bottom dish. Because of negative geotaxis and positive phototaxis, the female left the capsule to enter freely into the centre of the petri dish. Five minutes before insertion of the gelcap, the bait (i.e., the odour source) was introduced to ensure the presence of an odour gradient. A single bait was positioned at random at the inner edge of the petri dish. The upper edge of the inner dish was wrapped with Teflon tape to create an (almost) airtight compartment. Since nothing is known about the formation of odour gradients, their interaction and possible differences in diffusion rates of active odours, attractiveness could only be studied reliably in a no-choice situation with a single bait. Before and after use, petri dishes were cleaned with methanol, rinsed in hot water and dried in a stove at 45°C.

Preparation of baits.

Six baits were used to test their attractiveness to *U. lariophaga* females: young (0 - 20 h) *C. maculatus* eggs on a glass bead, *C. maculatus* eggs three days old on a glass bead, young (0 - 20 h) *C. maculatus* eggs on a cowpea bean, a clean glass bead, a clean cowpea bean and a cowpea bean with *C. maculatus* larvae.

Clean, ellipsoid glass beads (diam. 18 mm, height 7 mm) and clean cowpea beans served as controls and as carriers for the host eggs. Each bait category was kept in a separate incubator (30 ± 0.5°C, 60 ± 10% r.h., unless stated otherwise) to prevent cross-contamination of odours.

Young *C. maculatus* eggs on glass beads were obtained by placing about 20 freshly emerged beetles (males and females) overnight in a small petri dish (diam. 5 cm) with five glass beads. Egg loads of the beads varied between 5 and 15.

Three days old *C. maculatus* eggs on beads were prepared by transferring beads with young eggs, produced as described above, into a clean, small petri dish. Dishes were placed open in an empty incubator (25 ± 0.5°C) to get rid of adhering odours. After one day, the lid was replaced and the dishes were transferred to another incubator (same temperature) for two more days before being used.

Young *C. maculatus* eggs on beans were obtained following the same procedures as with beads, but using 10 beetles (males and females) on 20 cowpeas. Egg loads of beans used in the experiments varied between 5 and 15.

Beans with *C. maculatus* larvae were prepared by putting ten cowpea beans with young beetle eggs in a clean petri dish. Beans were incubated at 25 ± 0.5°C during 14 days prior to use.

Experiments.

The study was divided into two experiments. In the first experiment (November-December 1991) the attractiveness of six different baits to naive *U. lariophaga* females was tested (Table 4.1), to answer the following questions of interest:

1a1 Are baits with beans more attractive than baits with beads?
1a2 Are baits with eggs more attractive than baits without?
1a3 Is the difference in attractiveness between baits with and without eggs the same for beads and beans? This is the interaction between eggs/no eggs and beads/beans.
1b Are beads with 3 days old eggs as attractive as beads with young eggs?
1c Are beans with larvae as attractive as beans with eggs?

If significant interaction was found (see *Testing procedures*), the main effect questions 1a1 and 1a2 were divided into separate questions.

Oviposition experience has a positive effect on olfaction responses in parasitoids (Vet & Papaj, 1992); it can also evoke an arrestment behaviour (Gardner & Van Lenteren, 1986). The influence of oviposition was studied in the second experiment (January - February 1992) in five different treatments (Table 4.1), based on the following questions of interest:

2a1 Are baits with eggs more attractive than baits without eggs?
2a2 Are experienced females attracted differently to baits compared to naive females?
2a3 Does interaction occur between eggs/no eggs and naive/experienced females?
2b Has duration of gelcap stay influence on attraction (using experienced wasps and beans with eggs)?

In case of interaction, the main effect questions 2a1 and 2a2 were again divided into separate ones. Treatments (exposure to a bait) were organized in an unbalanced block design with week of experimentation as blocking factor. The block design was incomplete for experiment I (not all treatments occurred in each week) and complete for experiment 2. The sequence of treatments on a particular day was randomized with each treatment at least carried out once a day between 10.00 h and 20.00 h. The total number of replicates per treatment ranged between 27 and 72 (Table 4.1). Experiments were conducted in a climate chamber (30 ± 1°C, 30 ± 10% r.h.). Light intensity at the location of the petri dishes was 3700 Lux.

Parameters determined.
Once in the petri dish, the female was observed during a period of up to 15 minutes. The observation ended if it arrived at the bait or commenced flying. For each parasitoid, the following parameters were determined: arrival or not at the bait within 15 minutes, time of arrival in seconds (if any), flight or not within 15 minutes, and time of flight in seconds (if any). An overview of the numbers of arriving, flying and not arriving nor flying (i.e. still searching), *U. lariophaga* females is given in Table 4.1.

Statistical methods

Introduction and motivation.
Attractiveness of the baits was studied by comparing the determined parameters between the covariates (explanatory variables). The covariates were treatment and week of experimentation, the latter was included because changes in searching activity were found between the different weeks in which the experiments were carried out (see *Testing procedures).* The most important measure of attractiveness of a bait was time of arrival: the earlier the arrival time, the more attractive the bait. Although time of flight was probably a less clear indicator of attractiveness, we assumed that with attractive baits parasitoids would be less inclined to fly than with less attractive ones, giving later flight times. We have emphasized the analysis of arrival times.

Table 4.1 Overview of results of the treatments in experiments 1 and 2. Numbers of naive and oviposition experienced (exp) *Uscana lariophaga* females arrived, not arrived nor flying, and flying within 15 minutes after release when exposed to various baits in a diffusion olfactometer.

Treatments	Baits	Females	Weeks	Number of females			
				Total	Arrived	Not arrived and not flying	Flying
Experiment 1 (November/December 1991)							
1	beads, clean	naive	1-4	40	2	23	15
2	beads + young eggs	naive	1-4	37	27	2	8
3	beads + old eggs	naive	1-4	36	28	6	2
4	beans, clean	naive	1-5	72	31	24	17
5	beans + young eggs	naive	1-5	68	54	6	8
6	beans + larvae	naive	3-5	35	15	8	12
Experiment 2 (January/February 1992)							
7	beans, clean	naive	6-9	48	5	14	29
8	beans + young eggs	naive	6-9	44	26	4	14
9	beans, clean	exp	6-9	33	2	11	20
10	beans + young eggs	exp	6-9	27	18	5	4
11	beans + young eggs	exp	6-9	40	21	5	14

A X^2 analysis of numbers would have the drawbacks of not using the information of times and of not being able to adjust for covariates besides treatment. A classical analysis of times would be analysis of variance. However, because of the occurrence of so-called 'censored observations' (see next section), this method could have led to erroneous conclusions (Bressers *et al.*, 1991). A method that handles the analysis of these observations accurately and uses the full information of times, is survival analysis. From the available survival models Cox's proportional hazards model was chosen, because it does not make *a priori* assumptions about the form of the survivor function (Cox, 1972). In the next sections, we have given a rather detailed description of the model. We considered this useful, because the Cox proportional hazards model and survival analysis in general, although a powerful tool in data analysis, is not well known in the field of entomology. The model combines easy adjustment for covariates and correct treatment of censoring, with flexibility through baseline hazard estimation. The assumptions of the model are not too restrictive. For some examples of the use of the model, we refer to Haccou & Hemerik (1985), Haccou *et al.* (1991) and Hemerik *et al.* (1993).

Survival analysis.
Survival analysis deals with duration until a specific event occurs. Traditionally, such an event is called a 'failure'. We defined failure as the end of searching activity, which in our experiments had two causes: arrival at the bait and flight. A situation with more than one cause of failure is called a 'competing risk problem' (Prentice & Kalbfleisch, 1978).

For each parasitoid a time T until failure was measured. Parasitoids which neither arrived nor flew within the observation period, had censored times of failure. When looking at failure cause *arrival*, flight resulted in a censored observation. For failure cause *flight*, arrival gave a censored time. We were interested in the probability distribution of T, especially the survivor function $S(t)$. This is the probability that a parasitoid would fail at time t or beyond, i.e., the probability that there was still searching activity at time t. The relationship between survivor function and covariates was studied. The covariates probably affected arrival and flight times differently, so separate models were needed.

Proportional hazards model.
In the Cox proportional hazards model attention is focused on the hazard function $\lambda(t)$. At time $T=t$ a parasitoid experienced a certain hazard $\lambda(t)$, which is the probability of failure at time t per unit of time, given no failure had occurred yet. It can be interpreted as the instantaneous failure rate. Each cause of failure had its own hazard function: $\lambda_a(t)$ for *arrival* and $\lambda_f(t)$ for *flight*. In the model hazards are assumed to be proportional, as can be seen in the mathematical formulation of the (arrival) hazard for experiment 1:

$$\lambda_a(t;z) = \lambda_{a,0}(t)\exp(\Sigma_i \beta_i \mathrm{t}_i + \Sigma_j \gamma_j w_j) \qquad (1)$$

with $\lambda_{a,0}(t)$ the so called baseline hazard function, $z = (t_1,t_2,t_3,t_5,t_6,w_2,w_3,w_4,w_5)$ the covariate vector, with indicator (0/1) variables coding for treatment (t_i) and week of experimentation (w_j), and β_i and γ_j the corresponding unknown regression coefficients. The unspecified baseline hazard function $\lambda_{a,0}(t)$ is estimated from the data and gives the model great flexibility. It is the hazard function for the reference category, which has all covariates at the value 0. In experiment 1, treatment 4 in week 1 was chosen as reference category. The arrival hazard function for treatment 1 in week 3 for example, can thus be written as $\lambda_{a,0}(t)$ times $\exp\{\beta_1+\gamma_3\}$, with hazard ratio $\exp\{\beta_1+\gamma_3\}$. The regression coefficients β_i and γ_j can be interpreted through the hazard ratio. For example, suppose that for treatment 1 $\beta_1 = -1$. The hazard ratio is then e^{-1}, which means that the instantaneous probability of arrival for treatment 1 is e^{-1} (≈ 0.37) times the probability of the reference treatment, irrespective of time. So treatment 1 is less attractive. Thus, when looking at *arrival,* positive regression coefficients indicate more attraction with respect to the reference category. As to failure cause *flight,* a negative regression coefficient has to be interpreted as an indicator of more attraction.

For the second experiment, a proportional hazard model for *arrival* with five treatments and four weeks was defined; reference category was treatment 7 in week 6. Similar models were defined for the flight hazard.

Testing procedures.

Hypotheses about treatment were tested in two steps. First the overall effect of treatment on *arrival* and *flight* was studied using likelihood ratio tests. The null hypothesis of no difference between treatments was formulated as $\beta_1=\beta_2=\beta_3=\beta_5=\beta_6=0$. Results were presented in an analysis of deviance table (Aitkin *et al.,* 1990). If a significant result for treatments was found, we proceeded with Wald-tests (SAS Institute Inc., 1991), corresponding to the earlier formulated questions of interest.

Different assumptions were made when using the model. Proportionality of hazards was an assumption which was roughly checked by looking at Kaplan-Meier estimates of cause-specific survivor functions for the various treatments. Another assumption was independent occurrence of censors. Kalbfleisch & Prentice (1980) described a graphical test for checking the suitability of the model by making residual plots. Testing our models in this way did not reveal clear violations of the model assumptions. Fitting the model was done by maximization of the partial likelihood, resulting in maximum likelihood estimates of the baseline hazard and regression coefficients (Kalbfleisch & Prentice, 1980). For fitting we used SAS (PROC PHREG) (SAS Institute Inc., 1991) and a computer program from Haccou & Hemerik (1985).

Instead of the baseline hazard functions we estimated the cumulative baseline hazard functions $\Lambda_a(t)$ and $\Lambda_f(t)$. These are the integrals from 0 to t of, respectively, the hazards $\lambda_{a,0}$ and $\lambda_{f,0}$ (Figure 4.2). From the total cumulative hazard $\Lambda(t)$ $(=\Lambda_{a,0}(t)+\Lambda_{f,0}(t))$, the survivor function $S(t)$ can be derived easily (see e.g., Aitkin *et al.*, 1990). The cause-specific hazard functions are restored by taking slopes of $\Lambda_{a,0}(t)$ and $\Lambda_{f,0}(t)$.

During the analysis, we found in the first experiment a strong effect of week of experimentation on failure cause *arrival* (Table 4.2). As temperature, relative humidity and light intensity were held constant, changes in atmospheric pressure might be responsible, as suggested by Steinberg *et al.* (1992). To evaluate the influence of barometric fluctuations, the following covariates were calculated for the days of experimentation (t=time of experimentation): the mean atmospheric pressure during 6 and 12 hours before t ($m6$ and $m12$), the change of pressure during 6 and 12 hours before t as measured by the regression coefficient of pressure on time ($r6$ and $r12$), and by the maximum difference during these periods ($d6$ and $d12$). The absolute values ($ar6$, $ar12$, $ad6$ and $ad12$) of pressure changes over time were also calculated. Each covariate was separately entered into the model, replacing week. Only the effect on failure cause *arrival* was studied.

Results

Summary of frequencies.

In both experiments, the numbers of female *U. lariophaga* parasitoids that arrived, varied considerably between the different treatments (Table 4.1). The lowest arrival score (2%, n=40) was observed for clean beads; the highest (79.3%, n=68) for treatment 5 (beans with young eggs). In experiment 2, the number of flyers was higher than in experiment 1; consequently, fewer females arrived. Experienced females, which stayed a short time in the gelcap, had the highest arrival score (66.7%, n=27), using beans with fresh eggs; naive females, using clean beans, scored lowest (10.4%, n=48).

Survivor function.

Kaplan-Meier estimated for *arrival* are presented in Figure 4.1a and 4.1b; those for *flight* in Figure 4.1c and 4.1d. Each plotted symbol represents at least one failure. In these graphs the proportion of the parasitoids which did not fail, i.e., which did not arrive (Fig. 4.1a, 4.1b) or did not fly (Fig. 4.1c, 4.1d), is pictured as a function of time. No correction was made for the week of experimentation. The figures serve as a graphical data summary. From the curves, distortions of the proportionality assumption of the model can also be read, since proportionality of hazards implies equality of curves, except for a constant power.

The graphs for *arrival* showed more or less the same general pattern. It took some time for the first females to arrive (time was needed to walk to the bait), after which a more or less constant, but treatment dependent, number arrived per time unit. In the end a decreasing number still failed. In both experiments, treatments involving eggs showed earlier arrival times, corresponding to more steeply dropping curves. Oviposition experience did not seem

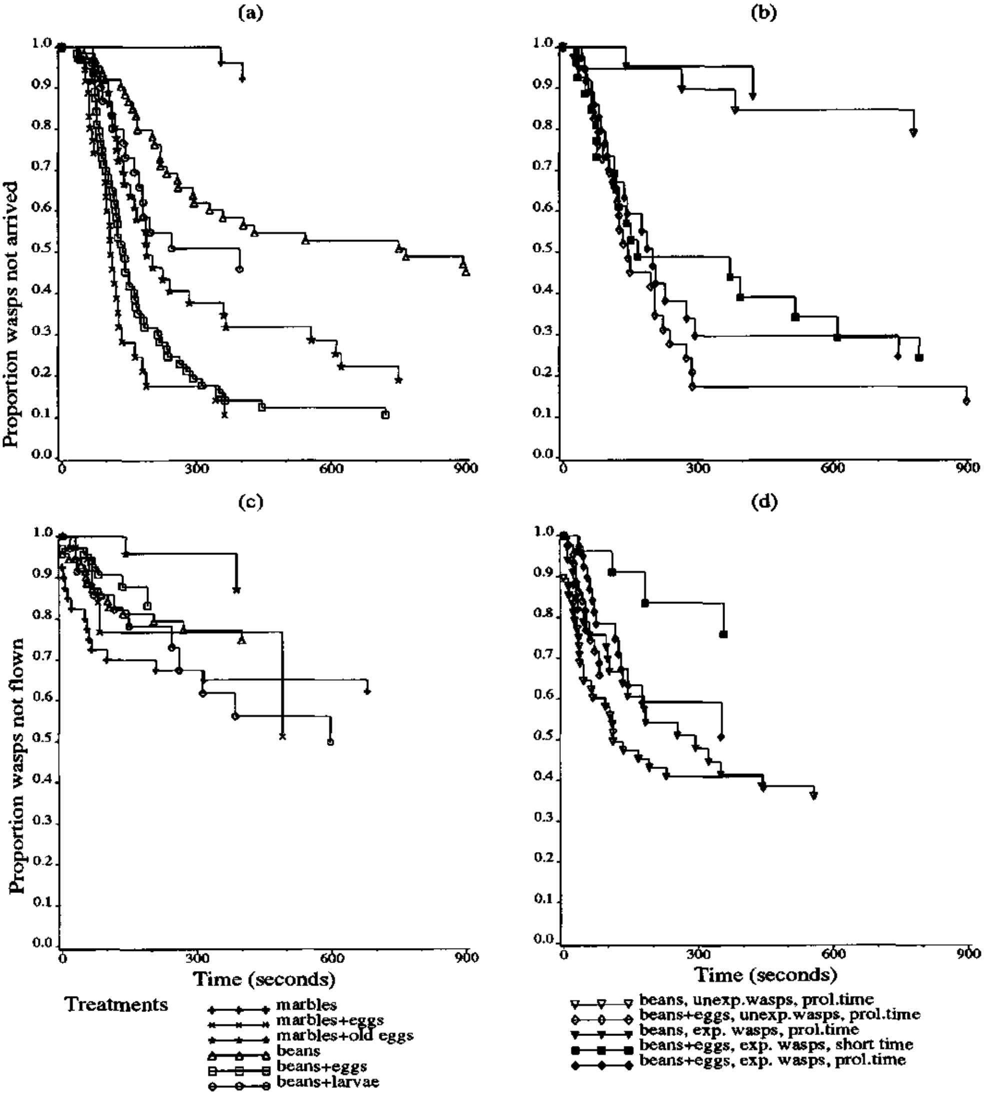

Figure 4.1 Kaplan-meier estimates of the survivor functions for *arrival* (a,b) and *flight* (c,d) of *Uscana lariophaga* females for the treatments in experiment 1 (a,c) and experiment 2 (b,d). More attractive treatments have more steeply dropping *arrival* curves (a,b). See text for further explanation.

to matter much (Fig. 4.1b). With treatment 1 (clean beads), *U. lariophaga* females did almost not arrive at all. Remarkable were clean beans, which showed a moderate number of arrivals in experiment 1 (treatment 4, Fig. 4.1a), but had few arrivals in experiment 2 (treatment 7 and 9, Fig. 4.b). Notice however, the high proportion of flyers with this bait (Fig. 4.1d).

The curves concerning flight revealed a different picture. Flight occurred immediately after start of the treatments. With treatment 3 parasitoids showed the least tendency to fly (Fig. 4.1c). In experiment 2, parasitoids with treatment 10 did not fly often (Fig. 4.1d). Curves in Figure 4.1d had about the same shape. In experiment 1, however, treatment 1 had many early flights, resulting in a slightly different shape of the curve.

Proportional hazards: regression coefficients.

In Table 4.2 the results from the likelihood ratio tests on the overall effects of treatment and week on *arrival* and *flight* are summarized. All tests were performed at a significance level of 0.05, unless stated otherwise. In all four models a significant effect of treatment was found, but effects were much more evident on arrival time than on flight time. In experiment 1 an effect of week could be demonstrated, strong on *arrival*, but only moderate on *flight*.

Table 4.2 Analysis of deviances for the 4 proportional hazards models in experiments 1 and 2. Changes of deviance are likelihood ratio statistics and have approximately X^2 distributions under corresponding null hypotheses (Aitkin *et al.*, 1990); α=0.05. See *Testing procedures* for details. (df=degrees of freedom).

Source of variation	df	Deviance change *arrival*	P value	Deviance change *flight*	P value
Experiment 1					
Week	4	27.3	< 0.001	10.5	0.03
Treatment	5	110.6	< 0.001	14.3	0.01
Experiment 2					
Week	3	1.6	0.7	1.8	0.62
Treatment	4	51.6	< 0.001	15.8	0.003

In Table 4.3, the estimated regression coefficients, standard errors and hazard ratios for the treatments in experiments 1 and 2 are given. The results of the Wald-tests are presented in Table 4.4. Based on these results, we could answer the questions raised earlier. For experiment 1, we concluded the following with respect to failure cause *arrival:*

1. Interaction occurred between no-eggs/eggs and beads/beans (P=0.0004, 1a3). *Uscana lariophaga* females were more attracted by clean beans than by clean beads (P=0.002, 1a1). It could not be demonstrated that beans with eggs were more attractive than beads with eggs (P=0.99).
2. Parasitoids were more attracted by baits with eggs than by baits without eggs, both for glass beads and beans (both P < 0.0001, 1a2).

Table 4.3 Estimated regression coefficients $\hat{\beta}$, standard error (SE) and hazard ratios (hr) for the failure causes *arrival* and *flight* of the treatments in experiments 1 and 2. Treatment numbers refer to Table 4.1. See *Proportional hazards model* for details.

Treatment	$\hat{\beta}$ *arrival*	SE	hr	$\hat{\beta}$ *flight*	SE	hr
Experiment 1						
1	-2.08	0.73	0.12	0.52	0.38	1.67
2	1.92	0.29	6.83	0.32	0.46	1.37
3	1.04	0.28	2.84	-1.39	0.76	0.25
4	Reference					
5	1.29	0.23	3.64	-0.46	0.44	0.63
6	-0.17	0.33	0.84	0.45	0.43	1.57
Experiment 2						
7	Reference					
8	2.12	0.49	8.36	-0.62	0.33	0.54
9	-0.76	0.84	0.47	-0.25	0.29	0.78
10	1.73	0.51	5.64	-1.66	0.54	0.19
11	1.81	0.50	6.13	-0.67	0.33	0.51

Table 4.4 Results of Wald-tests for treatment contrasts in experiments 1 and 2; α=0.05. See *Testing procedures* for details. (exp= oviposition experience).

Question	Type of test	P-value *arrival*	P-value *flight*
Experiment 1			
Interaction (1a3)	two-sided	0.0004	0.67
Eggs (main effects) (1a2)	one-sided		0.15
with beads		< 0.0001	
with beans		< 0.0001	
Beans (main effects) (1a1)	one-sided		0.03
without eggs		0.002	
with eggs		0.99	
Old eggs (1b)	two-sided	0.001	0.03
Larvae (1c)	two-sided	< 0.0001	0.06
Experiment 2			
Interaction (2a3)	two-sided	0.61	0.68
Eggs (main effect) (2a1)	one-sided	< 0.0001	0.02
Exp (main effect) (2a2)	two-sided	0.23	0.53
Gelcap stay (2b)	two-sided	0.80	0.08

3. The attractiveness of beads with old eggs and beads with young eggs differed significantly (P=0.001, 1b); beads with young eggs were more attractive.
4. The attractiveness of beans with larvae and beans with eggs differed significantly (P<0.0001, 1c); beans with eggs were more attractive.

We concluded the following for experiment 2:

1. No interaction occurred between no-eggs/eggs and naive/experienced females (P=0.61, 2a3).
2. Beans with eggs were more attractive than beans without eggs (P < 0.0001, 2a1). Oviposition experience did not effect the olfactive response of *U. lariophaga* females (P=0.23, 2a2).
3. There was no difference between experienced females with short and long gelcap stay (P=0.80, 2b).

Regarding failure cause *flight,* the effects were much less prominent. In the first experiment, it appeared that baits with beans had less flight than baits with beads (P=0.03), With treatment 3 (beads with old eggs), parasitoids were less inclined to fly than with beads with young eggs (P=0.03). In the second experiment baits with eggs showed less flight than baits without eggs (P=0.02).

Proportional hazards: cumulative baseline hazard.

The cause-specific estimates of the cumulative baseline hazards for *arrival* and *flight* are presented in Figure 4.2a (experiment 1) and 4.2b (experiment 2). Smooth curves were drawn through the points. As an example, in experiment 1 at t=300, $\Lambda_a(t)$ was estimated as 0.20 and $\Lambda_f(t)$ 0.25, giving an estimated total cumulative hazard Λ (300) of 0.45. This corresponded to an estimated S(300), the probability that parasitoids were still searching at t=300, of $e^{-0.45}$ (=0.64) (see Aitkin *et al.*, 1990).

In both experiments, the baseline hazard for failure cause *arrival,* the slope of its cumulative hazard function, increased slowly after t=0 and reached a more or less constant value after about 60 seconds (somewhat earlier in experiment 2). Until about t=300 seconds it remained constant (higher in experiment 1 than in experiment 2), indicating that in the first experiment the reference treatment had higher instantaneous probabilities of arriving in the reference week. Afterwards, it decreased steadily and after about 500 seconds, in both experiments only a few parasitoids still arrived. For failure cause *flight,* in both experiments the baseline hazard was about constant from the start of the treatment until about t=150 seconds, after which it slowly decreased. In experiment 2, the flight hazard of the reference category was much higher than the arrival hazard. It was also higher than the flight hazard in experiment 1.

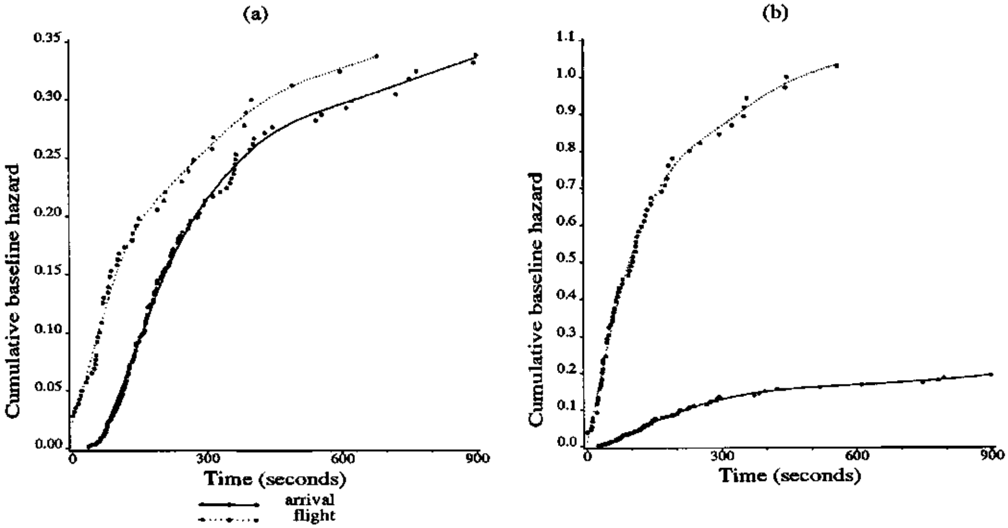

Figure 4.2 Estimates of the cumulative baseline hazards for *arrival* and *flight* of naive *Uscana lariophaga* females in experiments 1 (a) and 2 (b) (note difference in scale). See text for further explanation.

Atmospheric pressure.

In Table 4.5, changes in deviance for adding a covariate into the model are given, together with the P-values (using a X^2-approximation) and estimates of the regression coefficients. Because of the exploratory approach followed, we performed the tests at a significance level of 0.05/11= 0.0045 (11 covariates were tested). This Bonferroni-method prevents null hypotheses being rejected too quickly (see e.g., Snedecor & Cochran, 1989).

None of the covariates explained as much as covariate week itself (deviance 27.3, compared to a maximum of 8.9 for *ar*12). Only covariate *ar*12 met the significance level 0.0045. The estimate of the regression coefficient of *ar*12 was positive (0.8), which suggests that more parasitoids arrived with larger absolute changes in barometric pressures. We concluded cautiously that there might be an effect of absolute atmospheric pressure changes in arrival behaviour of parasitoids.

Table 4.5 Effect of changes in atmospheric pressure on failure type *arrival* of naive *Uscana lariophaga* females in experiment 1; α=0.0045. See *Testing procedures* for details.

Covariate	Deviance change	P-value	β
week	27.3	< 0.001	
p	2.0	0.16	0.010
*m*6	2.4	0.12	0.011
*m*12	2.9	0.09	0.013
*r*6	1.5	0.21	-0.247
*r*12	1.1	0.28	-0.233
*d*6	2.1	0.15	-0.049
*d*12	0.6	0.45	-0.014
*ar*6	6.4	0.01	0.679
*ar*12	8.9	0.003	0.836
*ad*6	7.0	0.008	0.126
*ad*12	7.4	0.007	0.071

Discussion

Experiment 1.

This experiment proved that baits with host eggs were more attractive to naive *U. lariophaga* females, i.e., had shorter arrival times, than baits without eggs (including beans with larvae). Clean cowpea beans were more attractive than clean beads. No differences could be found between beads with eggs and beans with eggs, nor between clean beans and beans with larvae.

Attraction of an egg parasitoid by its host's eggs has only rarely been described (e.g., Ferreira *et al.,* 1979; Wysoki & De Jong, 1989). Pak *et al.* (1991) reported that olfaction in *Trichogramma* spp. only occurred at very short distances and that they have poor eyesight. Therefore, our results could not be explained by visual orientation of *U. lariophaga* females, since they discriminated between baits with and without eggs over distances up to 7 cm (wasp size approx. 0.4 mm).

We could only speculate on the source and nature of the odours that attracted *U. lariophaga* females to eggs of *C. maculatus.* Eggs *per se* probably do not have any odour (e.g., Laing, 1937; Kaiser *et al.,* 1987, 1989). They referred to eggs of moths, which may become contaminated with scales during oviposition (Vinson, 1984b). These scales emanate volatiles, acting as kairomones for parasitoids. The host eggs used in our study were laid by beetles kept in closed petri dishes. It is possible that the eggs absorbed volatile beetle odours, or that faeces or scales of the beetles adhered to the eggs. Bruchids are known to use sex pheromones (Pajni & Gupta, 1990; Cork *et al.*, 1991), copulation release pheromones (Tanaka *et al.*, 1981) and marking (oviposition deterrent) pheromones (Credland & Wright, 1990; Messina *et al.*, 1992) in intra-specific communication. Bruchids also attach their eggs to beans with a glue-like substance (Biémont *et al.*, 1982). The glue around the eggs of the green stink bug *(Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae)) contains a kairomone for the egg parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) (Bin *et al.*, 1993). Beetle odours could also contaminate the beads and beans on which the eggs were deposited.

The fact that old eggs were still attractive suggests that at least some components of the host odours were relatively highly persistent. Credland & Wright (1990) found that an ether-extractable fraction from oviposition deterrents of *C. maculatus* remained biologically active for at least 30 days.

The attractiveness of baits with host eggs should be evaluated in the context of the general host location strategy of a parasitoid. It is plausible that *U. lariophaga* females responded to cowpea odours, since cowpea is the host-plant of *C. maculatus*. This response could be used in host habitat location, allowing *U. lariophaga* females to locate cowpea fields and stores. It would be interesting to test also the parasitoid's response to green (parts of) cowpea plants (e.g. the pods), which could guide the parasitoid in colonizing cowpea fields in the rainy season. However, plant volatiles of cowpea constitute only an indication for the presence of host eggs. They are, in the terminology of Vet & Dicke (1992), not a 'reliable stimulus', hence, they are not likely to produce a strong response. On the other hand, the sampling survey in Niger (Chapter 2) demonstrated that in 48 out of 50 samples, bruchids were present. In the current study, the parasitoids' response to beans was relatively weak in comparison to eggs. A stimulus intimately and reliably associated with its host, will elicit a strong response in parasitoids. Serving as a guiding cue it will most likely result in a quick and successful encounter with the host (Vet & Dicke, 1992). Odours associated with the host's eggs, constitute a reliable orientational cue for egg parasitoids and could therefore evoke a strong response in foraging *U. lariophaga* females. Our results supported this assumption.

Experiment 2.

From experiment 2, it could be concluded that a single oviposition experience did not improve significantly the searching capabilities of *U. lariophaga* females. Experienced females seemed to perform even less, although the differences were not significant. Some authors reported that oviposition experience enhanced host seeking behaviour in Trichogrammatidae, for example Gross *et al.* (1981). This issue will be considered again in Chapter 7 of this thesis. On the other hand, Kaiser *et al.* (1989) found that the effect of an oviposition experience only lasted for a few minutes.

In egg parasitoids, due to their small body size, foraging flights are more governed by chance and wind direction than by directional movements towards chosen stimuli or targets. From this, and from their short life span, it follows that egg parasitoids are faced with relatively few foraging decisions in their total life span, and therefore learning in egg parasitoids is thought to play little or no role. Especially high innate responses to reliable stimuli are unlikely to be affected by experience (Vet *et al.*, 1990; 1995).

Various authors have mentioned an arrestment reaction by oviposition experience in egg parasitoids (e.g., Gardner & Van Lenteren, 1986; Noldus *et al.*, 1991) (see also Chapter 7). In order to explain the results of treatment 10, we hypothesized that the strong 'egg effect' overruled an arrestment reaction, or arrestment, for one reason or another, did not occur.

Flyers.
Trichogrammatidae are known to be positively phototactic (for references, see Brower, 1991). Light intensities used during the experiments could not have been the cause of the observed flying response, since this behaviour was variable during the weeks in which the treatments were carried out (Table 4.2). An overall treatment effect existed on failure type *flight* in both experiments (Table 4.2), but compared with *arrival,* the effects of treatments were less prominent. Although it seemed reasonable to assume that an unattractive bait could induce a parasitoid to fly, resulting in a high hazard rate for failure type *flight,* our results did not support this assumption unequivocally. In experiment 1, no difference could be found between baits with and without eggs when looking at *flight,* but in experiment 2 baits with eggs had less flight (Table 4.4). In experiment 1, for failure cause *arrival,* young eggs were more attractive than old eggs (using beads). However, for *flight* the opposite was observed, contrary to what was expected (Table 4.3 and 4.4). With clean beans females were less inclined to fly than with clean beads, which agreed with the observation that beans were significantly more attractive than clean beads, when looking at *arrival.* More research is needed to identify the factors influencing the flying behaviour in *U. lariophaga* females.

Atmospheric pressure.
Changes in atmospheric pressure could have been the cause for the variations in searching activity of the parasitoids. Steinberg *et al.* (1992) found a daily variation in olfactory response in the larval parasitoid *Cotesia glomerata* (Linnaeus) (Hymenoptera: Braconidae). Increasing searching activity was correlated with increasing atmospheric pressure, and decreasing activity with fluctuating or decreasing barometric flux. Our preliminary results differed from their observations. We could not find a day-to-day effect and, in our experiments, parasitoids responded differently to changes in atmospheric pressure. However, the positive effect of (absolute) changes in atmospheric pressure on the searching activity of *U. lariophaga* females (Table 4.5), was relatively weak. An ecological interpretation of this finding is not evident. To investigate the influence of variations in barometric pressure on the olfactive response of

U. lariophaga parasitoids we recommend that further experiments in a complete block design, with week or day as blocking factor, be conducted.

Alternatively, in the course of the experiments from November 1991 until February 1992, the responses of *U. lariophaga* may have been influenced by odour contaminations on the glass of the olfactometer. At that time, washing and drying at 45°C was thought to be sufficient to remove any odour traces on the glass. However, later experiments have shown that odour traces may still have accumulated on the walls of the olfactometer, interfering with the responses of the wasps. More on this topic will be discussed in Chapter 5.

Acknowledgements

Gert Ormel devoted most of his study-leave to the research presented here. Gerrit Gort introduced us to the proportinal hazards models and did most of the analyses. Wasps were identified by Andrew Polaszek (Int. Inst. Entomol., London). Meteorological data were provided by Mr. Bimie (WAU, Dept. of Meteorology). The support and comments of Lia Hemerik and Arnold van Huis are gratefully acknowledged. Thanks are also due to Gerard Pesch for technical assistance with the insect rearing.

References

Aitkin, M.; Anderson, D.; Francis, B.; Hinde, J., 1990. Statistical Modelling in GLIM. Clarendon Press, Oxford, 374 pp.

Biémont, J.C.; Chauvin, G.; Germain, J.F., 1982. L'oeuf de *Bruchidius atrolineatus* (Pic) et son système de fixation. Can. J. Zool. 60: 2610-2615.

Bin, F.; Vinson, S.B.; Strand, M.R.; Colazza, S.; Jones, W.A., 1993. Source of an egg kairomone for *Trissolcus basalis*, a parasitoid of *Nezara viridula*. Physiol. Entomol. 18: 7-15.

Bourarach, J.; Hawlitzky, N., 1984. Attractivité des oeufs de *Spodoptera littoralis* (Bsd) et *Helicoverpa armigera* (Hb) (Lep.: Noctuidae) pour *Trichogrammatoidea lutea* (G) et *Trichogramma rhenana* Voegelé et Russo (Hym.: Trichogrammatidae). Actes l'Inst. Agron. Vétéri. 4: 137-141.

Bressers, M.; Meelis, E.; Haccou, P.; Kruk, M., 1991. When did it really start or stop: the impact of censored observations on the analysis of duration. Beh. Processes 23: 1-20.

Brower, J.H., 1991. Influence of light on dispersal of *Trichogramma pretiosum* in a warehouse. *Trichogramma* and other egg parasitoids. Proc. 3rd Int. Symp., 23-27 September 1990. San Antonio. Les Colloques de l'INRA 56: 55-58.

Cork, A.; Hall, D.R.; Blaney, W.M.; Simmonds, M.S.J., 1991. Identification of a component of the female sex pheromone *of Callosobruchus analis* (Coleoptera: Bruchidae). Tetrahedron Letters 32: 129-132.

Cox, D.R., 1972. Regression models and life tables. J. Royal Stat. Soc. B 34: 187-220.

Credland, P.F.; Wright, A.W., 1990. Oviposition deterrents of *Callosobruchus maculatus* (Coleoptera: Bruchidae). Physiol. Entomol. 15: 285-298.

Ferreira, L.; Pintureau, B.; Voegelé, J., 1979. Un nouveau type d'olfactomètre. Application à la mesure de la capacité de recherche et à la localisation des substances attractives de l'hôte chez les Trichogrammes (Hym.: Trichogrammatidae). Ann. Zool. Ecol. Anim. 11: 271-279.

Gardner, S.M.; Lenteren, J.C. van, 1986. Characterisation of the arrestment responses *of Trichogramma evanescens.* Oecologia 68: 265-270.

Gross, H.R.; Lewis, W.J.; Nordlund, D.A., 1981. *Trichogramma pretiosum:* effect of pre-release parasitization experience on retention in release areas and efficiency. Environ. Entomol. 10: 554-556.

Haccou, P.; Hemerik, L., 1985. The influence of larval dispersal in the Cinnabar Moth *(Tyria jacobaeae)* on predation by the red wood ant *(Formica polyctetia):* an analysis based on the proportional hazards model. J. Animal Ecol. 54: 755-769.

Haccou, P.; Vlas, S.J. de; Alphen, J.J.M. van; Visser, M.E., 1991. Information processing by foragers: effects of intra patch experiences on the leaving tendency of *Leptopilina* heterotoma. J. Animal Ecol. 60: 93-106.

Hemerik, L.; Driesen, G.; Haccou, P., 1993. Effects of intrapatch experiences on patch time, search time and searching efficiency of the parasitoid *Leptopilina clavipes.* J. Animal Ecol. 62: 33-44.

Huis, A. van; Appiah, S.O., 1995. *Uscana lariophaga,* West-African egg parasitoid of the cowpea bruchid beetle *Callosobruchus maculatus*: photoperiod, parasitization and eclosion interactions. Entomol. exp. appl. 76: 1-6.

Huis, A. van; Kaashoek, N.K.; Maes, H.M., 1991a. Biological control of bruchids (Col.: Bruchidae) in stored pulses by using egg parasitoids of the genus *Uscana* (Hymn.: Trichogrammatidae): a review. In: E. Fleurat-Lessard & P. Ducom, (Eds) Proc. 5th Int. Working Conf. Stored-Product Prot., 9-14 September 1990, Bordeaux. INRA. volume I, pp. 99-108.

Huis, A. van; Schütte, C.; Cools, M.H.; Fanget, Ph.; Hoek, H. van der; Piquet, S.P., 1994. The role of semiochemicals in host location by *Uscana lariophaga,* egg parasitoid of *Callosobruchus maculatus.* In: E. Highley, E.J. Wright, H.J. Banks & B.R. Champ (eds), Stored Product Protection. Proc. 6th Int. Working Conf. Stored-product Prot., CAB Int., Wallingford, volume II, pp. 1158-1164.

Huis, A. van; Wijkamp, M.G.; Lammers, P.M.; Klein Goldewijk, C.G.M.; Seeters, J.H.; Kaashoek, N.K., 1991b. *Uscana lariophaga* (Hymenoptera: Trichogrammatidae), an egg parasitoid of bruchid beetle (Coleoptera: Bruchidae) storage pests in West Africa: host-age and host-species selection. Bull. ent. Res. 81: 65-75.

Jackai, L.E.N.; Daoust, R.A., 1986. Insect pests of cowpeas. Ann. Rev. Entomol. 31: 95-115.

Kaiser, L.; Pham-Delegue, M.H.; Backchine, E.; Masson, C., 1989. Olfactory responses of *Trichogramma maidis* Pint. et Voeg.: effects of chemical cues and behavioral plasticity. Journal of Insect Behavior 2: 701-712.

Kaiser, L.; Pham-Delegue, M.H.; Hawlitzky, N.; Masson, C., 1987. Comportement de récherche et d'oviposition de *Trichogramma maidis* Pint. et Voeg. (Hym.: Trichogrammatidae): analyze quantitative en conditions contrôlées. Compte Rendu Acad. Sci., Paris, Série III, 305: 95-99.

Kalbfleisch, J.D.; Prentice, R.L., 1980. The statistical analysis of failure time data. Wiley & Sons, New York, 321 pp.

Laing, J., 1937. Host finding by insect parasites. 1. Observations on the finding of hosts by *Alysia mandicator, Mormoniella vitripennis* and *Trichogramma evanescens.* J. Animal Ecol. 6: 298-317.

Mendel, Z.; Dunkelbaum, E.; Robison, D., 1990. Sexual behavior of *Masucoccus josephi* (Homoptera: Margarodidae). J. Chem. Ecol. 16: 2341-2352.

Messina, F.J.; Kemp, J.L.; Dickson, J.A., 1992. Plasticity in the egg-spacing behavior of a seed beetle: effects of host deprivation and seed patchiness (Coleoptera: bruchidae). J. Insect Beh. 5: 609-621.

Noldus, L.P.J.J.; Lenteren, J.C. van, 1985. Kairomones for the egg parasite *Trichogramma evanescens* Westwood. 2. Effects of contact chemicals produced by two of its hosts, *Pieris brassica* L. and *Pieris rapae* L. J. Chem. Ecol. 11: 793-800.

Noldus, L.P.J.J.; Lenteren, J.C. van; Lewis. W.J., 1991. How trichogramma parasitoids use moth sex pheromones as kairomones: orientation behaviour in a windtunnel. Physiol. Entomol. 16: 313-327.

Nordlund, D.A.; Chalfant, R.B.; Lewis, W.J., 1985. Response of *Trichogramma pretiosum* to volatile synonomes from tomato plants. J. Entomol. Sci. 20: 372-376.

Pajni, H.R.; Gupta, S., 1990. Role of antennal sensillae in perception of sex pheromone in bruchids, *Callosobruchus maculatus* (F.) and *C. chinensis* (L.). Indian J. Exp. Biol. 28: 174-178.

Pak, G.A.; Berkhout, H.; Klapwijk, J., 1991. Do *Trichogramma* look for hosts? *Trichogramma* and other egg parasitoids. Proc. 3rd Int. Symp., 23-27 September, 1990. San Antonio. Les Colloques de l'INRA 56: 77-80.

Prentice, R.L.; Kalbfleisch, J.D., 1978. The analysis of failure times in the presence of competing risks. Biometrics 34: 541-554.

Renou, M.; Hawlitzky, N.; Berthier, A.; Malosse, C.; Ramiandrasoa, F., 1989. Mise en évidence d'une activité kairomonale des oeufs de la pyrale du mais sur les femelles de *Trichogramma maidis*. Entomophaga 34: 569-580.

SAS Institute Inc., 1991. SAS technical report P-217, SAS/STAT Software: the PHREG Procedure version 6. SAS Institute Incorporation, Cary, 59 pp.

Shu, S.; Jones, R.L., 1989. Kinetic effects of a kairomone in moth scales of European corn borer on *Trichogramma nubilale* Ertle & Davies (Hym.: Trichogrammatidae). J. Insect Beh. 2: 123-131.

Snedecor, G.W.; Cochran, W.G., 1989. Statistical methods. Iowa State Univ. Press, Ames, 503 pp.

Steinberg, S.; Dicke, M.; Vet, L.E.M.; Wanningen, R., 1992. Response of the braconid parasitoid *Cotesia (=Apanteles) glomerata* to volatile infochemicals: effects of bioassay set-up, parasitoid age and experience and barometric flux. Entomol. exp. appl. 63: 163-175.

Strand, M.R.; Williams, H.J.; Vinson, S.B.; Mudd, A., 1989. Kairomonal activities of 2-acylcyclohexane-1,3 diones produced by *Ephestia kuehniella* Zeller in eliciting searching behavior by the parasitoid *Bracon hebetor* (Say). J. Chem. Ecol. 15: 1491-1600.

Tanaka, K.; Ohsawa, K.; Honda, H.; Yamamoto, I., 1981. Copulation release pheromone, erectin, from the azuki bean weevil (*Callosobruchus chinensis* L.). J. Pesti. Sci. 6: 75-82.

Teal, P.E.A.; Tumlinson, J.H.; Heath, R.R., 1986. Chemical and behavioral analyses of volatile sex pheromone components released by calling *Heliothis virescens* (F.) females (Lepidoptera: Noctuidae). J. Chem. Ecol. 12: 107-126.

Vet, L.E.M.; Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. Ann. Rev. Entomol. 37: 141-172.

Vet, L.E.M.; Lewis, W.J.; Cardé, R.T., 1995. Parasitoid foraging and learning. In: R.T Cardé & W.J. Bell (eds), Chemical Ecology of Insects 2, Chapman & Hall, London, pp. 65-101.

Vet, L.E.M.; Lewis, W.J.; Papaj, D.R.; Lenteren, J.C. van, 1990. A variable-response model for parasitoid foraging behaviour. J. Insect Beh. 3: 471-490.

Vet, L.E.M.; Papaj, D.R., 1992. Effects of experience on parasitoid movement in odour plumes. Physiol. Entomol. 17: 90-96.

Vinson, S.B., 1984a. Parasitoid-host relationships. In: W.J. Bell & R.T. Cardé (eds), Chemical ecology of insects. Chapman & Hall, London, pp. 205-233.

Vinson, S.B., 1984b. How parasitoids locate their hosts: a case of insect espionage. In: T. Lewis (Ed.) Insect communication. Proc. 12th Symp. Royal Entomol. Soc. London, 7-9 Sept. 1983, Academic Press, London, pp. 325 -348.

Vinson, S.B., 1985. The behaviour of parasitoids. In: G.A. Kerkut & L.I. Gilbert (eds), Comprehensive Insect Physiology, Biochemistry and Pharmacology, Pergamon Press, Oxford, 9: 417-469.

Wysoki, M.; Jong, M. de, 1989. Attraction of *Trichogramma platneri* to eggs of some lepidopterous pests of avocado. Phytoparasitica 17: 315-318.

Host location in stored cowpea by *Uscana lariophaga*

5

Abstract

A good spatial distribution and an efficient host location behaviour are prerequisites for successful biological control of stored product pests. These attributes were investigated in the egg parasitoid *Uscana lariophaga* Steffan (Hym.; Trichogrammatidae), foraging for clusters of *Callosobruchus maculatus* Fab. (Col.; Bruchidae) eggs in experimental cowpea (*Vigna unguiculata* Walp.) storage containers. Females have an innate tendency to move upwards in a cowpea seed stock. Host clusters above the release point have an increased chance to be found by *U. lariophaga* compared to clusters below the site of release, but still, significant numbers of clusters below the release point are also being visited and parasitized. In host clusters with more than 50 eggs, parasitoids are arrested and may spend their entire egg load or foraging time, leaving other clusters unexploited. Host clusters with more eggs are more frequently parasitized, irrespective of the volume of host clusters or the egg density and distribution within those clusters. This increased chance of detection is thought to be the result of an increase in egg odour concentration or in the volume of the odour sphere around clusters with more eggs. This hypothesis was tested in a tube diffusion olfactometer.

This Chapter will be published, in a slightly different form, as:
Alebeek, F.A.N. van; Huis, A. van, 1997. Host location in stored cowpea by the egg parasitoid *Uscana lariophaga* (Hym., Trichogrammatidae). Zeitschrift für angewandte Entomologie (in press).

Chapter 5

Introduction

Movements of several beetle pests through stored wheat was investigated extensively e.g. by Surtees (e.g. 1962, 1964, 1965). Pest populations may form local 'hot spots', in response to temperature and humidity gradients, often near the centre and surface of the stored commodity (e.g. Howe, 1943; Surtees, 1962; Shazali & Smith, 1990). An innate tendency in parasitoids of storage pests to move upwards (e.g. through negative geotaxis) in a mass of stored seeds, is adaptive if pest populations tend to accumulate near the surface or if new infestations generally enter the commodity from above.

Dispersal of natural enemies of stored product pests is a prerequisite for successful biological control in storage systems, in order to locate loci of pest infestations in bulk masses. In recent studies evaluating the characteristics of successful parasitoids for biological control, searching behaviour has been found to be of crucial importance, even more so than the intrinsic rate of increase (Van Roermund, 1995; Van Steenis, 1995).

The egg parasitoid *Uscana lariophaga* Steffan (Hymenoptera; Trichogrammatidae) is being evaluated as a candidate for biological control of bruchid pests (*Callosobruchus maculatus* Fab. and *Bruchidius atrolineatus* Pic.) (Coleoptera; Bruchidae) in stored cowpea (*Vigna unguiculata* Walp.) in West Africa (Van Huis, 1991; Van Huis *et al.*, 1991a). Females of *U. lariophaga* have to find suitable habitats and hosts therein, facing the constraints of their size of 0.4 mm, and a longevity of only 2-3 days. In addition, the parasitoids have to cope with completely different situations during the year. In the rainy season, parasitoids forage in open cowpea fields with relatively low host densities. During the dry season, parasitoids have to search in stocks of dry beans or pods in closed, dark granaries with sometimes high host densities. In a storage habitat, *U. lariophaga* females search for hosts presumably predominantly by walking in the cowpea seed or pod stock. Upon host encounter, wasps demonstrate a specific sequence of parasitization behaviour as described in Van Huis *et al.* (1991b).

Our current research focuses on the foraging behaviour of the parasitoid, to investigate whether its host location behaviour is adapted to a stored cowpea environment. *Uscana lariophaga* recognizes volatiles from uninfested cowpea seeds, and is attracted by odours from freshly laid *C. maculatus* eggs (Chapter 4). Inside a cowpea granary, when the parasitoid is surrounded by cowpea seeds, plant odours often cannot provide directional cues. Therefore, in this article, we explore the question as to how *U. lariophaga* forages for clusters of *C. maculatus* host eggs within a three-dimensional mass of cowpea seeds. The spatial distribution of parasitized host clusters, and the chance of detection for host clusters of different sizes and densities are analysed. In addition, the role of egg odour concentration in the host location process is evaluated.

Materials and methods

General

Details on the rearing and origin of *C. maculatus* and *U. lariophaga* are given in Chapter 4. Cowpea variety 'Black Eyes' from California was used in the experiments. Parasitoid females were 0-16 h old, mated and fed with honey. One hour before the experiment, individual females were given an oviposition experience on a *C. maculatus* egg (0-20 h old). All experiments were carried out at 30±1°C and a RH of 20-40%.

Closed plastic containers of 1 litre (ø = 8 cm, height 20 cm) or 8 litres (ø = 23 cm, height 20 cm), filled with clean cowpea seeds, were used as experimental storage units (Fig. 5.1). Containers were kept in complete darkness. Because of the amount of cowpea seeds involved, beans were re-used throughout one experiment. Between tests, seeds were kept in a clean oven at 45°C for 24-48 h in order to remove possible volatiles left by bruchid egg clusters or walking parasitoids. A gelatine capsule containing one *U. lariophaga* female was placed in the centre of a container. The capsule could be opened from the outside by pulling threads attached to it through small holes in both sides of the container wall.

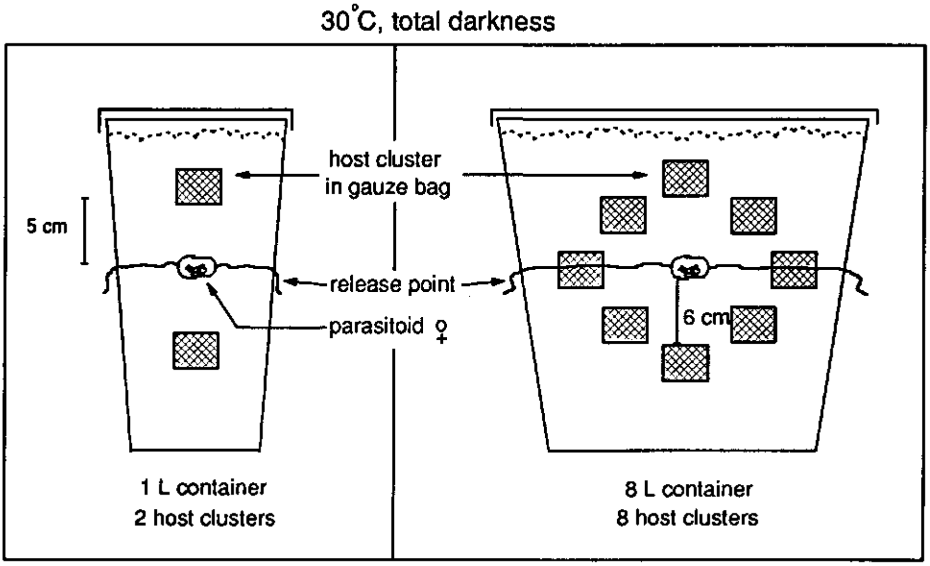

Figure 5.1. Schematic presentation of the experimental setup in the spatial orientation studies. In 8 litre containers, host clusters are here represented in a two-dimensional plane, but in reality, clusters are distributed at equal distances over a three-dimensional sphere around the release point. Containers are filled with clean cowpea seeds. Wasp size is not on scale, actual length of *U. lariophaga* is 0.4 mm.

Spatial orientation studies

To test spatial orientation and dispersal of *U. lariophaga*, individual females were released in experimental containers with clusters of host eggs around the release point. Host egg clusters were used as "baits", with parasitism of eggs indicating arrival of a wasp in a cluster.

Host clusters consisted of fresh *C. maculatus* eggs on 8 cowpea beans in small polyamine gauze bags (mesh width 2 mm). In a seperate test, volumes of host clusters were estimated by wrapping the beans into thin plastic foil and immersing them in a known volume of water. The volume of a cluster with 8 beans is 2.15 cm^3, including the air spaces between beans. Egg densities tested were mainly in the range of 1-70 eggs per cluster. All clusters in one container had approximately the same host density; densities varied over different containers. Precise egg numbers per host cluster could not be counted directly after oviposition, because the transparant eggs are difficult to find on cowpea seeds and because handling would damage the eggs. Therefore, after completion of each experiment, host clusters were recovered from the containers and incubated in glass tubes. Precise egg numbers (parasitized and unparasitized) could be counted four to five days later, as parasitized eggs became black and unparasitized eggs became white. Damaged eggs were ignored.

Orientation was investigated within 1 litre and 8 litre containers, and wasps were allowed to forage and move through the bean stock during 24 hours. In the 1 litre containers, two host clusters were offered, one 5 cm below and one 5 cm above the release point (Fig. 5.1), corresponding to approx. 125 female body lengths of *U. lariophaga* (0.4 mm). For the analysis, only containers with one cluster parasitized were used. The number of containers with either the top- or the bottom-cluster parasitized were compared with a Binomial distribution, assuming no preference in *U. lariophaga* females for going up- or downwards (Chi-square test, H_o: $p = 0.5$).

In 8 litre containers, 8 host clusters were distributed over a theoretical sphere (ø 13 cm) around the release point (Fig. 5.1). Distance between host clusters was 8 cm, the distance between each cluster and the release point was 6 cm. The distribution of parasitized clusters in containers with only one parasitized cluster (Fig. 5.2A) was analysed with a Goodness of Fit test (G-statistic), assuming random chances for clusters to be parasitized (H_o: chance of detection is 1/8 : 3/4 : 1/8 for top-clusters : the total of six in-between-clusters -i.e. upper, centre and lower clusters- : bottom-clusters respectively). The distribution of parasitized clusters in 8 litres containers with multiple clusters parasitized was described also (Fig. 5.2B), but could not be analysed statistically because parasitism of clusters is not independent within containers.

Host cluster location in cowpea stock

The experimental setup to study *U. lariophaga* host cluster location behaviour in stored cowpea was studied in 1 litre containers filled with clean cowpea seeds. A single host cluster was put 5 cm above the release point. Different containers had clusters with different numbers of *C. maculatus* eggs. Wasps were allowed to search and parasitize for 4 h, after which the host clusters were recovered.

Three different egg distributions within host clusters were created by varying the numbers of seeds and eggs in a cluster (see also Chapter 7, and Figure 7.1):

Series I. Even egg distribution, i.e. a fixed egg density (one egg per bean) and variable cluster size (2-64 beans) depending on the total number of eggs in the cluster.

Series II. Clumped egg distribution, i.e. a fixed cluster size (8 beans) and a variable egg density (all beans within one cluster carried approximately an equal numbers of eggs; between 1 and 15 eggs per bean depending on the total number of eggs in the cluster).

Series III. 'Random' egg distribution, i.e. a fixed egg density (one egg per bean) and a fixed cluster size (64 beans). This distribution deviates from real randomness because there is never more than one egg per bean, and the distribution within a cluster gradually becomes even at higher densities (32 and 64 eggs per cluster).

Egg densities aimed at were 2, 4, 8, 16, 32 and 64 eggs per cluster. Cluster volume ranged from 0.46 cm^3 for 2 beans, 2.15 cm^3 for 8 beans, 9.86 cm^3 for 32 beans, to 19.69 cm^3 for 64 beans (air spaces included). The density of 8 eggs per cluster in series I and II, and density 64 in series I and III are identical, and the same datasets of these densities were used in the analysis of the different series. After each experiment, host clusters were incubated in glass tubes until, 4-5 days later, parasitized eggs became black and all eggs could be counted.

Olfaction studies

Results of host location studies gave rise to the hypothesis that different amounts of egg odour emitted by host clusters, depending on the number of host eggs in those clusters, would explain the different chances of detection by *U. lariophaga*. Therefore, we tested whether *U. lariophaga* would respond to odour concentration differences brought about by different numbers of *C. maculatus* eggs in a tube diffusion olfactometer. The olfactometer consisted of an open glass tube (length 100 mm, internal Ø 8 mm) with an introduction hole in the centre of the tube (at 50 mm, Ø 2 mm). Disposable plastic caps were inserted in the open ends. One cap held freshly laid *C. maculatus* eggs (0 - 16 h old) as odour source on two small glass marbles, the other cap as a control contained two clean glass marbles (no-choice test). In the tube olfactometer, the caps were closed with fine polyamide gauze (mesh width 0.2 mm) allowing diffusion of egg odours, but preventing tested parasitoids to reach the odour source. Before re-using the olfactometer tubes and glass marbles, they were cleaned with n-hexane, rinsed with hot water, and dried in a clean stove at 50°C for 24 h. The experimental setup was placed on a white surface under a white cloth, with a light intensity of 1400 Lux.

The position of the parasitoid in the tube and its behaviour (walking, sitting still) were recorded continuously during 15 min. Three different *C. maculatus* egg densities were tested as odour source: 2, 8 or (approx.) 64 eggs. Because diffusion rates of the unidentified odours were unknown, two waiting periods (time between introduction of the odour source and release of the parasitoid, for the establishment of an odour gradient) were tested: 10 and 30 min. Treatment effects (egg density and/or waiting time) on the total time spent walking in the odour and control half of the tubes and on the arrival time at either end of the tube were tested with non-parametric procedures using SPSS software.

Results

Spatial orientation

In 1 litre containers, 69 out of 210 containers did not have any parasitism and 76 had both clusters parasitized. Of the remaining 65 containers, 56 (86%) had only the top cluster, and 9 (14%) only the bottom cluster parasitized, indicating a strong preference to move upwards (Chi-square, $p < 0.001$).

The same preference for top-clusters was observed in the 8 litres containers with 8 host clusters around the release point (Figure 5.2). In containers with only one out of eight clusters parasitized ($n = 52$), significantly more often the top cluster was parasitized (46% of all cases), compared to 37% of the containers with one of the in-between (upper-, centre- or lower-clusters together) clusters and 17% of the containers with the bottom cluster parasitized (Fig. 5.2A). This distribution deviates significantly from a random selection of host clusters (H_0: 1/8 : 3/4 : 1/8, Goodness of Fit test, $G = 41.24$, $p < 0.001$). In containers with several host clusters parasitized ($n = 54$), 81% of all top clusters were parasitized, whereas approx. 40% of all other clusters were parasitized (Fig. 5.2B) (no test applicable).

Apparently, the majority of wasps first visits the top-cluster. Only if time and/or egg load allow for, wasps may also parasitize eggs in the lower host cluster. The distribution of parasitized host clusters is thus partly guided by the parasitoid's egg load and/or the time spent handling host eggs in a cluster. If cluster size increases in 8 litre containers, the first cluster has a higher chance to be found (i.e. parasitized), but fewer additional host clusters in the same container are parasitized. In containers with host clusters of less than six eggs, handling time and or initial egg load allowed for parasitism in, on average, 3.0 clusters per container, with a maximum of six parasitized clusters per container. In containers with clusters of 50-120 eggs, on average, only 1.0 cluster per container showed parasitism, with a maximum of two clusters in one container.

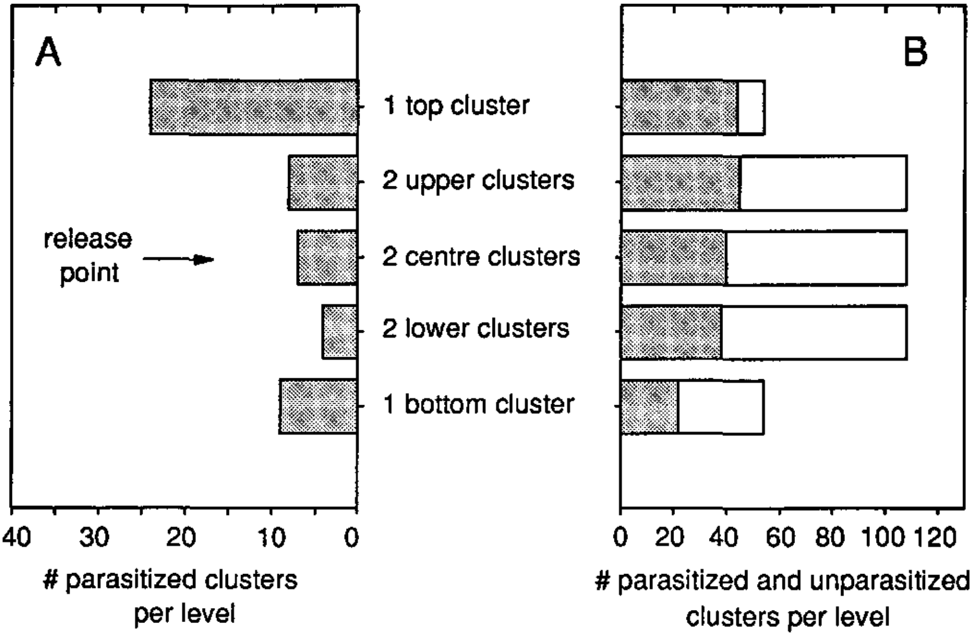

Figure 5.2 Spatial orientation of *U. lariophaga* females released in a 8 litre cowpea seed mass, with 8 host clusters situated at different levels around the release point. Distance between top- and bottom-cluster is 12 cm. **A.** Containers with only one host cluster parasitized (n = 52); the number of parasitized clusters (hatched bars) at different levels (Goodness of Fit test, $G = 41.24$, $P < 0.001$). **B.** Containers with several host clusters parasitized (n = 54); the number of parasitized (hatched bars) and unparasitized clusters (open bars) at each level (no test applicable).

Host location in cowpea stock

The percentage of containers in which the host cluster was found has been calculated for different host cluster sizes and for the three different egg distributions separately (Table 5.1). The number of *C. maculatus* eggs in a host cluster influences the chance that a cluster is found by an *U. lariophaga* female. Different egg distributions (i.e. even, clumped and random) within clusters did not significantly influence the chance of discovery by *U. lariophaga* (Table 5.1). Using these data, lumping the three egg distribution series together, linear regression yielded a significant positive correlation ($Y = 38.3 + 0.84\ X$, $n = 11$, $r^2 = 0.83$, $p < 0.05$) between the number of host eggs in a cluster (X) and the percentage clusters found (Y). Thus, clusters with more eggs have a higher chance of being parasitized.

Table 5.1 The percentage of clusters found (i.e. with parasitism) by *U. lariophaga*, for different cluster sizes in the three different egg distributions. n is the total number of containers in each cluster size.

	I even		II clumped		III random	
# eggs per cluster	% clusters found	n	% clusters found	n	% clusters found	n
1 - 5	41.1	73	44.4	36	33.8	74
6 - 9	44.4	36	44.4	36	47.2	36
10 - 20	--	--	58.3	36	--	--
21 - 35	66.7	36	75.0	36	58.3	36
36 - 70	82.9	35	91.4	35	82.9	35
total[1]	55.0 a	180	62.2 a	179	50.6 a	181

1: Means followed by the same letter are not significantly different (*G*-test for homogeneity of percentages).

Olfaction studies

Total walking time was higher in the treated half of the tube than in the control half for all treatments, significantly so in four out of six treatments (Table 5.2), indicating an arrestment effect of egg odour. For both waiting times (time between introduction of odour source and test of the parasitoid, i.e. 10 and 30 min.), mean walking time in the odour side increased with increasing egg density but, due to the high variability, this effect was not significant (Kruskal-Wallis one-way ANOVA). The ratio of average time spent in the treated side to time spent in the control side is always more than 1, and is highest for the highest egg density, for both 10 and 30 min waiting time (Table 5.2). Waiting time did not influence the total amount of time walked in the odour half (Mann-Whitney U - Wilcoxon Rank Sum W Test). Arrival times at the treated end of the tube were not significantly influenced by egg density nor by waiting times (results not shown here).

Discussion

Orientation studies

Uscana lariophaga predominantly moves upwards in cowpea seeds: top clusters were significantly more often parasitized than clusters below the release point. However, the parasitoid is also capable of (re)descending to locate host clusters below, if foraging time and/or the parasitoid's egg load allow for. In a cluster with many eggs, *U. lariophaga* females are arrested and spend their complete egg load, leaving other host clusters unexploited (see also Chapter 6).

Table 5.2. Mean total time (s) (range between brackets) spent walking per *U. lariophaga* female in the control half and odour half of a tube diffusion olfactometer, under different waiting times and *C. maculatus* egg densities. Waiting time is the period between introduction of the odour source and introduction of the parasitoid to be tested.

waiting time (min)	egg density	# wasps tested	total time (s) spent walking in: control side	odour side	test[1]	ratio T/C[2]
10	2	33	227 (0-557)	342 (0-775)	*	1.51
10	8	32	292 (0-773)	398 (0-872)	ns	1.36
10	64	34	249 (0-738)	430 (0-866)	*	1.73
30	2	33	266 (0-861)	307 (0-892)	ns	1.15
30	8	34	237 (0-618)	370 (0-769)	*	1.56
30	64	32	235 (0-698)	412 (0-867)	*	1.75

1: Odour versus control, Wilcoxon Matched-pairs Signed-ranks Tests, ns = not significant. * = $P < 0.05$.

2: T/C = Mean time spent in treated half : mean time spent in control half.

Maximum dispersal distances can be deduced from containers with a maximum of 6 host clusters visited. Results show that distances of at least 50 cm in 24 h can be travelled, which is relatively much, given the size (0.4 mm) of *U. lariophaga*.

The upward orientation of *U. lariophaga* corresponds to the behaviour of other parasitoids attacking stored product pests. When *Trichogramma* spp. were released on top of an inshell peanuts column or a wheat column, to test their penetration ability towards host egg clusters at different levels, parasitism decreased significantly over increasing depth (Brower, 1990; Schöller, 1994), while dispersal in the light was identical to that in darkness (Brower, 1990). *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae), a larval parasitoid of several stored product beetles, moved predominantly upwards and, if released on top, remained near the wheat surface (Press, 1988, 1992). *Eupelmus vuilleti* (Craw) (Hymenoptera; Eupelmidae), a larval parasitoid of bruchids in stored cowpea, also predominantly moved upwards in an uninfested cowpea column (Cortesero, 1994). In this study, *E. vuilleti* located hosts over 77 cm within 48 h after release, and did so much more efficiently in the upward direction than downwards. Kairomones of the larvae, their faeces, and pupae of bruchids attract *E. vuilleti* females (Cortesero, 1993). *Dinarmus basalis* (Rond) (Hymenoptera; Pteromalidae), another larval parasitoid of bruchids, also moves upwards in a column filled with cowpea seeds more readily than downwards. If released on top of a column with clean seeds (no hosts present), almost no wasps descended downwards (A. Douma, Niamey, pers. comm.).

Host location studies

The positive correlation between the number of eggs in a host cluster and the chance that such a cluster is found (i.e. parasitized) by *U. lariophaga* (Table 5.1), was also found in other experiments with 4 h foraging time (see Chapter 8). Such a correlation could not be demonstrated in a study with 24 h foraging time duration (see Chapter 6). In 24 h experiments, 36%-44% of the containers had more than one cluster parasitized, indicating that wasps apparently had plenty of time to search for host clusters within the cowpea stock. This may have obscured differences in encounter rates between clusters with fewer or more host eggs.

In random walking predators or parasitoids, an increase in the rate of detection of host clusters can result from a larger volume of a cluster, or from an increased host density within the cluster (e.g. Van Roermund *et al.*, 1996). However, in *U. lariophaga* the increased detection chance of host clusters with more eggs is not brought about by a larger volume of those clusters, as can be seen in series II (clumped egg distribution), where the cluster volume of 8 beans (2.15 cm^3) remains constant (Table 5.1). Nor is an increased chance for parasitism in a cluster the result of increased host density within the cluster; e.g. for densities of 6-9 eggs per 8 beans (in series II) or per 64 beans (in series III) the detection chance is equal (Table 5.1). Therefore, we hypothesize that the increase in detection chance is brought about by increased amounts of egg odour emitted by clusters with more eggs. This can either cause a steeper odour gradient around the host cluster, facilitating directional movements (chemotaxis, see Bell *et al.*, 1995) of the wasp; or it causes an increase in the volume or diameter of the odour "sphere" around the host cluster, increasing the chance of encounter with this odour sphere. This hypothesis was tested in the olfaction studies with the tube olfactometer.

Olfaction studies

Callosobruchus maculatus egg odour caused a significant arrestment of *U. lariophaga* females in the odour side in four out of six treatments, confirming earlier reports (Van Huis *et al.*, 1994; Chapter 4). Furthermore, walking time in the treated side and the T/C ratio (Table 5.2) increased with increasing egg density, suggesting that the effect of egg odour becomes stronger with higher numbers of eggs. However, due to the high variability in these no-choice tests, egg density effects were not significant and it could not be proven that different concentrations of egg-odour determine the chances of detection of egg clusters with different host egg numbers. Choice tests, offering different egg densities at the ends of the tube olfactometer are more appropriate to demonstrate differences in attraction and should be repeated to verify our hypothesis.

We have indications that *C. maculatus* egg (and other?) odour adsorbed to the glass olfactometer tubes and glass marbles, and that these contaminations were not effectively removed by our cleaning procedure (see Materials and Methods). In another experiment (Van Huis, pers. comm.), we compared new glass marbles and olfactometer tubes, that had never been used before, with previously used marbles and tubes that were cleaned as described earlier. In new tubes, using new marbles as controls, *U. lariophaga* females were significantly attracted to *C. maculatus* egg odour. However, in the parallel test with either old, cleaned tubes or old, cleaned glass marbles, this attraction was no longer significant.

Accumulation of odour traces on the wall of the olfactometer and interference with the parasitoids response was also mentioned by Van Huis *et al.* (1994). In the course of previous experiments, as mentioned in Chapter 4, we observed an increase in the number of *U. lariophaga* females trying to fly out of a diffusion olfactometer. Only a small part of this variation could be explained by changes in barometric pressure. An alternative hypothesis would be the accumulation of egg odour traces on the glass wall of the olfactometer, preventing wasps from location the odour source.

Parasitoid or predator responses depending on the concentration of host cues have been reported before. In *Chelonus* sp. (Hymenoptera, Cheloninae), a larval parasitoid of the pink bollworm, the proportion of females responding to wing scale extracts of three lepidopterous hosts was dependent on the concentration of the chemicals (Chiri & Legner, 1982). Following the release of *Dinarmus basalis* females at the bottom of a 66 cm high column filled with cowpea seeds, the number of females found on top in 24 h increased with the number of *Bruchidius atrolineatus* (Pic) (Coleoptera; Bruchidae) host larvae present on top (A. Douma, Niamey, pers. comm.) (Huignard, 1996). It is not clear whether this is the result of attraction or an arrestment near the hosts. In the predatory mite *Typhlodromus pyri*, the percentage of mites responding in an Y-tube olfactometer appears to increase with increasing numbers of prey offered (Dicke, 1988). Also, the preference for odours from one prey species could be nullified or even reversed by increasing the numbers of the least preferred prey species (Dicke, 1988; Dicke *et al.*, 1988).

Uscana lariophaga females find host clusters with more eggs more readily than smaller clusters. In addition, they show a strong arrestment response, leading to aggregation in high density host clusters. At the population level within a multiple patch habitat, this aggregation behaviour may lead to an overall density dependent parasitism of host patches (e.g. Hassell & May, 1974; Hassell *et al.*, 1977; Walde & Murdoch, 1988). This would provide stability to the bruchid-parasitoid interaction, and corresponds to the observations of coexistence of bruchid and *Uscana* populations over several months (Lammers & Van Huis, 1989).

Results of the current tests demonstrate that *U. lariophaga* is not limited in her dispersal abilities, and that she is able to locate several host clusters within a cowpea seed stock. *Uscana lariophaga* uses host egg odours to locate host clusters, and detects host clusters with more eggs more readily than clusters with fewer eggs.

Acknowledgements

I thank Karin Koning, Edwin de Korte and Martijn Bezemer for their assistance in the orientation and host location studies, Emana Getu Dagage and Sandor van Voorst for their assistance in the olfaction experiments, and Mariët Cools and Gerard Pesch for maintaining the rearings. Joop van Lenteren, Arnold van Huis and Marcel Dicke gave valuable comments on the manuscript. G. Gort (WAU Department of Mathematics) advised on the statistical analyses. The financial support by the European Union (project TS2 029 F ESF and TS3 CT 930201) is gratefully acknowledged.

References

Bell, W.J.; Kipp, L.R.; Collins, R.D., 1995. The role of chemo-orientation in search behaviour. In: R.T. Cardé & W.J. Bell (eds), Chemical Ecology of Insects 2. Chapman & Hall, New York, pp. 105-152.

Brower, J.H., 1990. Host location ability of *Trichogramma pretiosum* Riley in inshell peanuts under laboratory conditions. J. Agric. Entomol. 7: 265-273.

Chiri, A.A.; Legner, E.F., 1982. Host-searching kairomones alter behaviour of *Chelonus* sp. nr. *curvimaenlatus*, a hymenopterous parasite of the pink bollworm, *Pectinophora gossypiella* Sanders. Environ. Entomol. 11: 452-455.

Cortesero, A.M., 1993. Response of the parasitoid *Eupelmus vuilleti* to the odours of the phytophagous host and its host plant in an olfactometer. Entomol. exp. appl. 69: 109-116.

Cortesero, A.M., 1994. La récherche de l'hôte chez *Eupelmus vuilleti* (Crw.). Analyse des rélations tritrophique entre la plante (*Vigna unguiculata* Walp), l'hôte (*Bruchidius atrolineatus* Pic) et le parasitoide. Doctoral Thesis, Université François Rabelais, Tours.

Dicke, M., 1988. Prey preference of the phytoseiid mite *Typhlodromus pyri*. 1. Response to volatile kairomones. Exp. Appl. Acarol. 4: 1-13.

Dicke, M.; Sabelis, M.W.; Jong, M. de, 1988. Analysis of prey preference in phytoseiid mites by using an olfactometer, predation models and electrophoresis. Exp. Appl. Acarol. 5: 225-241.

Hassell, M.P.; Lawton, J.H.; Beddington, J.R., 1977. Sigmoid functional responses by invertebrate predators and parasitoids. J. Animal Ecol. 46: 249-262.

Hassell, M.P.; May, R.M., 1974. Aggregation of predators and insect parasites and its effect on stability. J. Animal Ecol. 43: 567-594.

Howe, R.W., 1943. An investigation of the changes in a bin of stored wheat infested by insects. Bull. ent. Res. 34: 145-158.

Huignard, J. (ed), 1996. Biological control of bruchid insect pests of cowpea in West Africa. Contract no. TS 3 CT 930201. Progress Report European Union, June - December 1995, Université François Rabelais, Tours.

Huis, A. van, 1991. Biological methods of bruchid control in the tropics: a review. Insect Sci. Applic. 12: 87-102.

Huis, A. van; Kaashoek, N.K.; Maes, H.M., 1991a. Biological control of bruchids (Col.: Bruchidae) in stored pulses by using egg parasitoids of the genus *Uscana* (Hym.: Trichogrammatidae): a review. In: F. Fleurat-Lessard & P. Ducom (eds), Proc. 5th Int. Working Conf. Stored-product Prot. Bordeaux, volume I, pp. 99-107.

Huis, A. van; Schütte, C.; Cools, M.H.; Fanget, Ph.; Hoek, H. van der; Piquet, S.P., 1994. The role of semiochemicals in host location by *Uscana lariophaga*, egg parasitoid of *Callosobruchus maculatus*. In: E. Highley, E.J. Wright, H.J. Banks & B.R. Champ (eds), Stored Product Protection. Proc. 6th Int. Working Conf. Stored-product Prot., CAB International, Wallingford, volume II, pp. 1158-1164.

Huis, A. van; Wijkamp, M.G.; Lammers, P.M.; Klein Goldewijk, C.G.M.; Seeters, J.H. van; Kaashoek, N.K., 1991b. *Uscana lariophaga* (Hymenoptera: Trichogrammatidae), an egg parasitoid of bruchid beetle (Coleoptera: Bruchidae) storage pests in West Africa: host-age and host-species selection. Bull. Entomol. Res. 81: 65-75.

Lammers, P.M.; Huis, A. van, 1989. *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae), egg parasitoid of the stored insect pests *Callosobruchus maculatus* (F.) and *Bruchidius atrolineatus* Pic. (Col.: Bruchidae): population studies in the field and in storage in Niger. Proc. Int. Conf. Int. Pest Manage. 3: 1013-1022.

Press, J.W., 1988. Movement of a weevil parasitoid, *Anisopteromalus calandrae* (Howard), within a column of wheat in relation to host location (Note). J. Agric. Entomol. 5: 205-208.

Press, J.W., 1992. Comparative penetration efficacy in wheat between the weevil parasitoids *Anisopteromalus calandrae* and *Choetospila elegans* (Hymenoptera: Pteromalidae). J. Entomol. Sci. 27: 154-157.

Roermund, H.W.J. van, 1995. Understanding biological control of greenhouse whitefly with the parasitoid *Encarsia formosa*. From individual behaviour to population dynamics. Doctoral Thesis, Wageningen Agricultural University, Wageningen.

Roermund, H.J.W. van; Lenteren, J.C. van; Rabbinge, R., 1996. Analysis of the foraging behaviour of the whitefly parasitoid *Encarsia formosa* in an experimental arena: a simulation study. Jl. Insect Beh. (submitted).

Schöller, M.; Reichmut, C.; Hassan, S.A., 1994. Studies on biological control of *Ephestia kuehniella* Zeller (Lep.: Pyralidae) with *Trichogramma evanescens* Westwood (Hym.: Trichogrammatidae) - host-finding ability in wheat under laboratory conditions. In: E. Highley, E.J. Wright, H.J. Banks & B.R. Champ (eds), Stored Product Protection. Proc. 6th Int. Working Conf. Stored-product Prot., CAB International, Wallingford, volume II, pp. 1142-1146.

Shazali, M.E.H.; Smith, R.H., 1990. Ecological succession of four insect pests of stored sorghum in a laboratory ecosystem. Angew. Zool. 1: 93-105.

Steenis, M. van, 1995. Evaluation and application of parasitoids for biological control of *Aphis gossypii* in glasshouse cucumber crops. Doctoral Thesis, Wageningen Agricultural University, Wageningen.

Surtees, G., 1962. Laboratory studies on dispersion behaviour of adult beetles in grain. I.- The grain weevil, *Sitophilus granarius* (L.) (Coleoptera, Curculionidae). Bull. ent. Res. 54: 147-159.

Surtees, G., 1964. Laboratory studies on dispersion behaviour of adult beetles in grain. VI.- Three-dimensional analysis of dispersion of five species in a uniform bulk. Bull. ent. Res. 55: 161-171.

Surtees, G., 1965. Ecological significance and practical implications of behaviour patterns determining the spatial structure of insect populations in stored grain. Bull. ent. Res. 56: 201-213.

Walde, S.J.; Murdoch, W.W., 1988. Spatial density dependence in parasitoids. Ann. Rev. Entomol. 33: 441-466.

Egg-limited functional response of *Uscana lariophaga* 6

Abstract

The functional response of the egg parasitoid *Uscana lariophaga* Steffan (Hymenoptera: Trichogrammatidae) to eggs of its host *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae) was investigated in storage containers filled with a cowpea (*Vigna unguiculata* Walp.) seed mass. Foraging time was limited to 4 or 24 h. These indirect experiments were supplemented by direct observations of the parasitoid's handling time, egg-laying capacity and initial egg load. The foraging process of *U. lariophaga* can be divided into two distinct stages: the process leading to detection of host clusters and, after arrival within a host cluster, the response of the parasitoid to the host density within the cluster. The chance that a cluster is found by *U. lariophaga* appeared independent of the number of host eggs per cluster, but was influenced by the available foraging time. Within a host cluster, *U. lariophaga* demonstrated a Holling II type functional response. Parasitoids were strongly arrested in host clusters, leading to high levels of parasitism. Direct observations proved that handling time was not a limiting factor, but that *U. lariophaga*'s initial egg load and egg maturation rate limited the plateau level of her functional response. As such, direct observations were essential for a correct interpretation of the mechanisms underlying the shape of the functional response.

This chapter will be published, in a slightly modified form, as:
Alebeek, F.A.N. van; Koning, C.M.; Korte, E.A.P. de; Huis, A. van, 1996. Egg-limited functional response of *Uscana lariophaga*, egg parasitoid of bruchid beetle pests in stored cowpea. Entomologia Experimentalis et Applicata (in press).

Introduction

In West Africa, stored cowpea seeds (*Vigna unguiculata* Walp.) are heavily attacked by bruchid seed beetles (*Callosobruchus maculatus* Fab. and *Bruchidius atrolineatus* (Pic)). In a survey of cowpea stored for 3-4 months in Niger, on average 48-80% of the cowpea seeds were attacked by bruchids (see Chapter 2). In search for appropriate control methods suitable for subsistence farming conditions, the egg parasitoid *Uscana lariophaga* Steffan (Hymenoptera; Trichogrammatidae) was evaluated as a candidate for a conservation strategy of biological control (van Huis *et al.*, 1991a). In a conservation strategy, environmental manipulations aim at increasing the impact of native natural enemies on indigenous pest populations (Dent, 1991; Dutcher, 1993).

We focused on the searching behaviour of this parasitoid. Within the constraints of a longevity of a few days and a size of 0.4 mm, *U. lariophaga* females have to find suitable habitats and hosts therein. In addition, the parasitoids have to cope with completely different situations during the year. In the rainy season, parasitoids forage in open cowpea fields with relatively low host densities. During the dry season, parasitoids have to search in dry beans or pods in closed, dark granaries with sometimes high host densities. The reaction of an individual parasitoid to different host egg densities (functional response) will be reported here.

Three basic types of functional response have been defined (Holling, 1959a,b, 1961). A parasitoid's capability to locate hosts at low densities has become one of the selection criteria for testing candidates for biological control (van Lenteren, 1986; Pak, 1988; Minkenberg, 1990; see Chapter 3). In addition, the functional response is an essential element of population models simulating the parasitoid's impact on the host population. When recording the behaviour of individual parasitoids in functional response experiments, information on host discrimination, handling times, and patch time allocation can be obtained (van Lenteren & Bakker, 1978). However, to measure the functional response of *U. lariophaga*, we have to take into account that they forage in a three dimensional cowpea seed mass. Therefore, the functional response was tested in containers filled with beans, thereby losing the ability of direct behaviourial observations which could be carried out in a 2-dimensional set-up. The functional response experiments were supplemented with direct observations on the handling time, initial egg load and egg laying capacity of the parasitoid.

Materials and methods

General

The rearing of both *C. maculatus* and *U. lariophaga* was started in December 1990 with material originating from the Niamey region in Niger. Beetles were reared in Petri-dishes on cowpea seeds (*Vigna unguiculata* Walp.), variety 'California Black Eyes', at densities of 2-4 larvae per bean. The rearing was held at a L12:D12 photoperiod, with temperatures 35±1°C during the day and 25±1°C at night. Fresh *C. maculatus* eggs (0-24 h old) laid on cowpea seeds were used in all experiments. Parasitoids were reared on *C. maculatus* eggs (0-48 h old) on cowpea seeds in glass tubes, and fed with honey. Temperature was 30±1°C, and photoperiod L12:D12.

Unless stated otherwise, all wasps used in the experiments were mated, fed and 0-24 h old. One hour before the experiment, individual females were given one oviposition experience on a fresh *C. maculatus* egg. A gelatine capsule containing one *U. lariophaga* female was placed in the centre of a container (see Chapter 5, Fig. 5.1). In order to release the wasp in the bean stock, the capsule could be opened from outside by pulling threads attached to this capsule through small holes in the sides of the container.

Host clusters

Host clusters consisted of fresh *C. maculatus* eggs on eight cowpea seeds in small polyamine gauze bags (mesh width 2 mm). Clusters in one container had approximately the same host density; densities varied over different containers. Egg densities tested ranged from 2 to 120 eggs per cluster of eight seeds (see below). This closely fits egg densities observed in a survey of cowpea storage units in Niger, where egg densities encountered were within a range of 0-150 eggs per 8 seeds, with the median at 2.5 eggs and the average at 12 eggs per 8 seeds (Chapter 2). Precise egg numbers per host cluster could not be counted directly after oviposition, because handling would damage the eggs. Therefore, after completion of each experiment, host clusters were recovered from the containers and incubated in glass tubes. Four days later, parasitized eggs became black and unparasitized eggs became white in colour and precise egg numbers (parasitized and unparasitized) could be counted. Damaged eggs were ignored.

Functional response experiments

All experiments were carried out at 30±1°C and a RH of 20-40%. Closed plastic containers filled with clean cowpea seeds and placed in total darkness, were used as experimental units. Because of the amount of cowpea seeds involved, beans were re-used throughout one experiment. Between tests, seeds were kept in a clean oven at 45°C for 24-48 h in order to remove possible volatiles left by bruchid egg clusters or walking parasitoids. Three series of functional response experiments were carried out, one after another, reflecting an evolution in thinking about an appropriate experimental setup to test the functional response of *U.*

lariophaga under "quasi-natural" conditions. Information from the first and second experiment helps to understand the setup and results from the last experiment. Each experiment was run with new, clean cowpea seeds at the start.

Experiment 1

Containers (n=210) of 1 litre (ø = 8 cm, height 20 cm), filled with clean cowpea seeds, were used as experimental units (see Chapter 5, Fig. 5.1). Two host clusters with approximately the same egg density were offered per container; one cluster 5 cm above and one 5 cm below the release site of the wasp in the cowpea seed mass. Egg densities tested were mainly in the range of 1-50 eggs per cluster. Due to problems in the *C. maculatus* rearing at that time, few clusters with higher densities (50-120 eggs) could be obtained.

Wasps were allowed to forage for 24 h (fixed time). During the experiment, wasps could not leave the container, and therefore repeated visits to the same host cluster may have occurred. This may result in over-exploitation of the clusters and an overassessment of the response at lower densities (e.g. van Lenteren & Bakker, 1976). At the end of the experiment, it appeared that in 36% of the cases both clusters per container were parasitized. This suggests that *U. lariophaga* females had ample time to exploit the first host cluster, and subsequently to forage the other cluster. Therefore, a second experiment was designed with a larger container and multiple host clusters, to reduce the chance that the searching female returns to a previously exploited cluster.

Experiment 2

Containers (n=125) of 8 litre (ø = 23 cm, height 20 cm), filled with clean cowpea seeds were used. Within the cowpea seed mass, 8 host clusters were distributed over a theoretical sphere (ø 13 cm) around the release point (see Fig. 5.1). Female parasitoids were allowed to search and parasitize for 24 h. The larger volume and multiple patches offered alternative foraging space to parasitoids leaving a visited cluster. As such, parasitism within a single cluster constitutes a variable-time experiment, in which the parasitoids themselves decided if and when to leave (Walde & Murdoch, 1988). In order to achieve a homogeneous, three-dimensional arrangement of the host clusters, an open cubical structure of metal wire was used. Host clusters were attached to each corner of the cubical, and it was positioned with one corner facing downwards and another one facing upwards. Distance between host clusters was 8 cm, the distance between each cluster and the release point was 6 cm. Egg densities tested were around 4, 16 and 64 eggs per cluster. They were precisely determined at the end of each experiment (see above) and ranged between 2 and 120 eggs. Three replicates of each egg density were observed every day, until approx. 40 replicates were obtained.

At the end of the experiment, 44% of all containers (n=125) had more than one cluster parasitized, especially containers with low density clusters. At lower densities, *U. lariophaga* foraged widely within the container and, despite the larger container and more host clusters compared to Experiment 1, repeated visits to the same host cluster may still have occurred. Therefore, a third experiment was done with reduced foraging time.

Experiment 3

This was a copy of Experiment 2, except that the available foraging time for the *U. lariophaga* females was reduced to 4 h only. Densities tested were 4 and approximately 64 eggs per cluster (in total 78 observations). Now, only 3 out of 63 containers showed more than one cluster with eggs parasitized. Repeated visits to the same host cluster therefore seem unlikely. In this experiment, parasitism within a single patch can again be regarded as a variable time experiment (with a maximum of 4 h), in which the parasitoids themselves decided if and when to leave (Walde & Murdoch, 1988).

Direct observations

Several factors might explain the shape of the functional response curve, among which the handling time, egg laying capacity, initial egg load and egg maturation rate. Since these could not be observed in our experiments in containers, the functional response experiments were supplemented with direct observations and measurements of parasitoid behaviour.

Handling time

The handling time of parasitized and unparasitized eggs was estimated by detailed behaviourial observations of individually parasitizing females, using an event recorder with The Observer software (Noldus, 1991) for time registration. Each female was allowed to forage on the surface of cowpea cotyledons carrying 18-21 h old *C. maculatus* eggs, while recording the sequence and duration of behavioral traits (van Huis *et al.*, 1991b). Handling time was defined as the time from first contact with a host until the female abandoned the host. Ten ovipositions from each of twenty females were recorded.

Egg laying capacity

Another determinant of the functional response is the egg laying capacity. For *U. lariophaga* this was determined by offering individual females a surplus (> 150) of fresh *C. maculatus* eggs on about 20 beans in a small glass tube (ϕ = 2 cm, length 7 cm) during 4 h. Females (0-16 h old) (n=35) had one oviposition experience, and had access to a drop of honey. The egg laying capacity was also determined over 24 h oviposition time, by offering 40-60 fresh *C. maculatus* eggs on cowpea cotyledons in a petri dish (ϕ = 9 cm) to individual females (1-17 h old) (n=33). The number of parasitized eggs, colouring black after four days of incubation, is taken as the number of eggs laid per *Uscana* female, assuming perfect host discrimination and no immature parasitoid mortality.

Initial egg load

The initial egg load and egg maturation rate are also among the factors determining the upper limit (plateau level) of the functional response curve. The initial egg load of *U. lariophaga* was determined from 13-16 h old, mated females without prior oviposition experience (n=27). Individual wasps were placed in a droplet of water on a microscope slide and gently squeezed with a cover slide (18 x 18 mm). The number of eggs squeezed out of the ovarioles was counted under a light microscope at 40x and 100x magnification.

Analyses

In Experiment 1, out of 210 containers, 69 had no cluster parasitized, 76 had both clusters parasitized, 56 had only the top cluster parasitized and only 9 had the bottom cluster parasitized (see Chapter 5). From this, it follows that *U. lariophaga* has a strong tendency to move upwards (in containers with only one cluster visited, 86% had the top cluster parasitized, versus only 14% that had the bottom cluster parasitized), and that the top cluster is the first to be visited. If time and egg load allow for, the bottom cluster is the second to be visited. In the analysis of the functional response, we therefore took the data from the top-most cluster of each container, ignoring results from the other clusters in the same container. The other clusters were added as baits, to reduce the chance that, after leaving the first cluster, wasps would return to the same cluster again and would increase the level of parasitism during the second visit (see also *Discussion*).

As it appeared from the results of Experiment 1, *U. lariophaga* functional response can be characterized as an "all or nothing" type of response. Either host clusters are without parasitism, or rates of parasitism within clusters are close to 100%. Therefore, the foraging behaviour of *U. lariophaga* can be divided into two steps (see also *Discussion*):

a. the process that leads her to discover (or not) a cluster; and once arrived in a cluster,
b. the actual response of the wasp to the number of hosts within that cluster.

Results of the first step have been discussed in Chapter 5 of this thesis. Here, we discuss the second step. Therefore, functional response data were analyzed for visited clusters only (i.e. clusters with at least one egg parasitized). Top clusters without parasitism are included in Table 6.2 and Figure 6.2, but were ignored in regression and curve fitting (see also *Discussion*). Linear regression was applied over low density ranges, and a plateau level was calculated over the higher host densities, as a model of a Type I functional response (Holling, 1959a,b, 1961). In Experiment 1, too few data were available to allow for the calculation of a plateau level at higher host densities.

We could not replace parasitized hosts with unparasitized hosts during the trials, and thus clusters with (initially all) unparasitized hosts gradually became depleted (see: Houck & Strauss, 1985). For such cases, Rogers (1972) developed the "random parasite equation", which allows for host depletion over the time of the experiment. This equation (a Type II functional response) was also fitted on the results of Experiment 2 and 3. In order to estimate the attack rate (*a*) and the handling time (*Th*) from Rogers' random parasite equation, least squares non-linear regression was applied (Williams & Juliano, 1985; Juliano & Williams, 1987; Juliano, 1993), using Statgraphics software.

Results

Percentage of host clusters parasitized

The percentage of containers in which the top-cluster was found, i.e. demonstrated parasitism, is given in Table 6.1 for different cluster sizes. The percentage of top-clusters found was larger in Experiment 1 and 2 (both 24 h) than in Experiment 3 (4 h).

Table 6.1 The percentage of containers in which the top-cluster was found (i.e. showed parasitism) by *U. lariophaga*, for different cluster sizes in Experiment 1 and 2 (both 24 h) and Experiment 3 (4 h). n is the total number of containers in each cluster size

	Experiment 1		Experiment 2		Experiment 3	
# eggs / cluster	% clusters found	n	% clusters found	n	% clusters found	n
1 - 5	63.0	27	57.4	47	38.6	44
6 - 9	56.5	46	--	--	--	--
10 - 20	75.8	66	64.7	34	--	--
21 - 35	48.6	35	--	--	14.3	7
36 - 70	46.2	13	45.5	22	29.4	17
71 - 120	66.7	3	40.9	22	50.0	10
> 120	70.0	20	--	--	--	--
total[1]	62.8 a	210	54.4 a	125	35.9 b	78

1: Means followed by different letters are significantly different (*G*-test for homogeneity of percentages).

Functional response

In Experiment 1 (1 l, 24 h), 36% of the containers (n=210) had both clusters parasitized. In Experiment 2 (8 l, 24 h), 44% of all containers (n=124) had more than one cluster parasitized (see also Chapter 5). This especially happened with low density clusters (3-4 eggs), where 61% of the containers had more than one cluster (with a maximum of six clusters) parasitized, while in high density clusters (60-120 eggs) only 2% of the containers had more than one cluster parasitized. In Experiment 3 (8 l, 4 h), only 5% of 63 containers showed more than one cluster with eggs parasitized.

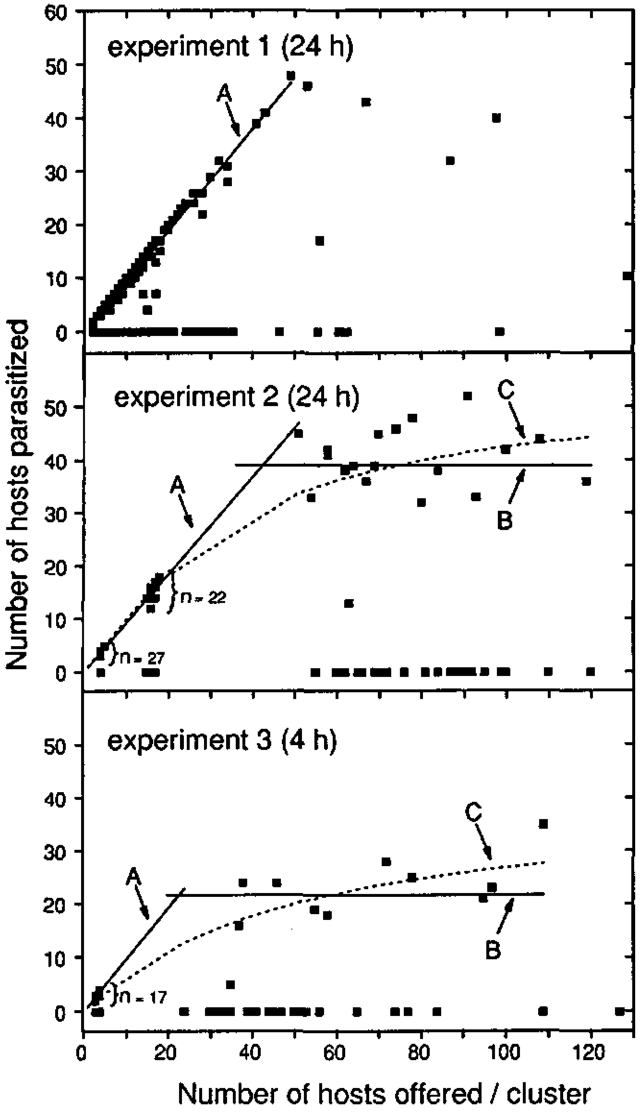

Figure 6.1 Functional response of *U. lariophaga* to different egg densities of *C. maculatus* in three different experiments (see text for details). Zero's were not used in curve fitting (see *Discussion*). Regression lines (A and B) assume a Type I functional response; curves C were fitted with Rogers' random parasite equation (Type II response). Details of regression lines, curves and parameters are given in Table 6.2.

Functional response data were analyzed for visited clusters only (i.e. the top clusters with at least one egg parasitized). Top clusters without parasitism are included in Table 6.2 and Figure 6.1, but were ignored in regression and curve fitting. Results from Experiment 1 (1 l, 24 h) pointed towards a Type I functional response (Fig. 6.1). In clusters of less than 50 eggs, on average 96% of the hosts were parasitized (Table 6.2, line A). However, due to few observations at higher densities, no clear plateau level could be established. Due to the limited volume of the containers and the 24 h duration of the trials, over-exploitation of low density clusters may have occurred, which would lead to an overestimation of the functional response.

Linear regression was also applied on the results of Experiment 2 (8 l, 24 h) and Experiment 3 (8 l, 4 h) (Fig. 6.1, line A). At low host densities, 92-95% of the eggs were parasitized (regression coefficients of lines A, Table 6.2). At higher host densities, a plateau level (line B) was calculated, but the variation in numbers of hosts parasitized is considerable.

Rogers random parasite equation was also fitted as a Type II functional response curve on the data from Experiment 2 and 3 (Fig. 6.1, Table 6.2, curve C). It appears that host densities tested were chosen in such a range, that a clear distinction between Type I and II (around the bending point of the curves, host density 15 - 40 eggs) cannot be made.

Table 6.2 Variables and correlation coefficients for the regression lines and Rogers random parasitoid equation curves fitted on the functional response data given in Figure 6.1

	Experiment 1 (2 clusters, 24 h)	Experiment 2 (8 clusters, 24 h)	Experiment 3 (8 clusters, 4 h)	Description of lines and curves
line A	$Y = 0.96 * X - 0.43$ $R^2 = 96.2\%$ $p < 0.001$ $n = 112$ $(x < 50)$	$Y = 0.92 * X + 0.08$ $R^2 = 98.0\%$ $p < 0.001$ $n = 49$ $(x < 40)$	$Y = 0.95 * X - 0.05$ $R^2 = 51.8\%$ $p = 0.001$ $n = 15$ $(x < 10)$	linear regression over low densities (Type I)
line B	--	$y = 39.1$ (± 8.1) $n = 19$ $(x > 40)$	$y = 21.6$ (± 7.2) $n = 13$ $(x > 20)$	plateau level, mean over high densities
curve C	--	$a = 1.64 * 10^{10}$ $Th = 0.43$ h $R^2 = 91.4\%$ $F = 869$, $df = 67$	$a = 0.34$ $Th = 0.10$ h $R^2 = 87.5\%$ $F = 210$, $df = 27$	Rogers' random parasite equation (Type II), non-linear least squares regression
zero's	$n = 78$	$n = 57$	$n = 50$	excluded from analysis

Y = number of hosts parasitized; X = number of hosts offered; R^2 = coefficient of determination; p = chance of regression coefficient being equal to 0; n = number of data points; a = attack rate in cm^2/s; Th = handling time in h; F = F-value from the ANOVA; df = degrees of freedom.

Direct observations

Mean handling and rejection time, egg laying capacity and initial egg load, as calculated from direct observations, are given in Table 6.3. The mean egg laying capacity of *U. lariophaga* females in small glass tubes in 24 h and 4 h corresponded closely with the plateau levels found in Experiment 2 and 3 respectively. In addition, the initial egg load of young females (25 eggs) corresponds well with the egg laying capacity in 4 h and the plateau level observed in Experiment 3 (Table 6.3).

Table 6.3. Direct observations on handling time, egg laying capacity and initial egg load of *U. lariophaga* females, with 0-20 h old *C. maculatus* eggs as host and, for comparison, plateau levels at higher host densities in functional response experiments 2 and 3

Observations	mean ± st.dev.		n
Handling time (s)			
of unparasitized hosts	180 ± 28		208
rejection of hosts	7 ± 8		539
Egg laying capacity (# eggs / ♀)			
in 24 h	39 ± 7	a[1]	30
in 4 h	20 ± 5	b	35
Initial egg load (# eggs / ♀)	25 ± 6	b	27
Plateau levels (# eggs / ♀)			
in 24 h (Experiment 2)	39 ± 8	a	19
in 4 h (Experiment 3)	22 ± 7	b	14

1: Kruskal-Wallis Test, means followed by the same character are not significantly different ($\alpha = 0.05$).

Discussion

Methodology

Determination of the functional response is fundamental to the analysis of host-parasitoid dynamics, to answer questions such as whether density dependent parasitism occurs. Density dependence would stabilize host-parasitoid interactions and is thought to be an important criterion for the selection of parasitoids in biological control (e.g. van Lenteren & Bakker, 1976).

Confusion exists because investigators use different experimental setups, frequently employ protocols which do not meet the assumptions underlying the models fitted to the data (Houck & Strauss, 1985), and apply statistical techniques that are inappropriate to identify compatible models or to estimate model parameters (Williams & Juliano, 1985; Juliano & Williams, 1987; Trexler *et al.*, 1988; Juliano, 1993). In our setup and analysis, we have tried to meet the requirements formulated by the above mentioned authors.

Analysis of top-clusters

In the analysis of the functional response, the data from the top-most cluster of each container were used, ignoring results from the other clusters in the same container. This choice for the top-most cluster is motivated by the results from Experiment 1, where out of 65 containers with only one parasitized host cluster, 86% had only the top cluster parasitized and only 14% had the bottom cluster parasitized. Apparently, the majority of wasps first visits the top-most cluster (see Chapter 5). At the beginning of each experiment, wasps had the same preparation and started with approximately the same initial egg load in their ovaria. After having parasitized the first cluster, wasps from clusters with different host densities leave with different egg loads remaining in their ovaria. Egg load is an important factor influencing foraging behaviour (for a review, see Minkenberg *et al.*, 1992). Thus, after the first visit, wasps from clusters with different host densities have different egg loads, and their exploitation of subsequent clusters can no longer be compared.

Additional motivation comes from the observation, that containers with more than one cluster parasitized occurred predominantly at low host densities (< 10 eggs/cluster). At lower densities, 24 h foraging time was sufficient to find and parasitize eggs in several clusters. At the higher host densities (> 40 eggs/cluster), containers had almost always only one (top) cluster parasitized. Here, wasps spent their complete foraging time and/or egg load in the first cluster visited, and we assume that most of them never even encountered the second cluster. Thus, at the level of single containers, wasps behave differently and as a result perceive different densities, depending on the density within single clusters. Taking the eggs of all clusters within one container together as one host density for that container neglects this difference in foraging behaviour, and obscures the fact that cluster size is an important factor influencing *U. lariophaga* foraging behaviour (see Chapter 5).

For the reasons stated above, the analysis of the functional response is based on the results from the top-most cluster, which are considered to be the first clusters visited by the parasitoid, and we ignore other clusters that were visited subsequently.

Omission of clusters without parasitism

As is most clearly demonstrated in the results of Experiment 1, *U. lariophaga* functional response appears to be an "all or nothing" type of response (Fig. 6.1). Either host clusters are without parasitism, or rates of parasitism within clusters are close to 100%. A considerable fraction of containers does not demonstrate any parasitism. Several possible explanations can be thought of:

+ misidentification of the parasitoids sex (males were released), or
+ the parasitoid was crushed between seeds after release, or
+ the parasitoid did not find those clusters, or
+ the parasitoid did enter a cluster, but left before parasitizing any eggs.

Knowing that *U. lariophaga* is attracted by host egg odours within 5 cm distance (van Huis *et al.*, 1994; Chapter 4 and 5), and that the egg acceptance/contact ratio is high (van Huis *et al.*, 1991b), the last explanation mentioned above seems rather unlikely. We therefore assume clusters without any parasitism as clusters that were not found by a parasitoid (for one of the first three reasons mentioned above). Therefore, our functional response experiments should be analyzed in two steps:

a. the process that leads a wasp to discover (or not) a host cluster; and
b. once arrived in a host cluster, the actual response of the wasp to the number of hosts within that cluster.

Finding host clusters

The process that leads to detection of host clusters was discussed in the previous chapter, and was found to be affected by the number of host eggs in a cluster. In the previous experiments, foraging time was limited to 4 h. No such relationship, between the number of host eggs in a cluster, and the chance that such a cluster was parasitized by *U. lariophaga*, was found in the current experiments (Table 6.1). For Experiment 1 and 2, the 24 h foraging time may explain this lack of relationship. In the 24 h experiments, 36%-44% of the containers had more than one cluster parasitized, indicating that wasps apparently had plenty of time to search for host clusters within the cowpea stock. This may have obscured differences in encounter rates between clusters with fewer or more host eggs. However, when foraging time is limited from 24 h (Experiment 2) to 4 h (Experiment 3), the fraction of host clusters found decreases significantly.

Functional response within visited clusters

Uscana lariophaga demonstrates a Type I functional response curve (as in Experiment 1) or a Type II curve (as in Experiment 2 and 3) (Fig. 6.1). Results from host densities between 15 and 40 are lacking, which makes it difficult to distinct between the two types. Type II functional responses have been widely identified for parasitoids in fixed time experiments (Walde & Murdoch, 1988). For *U. lariophaga*, the fit of the Type II curves (lines C) to the data is about as good as the Type I (lines A) at the lower densities (Table 6.2). However, the Type II curves deviate from the plateau level in the numbers of host parasitized and predict still increasing numbers of parasitized hosts at higher densities. This is clearly unrealistic in view of *U. lariophaga*'s limited egg load (see below). Type I functional responses have been found for other parasitoids in plant and field cages (e.g. Morales-Ramos & Cate, 1992; Wiedenmann & Smith, 1993).

Arrestment

If a cluster is found by *U. lariophaga* and at least one egg is parasitized, then it appears that, in clusters up to 40 eggs (in 24 h) or in clusters up to 25 eggs (in 4 h), 92% - 96% of the eggs will be parasitized (Table 6.2, regression coefficients of lines A). These high rates of parasitism indicate that the female wasp has a strong tendency to remain in a host cluster until almost all eggs have been parasitized or her egg load has been depleted. Host discrimination in *U. lariophaga* is almost perfect (van Huis *et al.*, 1991b), and rejection of parasitized eggs takes only 7 seconds. Assuming random search within a host patch, females will re-encounter substantial numbers of parasitized hosts before achieving 95% parasitism. This strong arrestment response in *U. lariophaga* females may lead to aggregation of parasitoids in high density host clusters, both in the field and storage situations.

Egg limitation

In the current experimental setup, *U. lariophaga* is egg-limited at the higher host densities, and this determines the plateau level in the functional response. Dissections demonstrated that young females carry on average 25 mature eggs in their ovarioles, which was the same as the number of hosts parasitized in small glass tubes in 4 h, and as the plateau level in the 4 h functional response (Experiment 3) (Table 6.3). Egg loads also corresponded well to the levels of 24 ± 4 and 22 ± 5 found in earlier dissections of 1-4 h and 1-19 h old *U. lariophaga* females (van Huis *et al.*, 1991b). Egg limited functional responses have been found for larval and pupal parasitoids (e.g. Collins *et al.*, 1981; van Roermund, 1995), and for Trichogrammatidae egg parasitoids also (e.g. Allen & Gonzalez, 1975; Hassell, 1982). *Uscana caryedoni* Viggiani demonstrates a type II functional response (Delobel, 1989). The maximum number of eggs laid in 24h functional response experiments was 49, which was higher than the mean lifetime fecundity (41 eggs) of females in a parallel test (Delobel, 1989). Thus, *U. caryedoni* also appears egg limited in her functional response.

The number of parasitized hosts increased to 39 after 24 h, both in the egg laying capacity experiment and in the functional response experiment (Experiment 2). Egg maturation rates can be estimated from the difference in ovipositions between the 24 h and the 4 h experiments, assuming that searching and handling time are not limiting. They are 0.74 and 0.88 egg/h in the egg laying capacity and the functional response experiments respectively.

Handling time

A type II functional response is traditionally explained as the result of increased total handling time, limiting the time left for searching (Holling, 1959b). This causes the gradual approach of the plateau level in the number of hosts parasitized. Handling time in *U. lariophaga*, i.e. time between first contact with a host and the time of leaving this host after parasitism, measured through direct observations, was on average 3 minutes per host. Rejection of parasitized hosts took less than 10 seconds (Table 6.3). This is much lower than the parameter value of Th, calculated from Experiment 2 and 3 (Table 6.2: 26 and 6 min. respectively). Unfortunately, the term 'handling' time introduced by Holling causes confusion. Both handling time (Th) and instantaneous attack rate (a) in Holling's disk equation and Rogers' random parasite equation are essentially merely parameters to describe the mathematical relationship. In most experimental studies, handling times calculated from the equations above fall widely beyond those measured by direct observation (for a review, see: Fernando & Hassell, 1980).

Thus, even at the highest host density and maximum number of hosts parasitized in our functional response experiments, *U. lariophaga* females still had significant time left for searching and re-encountering parasitized hosts. Actual handling time therefore cannot have been a limiting factor for the number of hosts parasitized in the functional response experiments. It is egg load and egg maturation rate that limit *U. lariophaga* females in their levels of parasitism.

Implications for biological control

Uscana lariophaga demonstrates a type I or a type II functional response. Thus, within a patch, parasitism is density independent or inversely density dependent. Both types are considered to cause instability in parasitoid-host relationships and are disadvantageous for biological control. However, *U. lariophaga* females also show a strong arrestment response, which can lead to aggregation in high density host clusters in the field and storage situation. At the population level within a multiple patch habitat, this aggregation behaviour may still lead to an overall density dependent parasitism of host patches (e.g. Hassell & May, 1974; Hassell *et al.*, 1977; Walde & Murdoch, 1988).

In the current experimental setup, *U. lariophaga* is egg-limited at the higher host densities, and this determines the plateau level in the functional response. All measures that increase the realized fecundity of *U. lariophaga* females, such as the provision of honey (van Huis *et al.*, 1991a), should increase the number of hosts parasitized per female, and thus potentially improve the biological control of bruchids with *Uscana*.

The functional response experiments have improved our insight in the way *U. lariophaga* responds to different densities of its host in a three-dimensional cowpea seed mass. Direct behaviourial observations (e.g. van Lenteren & Bakker, 1978) were not feasible in this setup. In such cases, we strongly recommend to supplement indirect functional response experiments with simple, direct observations of handling time, egg laying capacity and initial egg load. The use of independent information (see Casas & Hulliger, 1994), as in our case, allows for a correct interpretation of the actual mechanisms underlying the data from the functional response experiment.

Acknowledgements

I would like to thank Karin Koning and Edwin de Korte for their participation in the functional response experiments, and Marieke Bonhof, Lidy Rouwkema and Marcus van Es for sharing their results from the dissections and behaviourial observations. Nanda Kaashoek, Mariët Cools and Gerard Pesch skilfully maintained the insect rearings. Joop van Lenteren, Arnold van Huis, Herman van Roermund and Peter Mols gave valuable comments on the manuscript. This study was financially supported by the European Community (project TS2 029 F ESF and TS3 CT 930201) and coordinated by the Foundation for Biological Research (BION) (code 436.992).

References

Allen, J.C.; Gonzalez, D., 1975. The effects of temperature and host refuge on insect host-parasitoids models. Environ. Entomol. 4: 57-65.

Casas, J.; Hulliger, B., 1994. Statistical analysis of functional response experiments. Biocontrol Sci. Techn. 4: 133-145.

Collins, M.D.; Ward, S.A.; Dixon, A.F.G., 1981. Handling time and the functional response of *Aphelinus thomsoni*, a predator and parasite of the aphid *Drepanosiphum platanoidis*. J. Animal Ecol. 50: 479-487.

Delobel, A. 1989. *Uscana caryedoni* (Hym.: Trichogrammatidae): possibilités d'utilisation en lutte biologique contre la bruche de l'arachide, *Caryedon serratus* (Col.: Bruchidae). Entomophaga 34: 351-363.

Dent, D., 1991. Insect Pest Management. CAB International, Wallingford.

Dutcher, J.D., 1993. Recent examples of conservation of arthropod natural enemies in agriculture. In: R.D. Lumsden & J.L. Vaughn (eds), Pest Management: Biologically Based Technologies, Proc. Beltsville Symp. 18, Agric. Res. Serv., Am. Chem. Soc., Beltsville, pp. 101-108.

Fernando, M.H.J.P.; Hassell, M.P., 1980. Predator-prey responses in an acarine system. Res. Pop. Ecol. 22: 301-322.

Hassell, M.P., 1982. Patterns of parasitism by insect parasitoids in patchy environments. Ecol. Entomol. 7: 365-377.

Hassell, M.P.; Lawton, J.H.; Beddington, J.R., 1977. Sigmoid functional responses by invertebrate predators and parasitoids. J. Animal Ecol. 46: 249-262.

Hassell, M.P.; May, R.M., 1974. Aggregation of predators and insect parasites and its effect on stability. J. Animal Ecol. 43: 567-594.

Holling, C.S. 1959a. The components of predation as revealed by a study of small mammal predation on the European pine sawfly. Can. Entomol. 91: 293-320.

Holling, C.S. 1959b. Some characteristics of simple types of predation and parasitism. Can. Entomol. 91: 385-398.

Holling, C.S. 1961. Principles of insect predation. Ann. Rev. Entomol. 6: 163-182.

Houck, M.A.; Strauss, R.E. 1985. The comparative study of functional responses: experimental design and statistical interpretation. Can. Entomol. 117: 617-629.

Huis, A. van; Kaashoek, N.K.; Maes, H.M., 1991a. Biological control of bruchids (Col.: Bruchidae) in stored pulses by using egg parasitoids of the genus *Uscana* (Hym.: Trichogrammatidae): a review. In: F. Fleurat-Lessard & P. Ducom (eds), Proc. 5th Int. Working Conf. Stored-product Prot. Bordeaux, volume 1, pp. 99-107.

Huis, A. van; Schütte, C.; Cools, M.H.; Fanget, Ph.; Hoek, H. van der; Piquet, S.P., 1994. The role of semiochemicals in host location by *Uscana lariophaga*, egg parasitoid of *Callosobruchus maculatus*. In: E. Highley, E.J. Wright, H.J. Banks & B.R. Champ (eds), Stored Product Protection. Proc. 6th Int. Working Conf. Stored-product Prot., CAB International, Wallingford, volume II, pp. 1158-1164.

Huis, A. van; Wijkamp, M.G.; Lammers, P.M.; Klein Goldewijk, C.G.M.; Seeters, J.H. van; Kaashoek, N.K., 1991b. *Uscana lariophaga* (Hymenoptera: Trichogrammatidae), an egg parasitoid of bruchid beetle (Coleoptera: Bruchidae) storage pests in West Africa: host-age and host-species selection. Bull. ent. Res. 81: 65-75.

Juliano, S.A. 1993. Nonlinear curve fitting: predation and functional response curves. In: S.M. Scheiner & J. Gurevitch (eds), Design and Analysis of Ecological Experiments. Chapman & Hall, New York, pp. 159-182.

Juliano, S.A.; Williams, F.M., 1987. A comparison of methods for estimating the functional response parameters of the random predator equation. J. Animal Ecol. 56: 641-653.

Lenteren, J.C. van, 1986. Evaluation, mass production, quality control and release of entomophagous insects. In: K. Franz (ed), Biological Plant and Health Protection, Fortschritte der Zoologie no. 32. G. Fischer Verlag, Stuttgart, pp. 31-56.

Lenteren, J.C. van; Bakker, K., 1976. Functional responses in invertebrates. Neth. J. Zool. 26: 567-572.

Lenteren, J.C. van; Bakker, K., 1978. Behaviourial aspects of the functional responses of a parasite (*Pseudeucoila bochei* Weld) to its host (*Drosophila melanogaster*). Neth. J. Zool. 28: 213-233.

Minkenberg, O.P.J.M., 1990. On seasonal inoculative biological control. Doctoral Thesis, Wageningen Agricultural University, Wageningen.

Minkenberg, O.P.J.M.; Tatar, M.; Rosenheim, J.A., 1992. Egg load as a major source of variability in insect foraging and oviposition behavior. Oikos 65: 134-142.

Morales-Ramos, J.A.; Cate, J.R., 1992. Functional response of *Cactolaccus grandis* (Burks) (Hymenoptera; Pteromalidae) in field cages. Biol. Control 2: 193-202.

Noldus, L.P.J.J. 1991. The observer: a software system for collection and analysis of observational data. Beh. Res. Meth. Instr. Comp. 23: 415-429.

Pak, G.A., 1988. Selection of *Trichogramma* for innundative biological control. A study on behaviourial variations among strains and species of an egg-parasite genus. Doctoral Thesis. Wageningen Agricultural University, Wageningen.

Roermund, H.W.J. van, 1995. Understanding biological control of greenhouse whitefly with the parasitoid *Encarsia formosa*. From individual behaviour to population dynamics. Doctoral Thesis, Wageningen Agricultural University, Wageningen.

Rogers, D. 1972. Random search and insect population models. J. Animal Ecol. 41: 369-383.

Trexler, J.C.; McCulloch, C.E.; Travis, J., 1988. How can the functional response best be determined? Oecologia 76: 206-214.

Walde, S.J.; Murdoch, W.W., 1988. Spatial density dependence in parasitoids. Ann. Rev. Entomol. 33: 441-466.

Wiedenmann, R.N.; Smith, J.W., 1993. Functional response of *Cotesia flavipes* (Hymenoptera; Braconidae) at low densities of the host *Diatraea saccharalis* (Lepidoptera; Pyralidae). Environ. Entomol. 22: 849-859.

Williams, F.M.; Juliano, S.A., 1985. Further difficulties in the analysis of functional-response experiments and a resolution. Can. Entomol. 117: 631-640.

The effect of different host distributions on the functional response of *Uscana lariophaga*

7

Abstract

The functional response of the egg parasitoid *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae) was tested under three different host distributions (even, clumped and random) within clusters of *Callosobruchus maculatus* Fab. (Col.: Bruchidae) eggs. A Holling Type II functional response was found for all three distributions. At low host densities, less than 50% of the host clusters was parasitized. Also at low host densities, *U. lariophaga* females parasitized significantly fewer eggs in random egg clusters with many beans than in clusters with fewer beans and an even or clumped egg distribution. At higher egg densities, plateau levels of maximum number of hosts parasitized were the same for all three egg distributions. *Uscana lariophaga* appears to be adapted to search for even or clustered egg distributions, as can be found in the field and under storage conditions.

This chapter will be published, in a slightly different form, as:
Alebeek, F.A.N. van; Bezemer, T.M.; Huis, A. van; Lenteren, J.C. van, 1996. The functional response of *Uscana lariophaga* under different egg distributions of its host *Callosobruchus maculatus*. Entomologia Experimentalis et Applicata (in press).

Introduction

In search for biological control of bruchid (Coleoptera; Bruchidae) pests in stored cowpea in West Africa, the egg parasitoid *Uscana lariophaga* Steffan (Hymenoptera; Trichogrammatidae) is a promising candidate and its main characteristics are being evaluated (van Huis *et al.*, 1990, 1991; van Huis, 1991). We previously investigated the functional response of *U. lariophaga* to one of its hosts, *Callosobruchus maculatus* Fab. in cowpea stock (Chapter 6). This functional response was satisfactorily described by Rogers (1972) random parasitoid equation, as a Holling Type II functional response, and, over low densities, equally well with linear regression (as a Holling Type I functional response). *Uscana lariophaga* appeared to be egg-limited in the maximum number of hosts that could be parasitized in a given time (Chapter 6).

In the growing season, female parasitoids colonize open, green cowpea fields with relatively low host densities (see Fig. 1 in van Huis *et al.*, 1990). Host eggs may be distributed randomly at the field level, and more clustered at the plant and at the individual pod level (Huignard *et al.*, 1985; Germain *et al.*, 1987). During the dry season, parasitoids have to forage in masses of dry cowpea seeds or pods in a closed, dark storage structure with sometimes very high host densities (Chapter 2).

In the laboratory, *C. maculatus* generally distributes its eggs evenly over the available beans (Mitchell, 1990). However, geographical strains of *C. maculatus* show rather divergent egg laying strategies, ranging from virtually even to almost random egg dispersion (Messina & Mitchell, 1989; Messina & Dickinson, 1993).

Foraging behaviour of predators and parasitoids, and more specifically, the functional response, can be strongly affected by the host's distribution (Hassell & May, 1973; Hassell *et al.*, 1976; May, 1978; Houck & Strauss, 1985; Kareiva & Odell, 1987; Hassell & Pacala, 1990). We therefore investigated the effect of different *C. maculatus* egg distributions within host clusters on the functional response of *U. lariophaga* females.

Materials and methods

General

Details on rearing methods and origin of *C. maculatus* and *U. lariophaga* are given in Chapter 4. Cowpea (*Vigna unguiculata* Walp.) variety 'Black Eyes' from California was used in the experiments. All wasps used were 0-16 h old, mated and fed with honey (Mellona Santusa Honey, Quaker Oats b.v., Dordrecht, NL). One hour before the experiment, individual females were given an oviposition experience on a *C. maculatus* egg (0-20 h old). All experiments were carried out at 30±1°C in darkness.

Experimental units

Plastic containers of 1 litre (ø = 8 cm, height 20 cm), filled with clean cowpea seeds and placed in total darkness, were used as experimental units (see Chapter 5, Figure 5.1). A gelatine capsule containing one *U. lariophaga* female was placed in the centre of a container. The capsule could be opened from outside, in order to release the wasp in the bean stock. Because earlier observations indicated that *U. lariophaga* females are negatively geotactic, a single host cluster was situated 5 cm above the release site in the bean stock. Wasps were allowed to search and parasitize for 4 h, after which the host clusters were recovered. This period was chosen based on earlier experiments in larger containers with multiple host clusters, in which during 4 h foraging periods, only one cluster was visited and repeated visits to the same cluster were considered unlikely (Chapter 5 and 6). Dissection also showed that, in 4 h, parasitoids could lay a maximum number of eggs equal to their initial egg load (Chapter 6).

Host clusters

Host clusters consisted of fresh *C. maculatus* eggs (less than 20 h old) on cowpea seeds in a small polyamine gauze bag. Three series of egg distributions were tested simultaneously (Fig. 7.1):

Series I. Even egg distribution, i.e. a fixed egg density (one egg per bean) and variable cluster size (2-64 beans).

Series II. Clumped egg distribution, i.e. a fixed cluster size (8 beans) and a variable egg density (all beans within one cluster carried approximately an equal numbers of eggs, between 1 and 15 eggs per bean depending on the total number of eggs in the cluster).

Series III. 'Random' egg distribution, i.e. a fixed egg density (one egg per bean) and a fixed cluster size (64 beans). This distribution deviates from real randomness because there is never more than one egg per bean, and the distribution gradually becomes even at higher densities (32 and 64 eggs per cluster).

Egg clusters were composed in such a way that clusters with approx. 2, 4, 8, 16, 32 and 64 eggs were obtained. However, due to the considerable amount of work involved in preparing host clusters and in counting egg numbers, not all densities could be tested for each egg distribution. The initial trajectory of 2-8 eggs was thought to be of interest to demonstrate possible differences between different distributions, and higher densities (32 and 64 eggs) were though necessary to find possible plateau levels. Thus, time constraints forced us to omit density 16. In order to have some idea of parasitism at this density, we included density 16 in series II, omiting density 2 in the same series II (see empty cells in Figure 7.1).

The exact number of eggs per cluster was variable, and could only be determined at the end of the experiment, because any extra handling of beans with freshly laid eggs results in many damaged eggs. After each experiment, host clusters were incubated in glass tubes until, 4-5 days later, parasitized eggs became black and unparasitized eggs turned white, and could therefore be easily counted. The mean number of eggs per cluster in the three different series

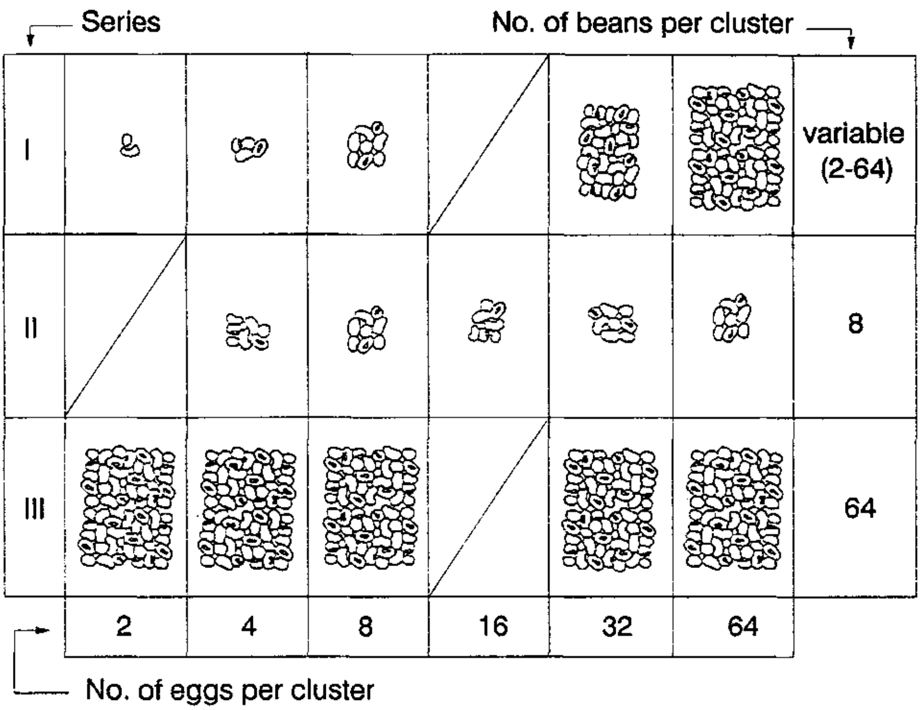

Figure 7.1 Schematic illustration of the different host clusters used in the three series of functional response experiments, indicating the number of seeds per cluster and the number of eggs aimed at. Empty boxes: density not tested. See Materials and methods for a detailed description of series I (even egg distribution), series II (clumped distribution) and series III (random distribution).

Table 7.1. Actual mean number of eggs per cluster ± standard deviation, range and number of replicates (n) in the three series of egg distributions (see Figure 7.1), for the different egg densities aimed at

Egg density aimed at:		2	4	8	16	32	64
Series I	mean ± st.d.	1.8 ± 0.4	3.8 ± 0.4	7.6 ± 0.6	--	30.7 ± 1.6	60.9 ± 3.2
Even	range (n)	1-2 (37)	3-4 (36)	6-9 (36)	--	26-34 (36)	48-64 (35)
Series II	mean ± st.d.	--	3.8 ± 0.5	7.6 ± 0.6	15.3 ± 1.1	30.0 ± 1.8	60.6 ± 2.8
Clumped	range (n)	--	3-5 (35)	6-9 (36)	13-18 (36)	24-32 (36)	51-64 (35)
Series III	mean ± st.d.	1.9 ± 0.3	3.8 ± 0.4	7.7 ± 0.5	--	30.4 ± 1.4	60.9 ± 3.2
Random	range (n)	1-2 (38)	3-4 (36)	6-8 (36)	--	26-33 (36)	48-64 (35)

is given in Table 7.1. The clusters with 8 eggs in series I and II, and clusters with 64 eggs in series I and III are identical (i.e. carry the same number of eggs on the same number of beans; see Figure 7.1), and the same data sets of these densities were used in the analysis of the different series. The three egg distributions (all densities) were tested once every day, until 35 replicates were obtained.

Analysis

Foraging behaviour of *U. lariophaga* has been divided into two separate steps: 1) the process that leads to the detection of host clusters; and 2) (after arrival in a cluster) the actual response of a female parasitoid to the host density *within* that cluster (see Chapter 6 for a discussion). The analyses in this Chapter are limited to the second step only, the functional response in clusters which have been found by *U. lariophaga*, i.e. clusters with at least one egg parasitized. Clusters without parasitism are included in Figure 7.2 and Table 7.2, but have not been used in the regressions and curve fitting.

Linear and non-linear regression were used to model Holling Type I and Type II functional responses respectively (see Chapter 6, for a motivation and references). Rogers (1972) random parasitoid equation for host depletion over time was fitted to our data. This equation has been developed for experiments in which hosts are not being replaced if parasitized during the experiment, as in our case. The attack rate (a) and the handling time (Th) from Rogers' random parasitoid equation were calculated by least squares non-linear regression using Statgraphics software.

Linear regression was done over the range of 2-10 eggs per cluster to compare rates of parasitism, and over 25-64 eggs per cluster to compare plateau levels between different series (Figure 7.2 and Table 7.2). Equality of slopes of linear regression lines was tested with an ANOVA followed by a comparison among regression coefficients with a GT2-test (Sokal & Rohlf, 1981). Differences between plateau levels were also tested with the GT2 method.

Walking behaviour

In order to explain possible differences between the three egg distributions, the walking behaviour of *U. lariophaga* females just after oviposition was studied. Individual females (0-16 h old, mated) were given one oviposition experience on a 1-20 h old *C. maculatus* egg. They were subsequently released in an experimental arena (5 x 8 cm), consisting of 28 cowpea cotyledons, arranged in four rows of seven cotyledons, slightly embeded in fine sand. No host eggs or traces were present in the arena. Wasp behaviour was monitored with a video camera, and the walking path was registered and analyzed using the VTMAS / Etho-Vision^R system (Version 1.50) (Noldus Information Technology, 1995).

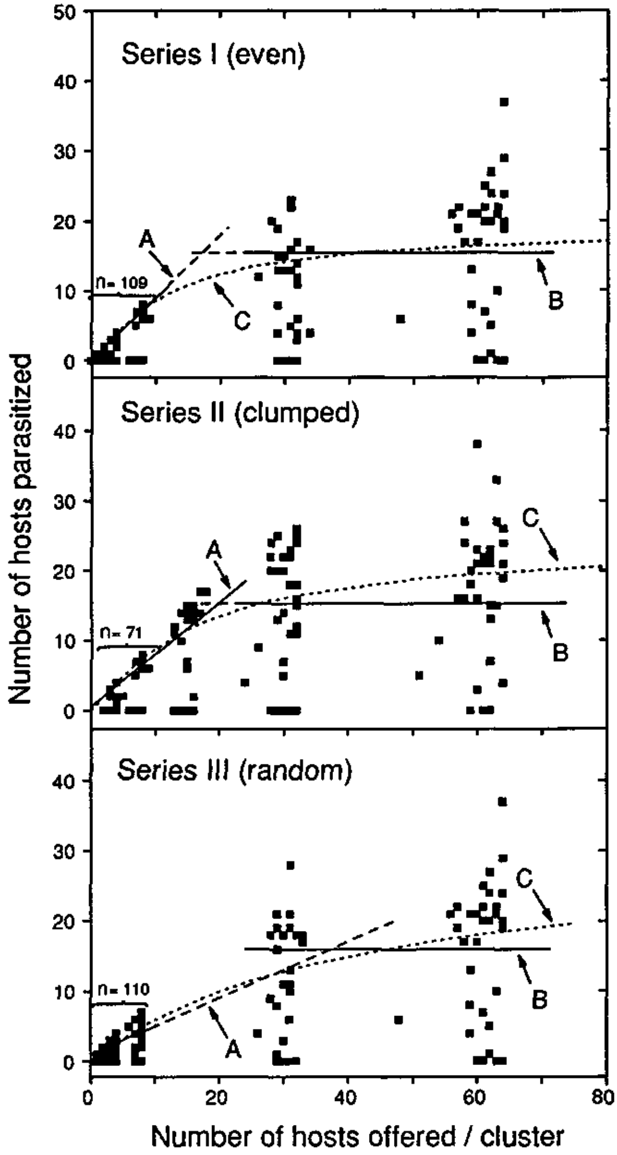

Figure 7.2 Functional response of *U. lariophaga* to different egg densities of *C. maculatus* in three different series (see text for details). Regression lines (A and B) assume a Type I functional response; curves C were fitted with Rogers (1972) random parasite equation (Type II response). Details of regression lines, curves and parameters are given in Table 7.2.

Results

Functional response experiments

At each density, considerable variation occurred in the number of parasitized hosts (Fig. 7.2). Over lower host densities, parasitism increased (almost) linearly. At higher host densities, parasitism levelled off, with considerable variation in the rates of parasitism (Fig. 7.2). Linear regression over low densities (Fig. 7.2, lines A) and Rogers random parasitoid equation (Fig. 7.2, lines C) were fitted to the data. Parameters of the lines and curves drawn in Figure 7.2 are presented in Table 7.2.

Table 7.2. Variables and correlation coefficients for the regression lines and Rogers (1972) random parasitoid equation curves fitted on the functional response data given in Figure 7.2

Line	Even distribution Series I	Clumped distribution Series II	Random distribution Series III	Description of lines and curves
A	$Y = 0.89 * X + 0.13$ $R^2 = 89.2\%$, $p < 0.001$ $n = 46$ $(x < 10)$	$Y = 0.75 * X + 0.56$ $R^2 = 67.6\%$, $p < 0.001$ $n = 53$ $(x < 20)$	$Y = 0.40 * X + 0.95$ $R^2 = 42.8\%$, $p < 0.001$ $n = 42$ $(x < 10)$	linear regression over low densities (Type I)
B	$y = 15.3$ (± 7.7) $n = 53$ $(x > 25)$	$y = 15.4$ (± 7.3) $n = 59$ $(x > 25)$	$y = 16.0$ (± 7.9) $n = 50$ $(x > 25)$	plateau over higher densities (Type I)
C	$a = 0.58$ (± 0.37) $Th = 0.17$ h (± 0.03) $R^2 = 55.0\%$ $F = 234$, $df = 99$	$a = 4.88$ (± 16.41) $Th = 0.17$ h (± 0.02) $R^2 = 53.4\%$ $F = 382$, $df = 112$	$a = 0.32$ (± 0.15) $Th = 0.14$ h (± 0.03) $R^2 = 59.0\%$ $F = 205$, $df = 91$	Rogers random parasitoid equation (Type II), non-linear regression
zero's	$n = 81$	$n = 67$	$n = 89$	excluded from analysis

y = number of hosts parasitized; x = number of hosts offered; R^2 = coefficient of determination; p = significance of regression coefficient being equal to 0; n = number of data points; a = attack rate in cm^2/s; Th = handling time in h; F = F-value from the ANOVA; df = degrees of freedom.

Regression coefficients (lines A) over visited patches of all egg distributions were significantly different from 0 ($p<0.001$) (Table 7.2) and from each other (ANOVA, $F=9.61$, $df=2,20$, $p<0.005$). Regression coefficients of series I (even) and II (clumped) and those of series II (clumped) and III (random) did not differ significantly (comparison among regression coefficients with a GT2-test; Sokal & Rohlf, 1981). Regression coefficients of the even distribution (series I) and the random distribution (series III) were significantly different ($MSD=0.457$, $df=3,20$, $p<0.01$).

Linear regression over densities above 25 eggs per cluster yielded slopes not significantly different from zero, i.e. a plateau level in the number of hosts parasitized was attained (Figure 7.2 and Table 7.2, lines B). Plateau levels were not significantly different between the three series (GT2-test, Sokal & Rohlf, 1981).

Parasitism in small clusters (few beans) with low host densities (series I, even, and series II, clumped) was high (regression coefficients: 89% and 75% respectively, Table 7.2). In low density clusters, *U. lariophaga* females apparently continue to search for unparasitized eggs, although they may have frequent encounters with already parasitized eggs. For clusters of 1-2 eggs (in series I and II, even and clumped respectively), 83% of the visited clusters had all eggs parasitized. For clusters of 3-5 eggs, this was 62%, and for clusters of 6-9 eggs, it was 30%. However, when eggs were distributed randomly in a larger cluster of beans (series III, random), parasitism was as low as 40% (regression coefficient of line A, Table 7.2). In this situation, *U. lariophaga* females apparently do not remain long enough in a host cluster until (almost) all host eggs have been parasitized.

Walking behaviour

The size of our *U. lariophaga* wasps (0.4 mm) relative to the pixel size in the video-computer system, and software problems in Version 1.50 of the Etho-Vision^R system, made it impossible to do a thorough analysis of walking tracks, in terms of walking velocity and relative turning angles on cowpea seeds. However, direct observations of the *U. lariophaga* females revealed significant differences in walking behaviour in the first few minutes after oviposition, compared to the behaviour ten minutes later. A representative example is presented in Figure 7.3.

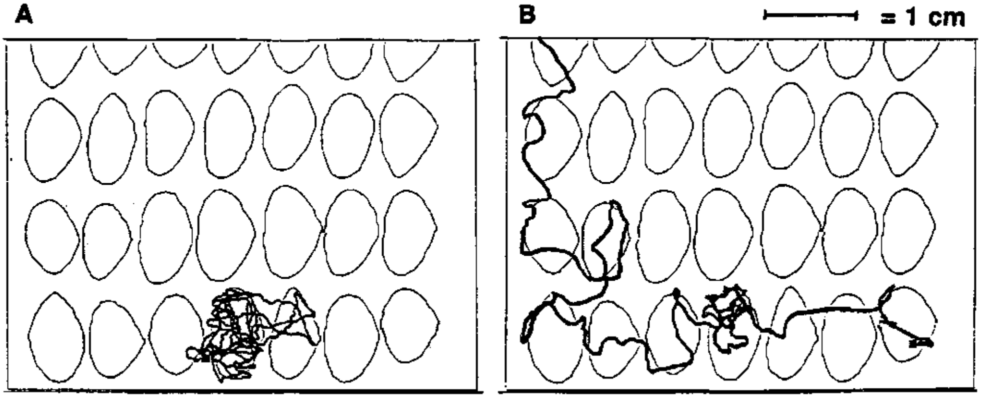

Figure 7.3 The characteristic walking behaviour of an *Uscana lariophaga* female in an arena with clean cowpea cotyledons, slightly embedded in fine sand. No hosts are present. **A.** Walking behaviour 0-2 minutes after oviposition in a *C. maculatus* egg. **B.** Walking behaviour of the same individual, 10-12 minutes after oviposition.

In the first two minutes, the walking is characterized by many sharp turns, and the *U. lariophaga* female, in this example, visited only three cotyledons near the point of release (Figure 7.3.A). Ten minutes later, the walking track of the same female had become much more straight, and, as a result, in the same span of time, 11 cowpea cotyledons were visited (Figure 7.3.B).

Discussion

The functional response curves (Fig. 7.2) and the high variability in the rates of parasitism found in this study closely resemble the ones found in earlier experiments (Chapter 6). Also, the plateau levels at high host densities (lines B) are the same as found before, and closely fit the number of mature eggs in the ovaria of young females (Chapter 6), confirming egg limitation as the mechanism causing the plateau levels. Over densities lower than 40 eggs per cluster, Rogers (1972) random parasitoid curves (a Type II functional response) described our data as well as (Type I) linear regression lines. However, the Rogers curves (lines C, Fig. 7.2) continue to increase at high host densities (>25 eggs per cluster), whereas linear regression over this density trajectory yields non-significant slopes, indicating a plateau level where *U. lariophaga* females become egg limited (Chapter 6).

Many factors have been found to influence rates of parasitism in insect parasitoids, for example temperature, humidity, illumination, characteristics of the walking substrate, the physiological condition of the parasitoid, the stage and species of host, and learning or conditioning to different hosts (for references, see Houck & Strauss, 1985). In the current experiment, we have carefully kept all these factors constant. Only the host's spatial distribution was manipulated to test its effect on *U. lariophaga*'s functional response.

An effect of host distribution on the rate of parasitism was reported by e.g. Madden & Pimentel (1965) and Cheke (1974). A random whitefly distribution within a patch resulted in lower rates of parasitism by *Encarsia* than when they were aggregated (Burnett, 1958a). In the current experiments, we also find lower regression coefficients for parasitism in the random distribution (series III), compared to the clumped distribution (series II) (Table 7.2). Increasing the arena size (i.e. distance between hosts) caused the functional response to change from a Type I into a Type III (Burnett, 1958b). The experimental protocol employed (size of the arena, lowest host densities tested, variable times and opportunity for wasps to leave the arena) influences the type of response that is found (van Lenteren & Bakker, 1976). Increasing the distance between hosts changed the functional response of *Trichogramma* spp. egg parasitoids from a Type II (inversely density dependent) into a Type I response (density independent) (Morrison *et al.*, 1980). This corresponds with our results, where the increase in distance between eggs from series I (even) to III (random) (see Fig. 7.1) changes Rogers curve from a typical Type II into an almost linear Type I relationship (Fig. 7.2, lines C in series I and III).

Rates of parasitism in series III (random) are significantly lower than in the even and clumped distribution (Table 7.2, regression coefficients of lines A are significantly different). In other words, *U. lariophaga* females found significantly fewer eggs in clusters with many beans and a random, low egg density (series III) than in clusters with an even or clumped egg distribution. *Uscana lariophaga* leaves clusters with a random egg distribution earlier than clusters with clumped or even egg distributions. Parasitoids leave a host cluster either because they have too many encounters with already parasitized hosts, or because they do not encounter (enough) unparasitized hosts in a given amount of searching time (e.g. Waage, 1978, 1979). *Uscana lariophaga* demonstrates a strong arrestment within host clusters, and continues to search for eggs in clusters that have already been largely exploited (Chapter 6). It seems that *U. lariophaga* leaves host clusters not because of (re-)encounters with parasitized eggs, but because of a lack of encounters with new, unparasitized eggs. Detailed behaviourial observations are necessary to test this hypothesis.

Our data reveal an arrestment response of *U. lariophaga* females in host clusters. Arrestment may occur when the giving up time (time since last contact with a host until the wasps decides to leave the host cluster) increases after contact with a host or host kairomones (e.g. van Roermund *et al.*, 1994). A lower rate of parasitism in low-density clusters with a random distribution could also be explained if the reaction distance of the parasitoid towards hosts is (much) smaller than the distance between hosts in a cluster. Then fewer hosts will be encountered in random clusters with larger distances between hosts than in even clusters (Fig. 7.1). However, from earlier work on *U. lariophaga*, it is known that *U. lariophaga* responds to host egg odours over distances of 5-7 cm (Chapter 4; van Huis *et al.*, 1994), which is much more than the maximum distance between eggs in our host clusters.

The most common explanation for arrestment is the occurrence of area restricted search (e.g. Yano, 1978; Gardner & van Lenteren, 1986). This may occur due to a change in walking behaviour immediately after oviposition, or in response to kairomones present on or near the host. Direct observations of foraging *U. lariophaga* females revealed that area restricted search does occur in *U. lariophaga* (Figure 7.3). In a clumped host egg distribution, the intensive searching behaviour closely around an egg that has just been parasitized, will result in increased encounter rates with subsequent host eggs. However, in a random egg distribution, at lower densities, this behaviour will result in time spent searching in areas where no hosts are available. As a result, after some time wasps may decide to leave a host cluster before all eggs have been encountered, and the resulting rates of parasitism in the random egg distribution will be lower.

Since several factors in the experimental design directly influence the outcome of functional response experiments, different studies can hardly be compared, and single estimates of "the" functional response of a parasitoid or predator are of relatively little value.

One approach to overcome these difficulties is to use detailed simulation models of foraging behaviour (e.g. Mols, 1993, van Roermund, 1995). Such models allow sensitivity analyses of different factors (e.g. experimental conditions) and behaviourial elements. After assessing the relative importance of those factors, their variability, and the magnitude of their effects, simulation models can be used to compare the effects of different treatments or characteristics of different parasitoids on the functional response. A complementary approach is to use comparative experiments with tightly controlled protocols to study the effect of a single variable on the functional response. In the current study we followed this latter approach with success. The functional response of *U. lariophaga* was lower under a random host distribution than under an even or a clumped distribution. The wasp demonstrates area restricted searching behaviour upon parasitism, which explains the results found. This behaviour may reflect an adaptation to the field conditions, where bruchid eggs appear to be clustered at the plant and pod level (Huignard *et al.*, 1985; Germain *et al.*, 1987). *Uscana lariophaga* is arrested in high host density clusters, which leads to aggregation in such patches at the population level. Such behaviour is also known from other Trichogrammatidae (e.g. Yano, 1978; Gardner & van Lenteren, 1986). The Type I or II functional response found here for *U. lariophaga*, should destabilize the host-parasitoid equilibrium. However, aggregation at high host densities may counteract this effect, and can help to stabilize the parasitoid-host equilibrium and lead to density-dependent parasitism in a multiple patch environment (e.g. Hassell & May, 1974; Hassell *et al.*, 1977; Hassell, 1978; Walde & Murdoch, 1988). In *Uscana semifumipennis*, density dependent parasitism (Type III functional response) was found in field data, and host availability was thought to influence this relationship (Siemens & Johnson, 1992).

Callosobruchus maculatus egg distribution patterns have extensively been investigated under laboratory conditions (e.g. Messina & Mitchell, 1989; Mitchell, 1990; Messina & Dickinson, 1993), but very little is known for storage situations. Sampling of *C. maculatus* egg densities and egg distribution patterns in granaries, especially early in the storage season when egg densities are relatively low, is a priority issue. Since *C. maculatus* egg distributions in the field are clustered, *U. lariophaga* foraging behaviour in host clusters appears adaptive, and therefore this parasitoid can be considered as a serious candidate for biological control of the bruchid pest.

Acknowledgements

The assistance of Martijn Bezemer in the experiments is gratefully acknowledged. Mariët Cools and Gerard Pesch maintained the insect rearings. Norbert Groot is thanked for his assistance in the observations of walking behaviour. Valuable comments on an earlier draft were given by Arnold van Huis, Joop van Lenteren, Peter Mols and Herman van Roermund.

References

Burnett, T., 1958a. Effect of host distribution on the reproduction of *Encarsia formosa* Gahan (Hymenoptera; Chalcidoidea). Can. Entomol. 90: 179-191.

Burnett, T., 1958b. Effect of area of search on reproduction of *Encarsia formosa* Gahan (Hymenoptera; Chalcidoidea). Can. Entomol. 90: 225-229.

Cheke, R.A., 1974. Experiments on the effect of host spatial distribution on the numerical response of parasitoids. J. Animal Ecol. 43: 107-114.

Gardner, S.M.; Lenteren, J.C. van, 1986. Characterisation of the arrestment responses of *Trichogramma evanescens*. Oecologia 68: 265-270.

Germain, J.F.; Monge J.P.; Huignard, J., 1987. Development of two bruchid populations (*Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (Fab.) infesting stored cowpea (*Vigna unguiculata* Walp) pods in Niger. J. Stored Prod. Res. 23: 157-162.

Hassell, M.P. 1978. The Dynamics of Arthropod Predator-Prey Systems. Princeton University Press, Princeton, 229 p.

Hassell, M.P.; Lawton, J.H.; Beddington, J.R., 1976. The components of arthropod predation. I. The prey death-rate. J. Animal Ecol. 45: 135-164.

Hassell, M.P.; Lawton, J.H.; Beddington, J.R., 1977. Sigmoid functional responses by invertebrate predators and parasitoids. J. Animal Ecol. 46: 249-262.

Hassell, M.P.; May, R.M., 1973. Stability in insect host-parasite models. J. Animal Ecol. 42: 693-726.

Hassell, M.P.; May, R.M., 1974. Aggregation of predators and insect parasites and its effect on stability. J. Animal Ecol. 43: 567-594.

Hassell, M.P.; Pacala, S.W., 1990. Heterogeneity and the dynamics of host-parasitoid interactions. Philos. Trans. Royal Soc. London, series B 330: 203-220.

Houck, M.A.; Strauss, R.E., 1985. The comparative study of functional responses: experimental design and statistical interpretation. Can. Entomol. 117: 617-629.

Huignard, J.; Leroi, B.; Alzouma, I.; Germain, J.F., 1985. Oviposition and development of *Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in *Vigna unguiculata* (Walp) cultures in Niger. Insect Sci. Applic. 6: 691-699.

Huis, A. van, 1991. Biological methods of bruchid control in the tropics: a review. Insect Sci. Applic. 12: 87-102.

Huis, A. van; Kaashoek, N.K.; Lammers, P.M., 1990. *Uscana lariophaga* (Hym.: Trichogrammatidae), egg parasitoid of two bruchid species of cowpea in West Africa. Proc. Exp. Appl. Entomol., Neth. Entomol. Soc. (N.E.V.), Amsterdam, 1: 101-106.

Huis, A. van; Kaashoek, N.K.; Maes, H.M., 1991. Biological control of bruchids (Col.: Bruchidae) in stored pulses by using egg parasitoids of the genus *Uscana* (Hym.: Trichogrammatidae): a review. In: F. Fleurat-Lessard & P. Ducom (eds), Proc. 5th Int. Working Conf. Stored-product Prot. Bordeaux, volume 1, pp. 99-107.

Huis, A. van; Schütte, C.; Cools, M.H.; Fanget, Ph.; Hoek, H. van der; Piquet, S.P., 1994. The role of semiochemicals in host location by *Uscana lariophaga*, egg parasitoid of *Callosobruchus maculatus*. In: E. Highley, E.J. Wright, H.J. Banks, and B.R. Champ (eds), Stored Product Protection. Proc. 6th Int. Working Conf. Stored-product Prot., CAB International, Wallingford, volume II, pp. 1158-1164.

Kareiva, P.; Odell, G., 1987. Swarms of predators exhibit "preytaxis" if individual predators use area-restricted search. Am. Naturalist 130: 233-270.

Lenteren, J.C. van; Bakker, K., 1976. Functional responses in invertebrates. Neth. J. Zool. 26: 567-572.

Madden, J.L.; Pimentel, D., 1965. Density and spatial relationships between a parasite and its housefly host. Can. Entomol. 97: 1031-1037.

May, R.M., 1978. Host-parasitoid systems in patchy environments: a phenomenological model. J. Animal Ecol. 47: 833-844.

Messina, F.J.; Dickinson, J.A., 1993. Egg-laying behaviour in divergent strains of the cowpea weevil (Coleoptera: Bruchidae): time budgets and transition matrices. Ann. Entomol. Soc. America 86: 207-214.

Messina, F.J.; Mitchell, R., 1989. Intraspecific variation in the egg-spacing behaviour of the seed beetle *Callosobruchus maculatus*. J. Insect Beh. 2: 727-742.

Mitchell, R., 1990. Behaviourial ecology of *Callosobruchus maculatus*. In: K. Fujii *et al.* (eds), Bruchids and Legumes: Economics, Ecology and Coevolution. Proc. 2nd Int. Symp. Bruchids Legumes, Okayama, pp. 317-330.

Mols, P., 1993. Foraging behaviour of the Carabid beetle *Pterostichus coerulescens* L. (= *Poecilus versicolor* Sturm) at different densities and distributions of the prey. Wageningen Agricultural University Papers no. 93-5, Wageningen, 95 pp.

Morrison, G.; Lewis, W.J.; Nordlund, D.A., 1980. Spatial differences in *Heliothis zea* egg density and the intensity of parasitism by *Trichogramma* spp.: an experimental analysis. Environ. Entomol. 9: 79-85.

Noldus Information Technology, 1995. Ethovision^{R}: Video Tracking, Motion Analysis & Behaviourial Recognition System. Reference Manual, Wageningen.

Roermund, H.J.W. van, 1995. Understanding biological control of greenhouse whitefly with the parasitoid *Encarsia formosa*, from individual behaviour to population dynamics. Doctoral Thesis, Wageningen Agricultural University, Wageningen.

Roermund, H.J.W. van; Hemerik, L.; Lenteren, J.C. van, 1994. Influence of intra-patch experiences and temperature on the time allocation of the whitefly parasitoid *Encarsia formosa*. J. Insect Beh. 7: 483-501.

Rogers, D. 1972. Random search and insect population models. J. Animal Ecol. 41: 369-383.

Siemens, D.H.; Johnson, C.D., 1992. Density-dependent egg parasitism as a determinant of clutch size in bruchid beetles (Coleoptera: Bruchidae). Environ. Entomol. 21: 610-619.

Sokal, R.R.; Rohlf, F.J., 1981. Biometry. The principles and practice of statistics in biological research (2nd ed). W.H. Freeman and Company, New York, 860 pp.

Waage, J., 1978. Arrestment responses of the parasitoid, *Nemeritis canescens*, to a contact chemical produced by its host, *Plodia interpunctella*. Physiol. Entomol. 3: 135-146.

Waage, J., 1979. Foraging for patchily-distributed hosts by the parasitoid, *Nemeritis canescens*. J. Animal Ecol. 48: 353-371.

Walde, S.J.; Murdoch, W.W., 1988. Spatial density dependence in parasitoids. Ann. Rev. Entomol. 33: 441-466.

Yano, E., 1978. A simulation model of searching behaviour of a parasite. Res. Pop. Ecol. 20: 105-122.

Dispersal and functional response of *Uscana lariophaga* in different habitats: cowpea pods and seeds

8

Abstract

The effect of two different habitats, stored cowpea (*Vigna unguiculata* Walp.) pods and seeds, on the dispersal and functional response of *Uscana lariophaga* Steffan (Hymenoptera; Trichogrammatidae) was investigated, with *Callosobruchus maculatus* Fab. (Coleoptera; Bruchidae) eggs as hosts. In the presence of directional light from the top, parasitoids moved faster through columns filled with pods than through columns (either filled with pods or seeds) in darkness. The different habitats, seed or pods, did not have a significant effect on dispersal. The maximal dispersal rate of colonizing *U. lariophaga* females may be up to several meter per day, and median net displacement rates are around 0.5 - 0.8 m per 24 h. The functional response of *U. lariophaga* could be described by Rogers (1972) random parasitoid equation as a Holling Type II curve and resembles the ones found in earlier experiments. At host densities below 40 eggs per cluster, *U. lariophaga* was more efficient at finding host eggs in a seed habitat than in a pod habitat, probably due to area restricted searching behaviour after oviposition. At higher host densities, *U. lariophaga* becomes egg limited, and neither walking behaviour nor the habitat structure significantly influence her foraging success.

This chapter will be published, in a slightly modified form, as:
Alebeek, F.A.N. van; Antwi, K.K.; Huis, A. van; Lenteren, J.C. van. Dispersal and functional response of *Uscana lariophaga* in two different habitats: stored cowpea pods and seeds. Submitted to Journal of Stored Products Research.

Introduction

In order to evaluate the suitability of the egg parasitoid *Uscana lariophaga* Steffan (Hymenoptera; Trichogrammatidae) for biological control of bruchid pests in stored cowpea in West Africa (van Huis *et al.*, 1990, 1991; van Huis, 1991), we investigated the functional response of *U. lariophaga* to different densities of one of its hosts, *Callosobruchus maculatus* Fab. (Coleoptera; Bruchidae) in cowpea stock (Chapter 6). This functional response could satisfactorily be described by Rogers (1972) random parasitoid equation as a Holling Type II functional response. The parasitoid *U. lariophaga* appeared egg-limited in the maximum number of hosts she could parasitize in a given amount of time, and is strongly arrested in high-density host clusters (Chapter 6). Subsequently, we investigated the effect of different *C. maculatus* egg distributions within host clusters on the functional response of *U. lariophaga* females. *Uscana lariophaga* females found significantly fewer eggs in low density clusters with a random egg distribution than in clusters with an even or clumped host distribution (Chapter 7). The egg parasitoid searches predominantly by walking (Van Huis *et al.*, 1994). Analysis of *U. lariophaga* walking behaviour on cowpea seeds revealed that female wasps demonstrated area restricted search just after parasitization (Chapter 7). This may reflect adaptation to bruchid egg distribution patterns under field conditions, which is clustered at the plant and pod level (Huignard *et al.*, 1985; Germain *et al.*, 1987).

In West Africa, traditional cowpea storage in granaries is either as pods or, after threshing, as loose seeds in bags. Cowpea storage in pods provides some protection against bruchids, because the pod wall acts as a physical and chemical barrier to the first instar bruchid larva drilling in from the egg (Caswell, 1973, 1974; Kitch *et al.*, 1991).

The storage habitat is a special kind of environment with discrete units (pods or seeds). The spatial structure of cowpea stored as pods is different from that of cowpea stored as seeds. In pods, there are fewer contact points between units and air spaces are relatively large compared with storage as seeds. Habitat structure and variation within habitats is known to have significant effects on pest populations (for a review, see: Margolies, 1993). Leaf morphology and structure (e.g. hairiness) are known to affect the effectiveness of natural enemies (for a review, see: Dicke, 1996). However, relatively little research has been undertaken to compare the effects of different habitat structures on the foraging behaviour and success of natural enemies, although significant effects have been found in some parasitoids (e.g. Thorpe, 1984; Casas, 1991).

In this chapter, I report on the effect of different storage habitat structures (i.e. cowpea stock as pods and as seeds) on *U. lariophaga* foraging behaviour, measured as her dispersal abilities and her functional response to different host densities.

Materials and methods

General

Details on the rearing and origin of *C. maculatus* and *U. lariophaga* are given in Chapter 4. All wasps used in the experiments were 0-16 h old, mated and fed with honey. One hour before the experiment, individual females were given an oviposition experience on a fresh *C. maculatus* egg. All experiments were carried out at 30$\pm$1°C.

Cowpea seeds were of the Black-eye variety from California. Cowpea pods were of variety TVx 3236, and obtained from Niger. Pods with eggs from host clusters in the functional response experiments were intact; the surrounding mass of pods to fill up the containers contained a fraction of broken and split pods.

Dispersal studies

Opaque PVC cylinders of 5 litre (ϕ = 22 cm, height 20 cm) were filled with either cowpea pods or cowpea seeds. Our observations indicate that *U. lariophaga* females are negatively geotactic and tend to move upwards (Chapter 5). Furthermore, wasps are attracted and arrested by volatile cues from *C. maculatus* eggs (Van Huis *et al.*, 1994; Chapter 4). Therefore, wasps were released at the bottom of a column, and their arrival at an odour bait on top of the column was monitored. At the bottom of each column, a small glass tube was placed with 10 *U. lariophaga* females. This tube was closed with a cork which could be pulled from outside by means of a thread, at the start of an experiment. The top of each cylinder was closed with a glass plate, with a central hole (ϕ = 2 cm). Over the hole in the glass plate, an odour bait was placed to attract the wasps. This bait consisted of approx. 60 *C. maculatus* eggs (20 h old) on 10 cowpea seeds in a small polyamine gauze bag. The bag was covered by a petri-dish lid. The underside of the glass plate was covered by a layer of insect-glue (Soveurode® aérosol, Sovilo, Siegfried, Switzerland). Glass plates were changed every hour after wasp release, and the wasps trapped in the glue were counted.

Three columns were used: one filled with seeds and standing in total darkness, and two columns filled with pods; one in total darkness and one standing in the light. This allows for comparison of two treatments: movement through pods and through seeds (both in the dark), and movement through pods in the light and in the dark. Since column walls were opaque, only light coming from above through the glass cover could penetrate the column filled with pods standing in the light. Light intensity at the pod column top level was 750 Lux; light intensity at 5 cm depth within the pod column was 6 Lux. Penetration of light in a column filled with seeds was very poor (0.2 Lux at 5 cm depth), and therefore this treatment was omitted.

Accumulated numbers of wasps trapped on top of each column (13 replicates for each type of column) were compared between treatments and, based on arrival times, maximum and median net displacement rates were calculated.

Functional response studies

Experimental units

Plastic containers of 5 litre (ϕ = 24 cm, height 18 cm), filled with clean cowpea seeds or pods and placed in total darkness, were used as experimental units. A gelatine capsule containing one *U. lariophaga* female was placed in the centre of a container. The capsule could be opened with threads from outside, to release the wasp in the stock (see Fig. 5.1). A single host cluster was situated directly above the release site in the stock. Wasps were allowed to search and parasitize for 4 h, after which the host clusters were recovered. This period was equal to earlier experiments and allows females to spent approximately their initial egg load, without over-exploitation of host clusters due to multiple visits (Chapter 5 and 6).

Host clusters

In each container, one host cluster was offered to the parasitoid. For the comparison of two habitat types (pods and seeds), we aimed at host clusters with identical surface areas, i.e. the same number of eggs per square centimetre. The surface of cowpea seeds was approximated by considering them as a cylinder with a half sphere at both ends. The length, largest and smallest diameter of 50 California Black-eye seeds were carefully measured, giving a mean surface area of 2 ± 0.3 cm^2 per seed. Pods were considered as long cylinders with a cone at both ends. The length, largest and smallest diameter of 50 pods from Niger were measured, giving a mean surface of 25 ± 5 cm^2 per pod. Based on these measurements, a host cluster of 65 seeds has approximately the same surface area as a cluster of 5 pods.

In containers filled with seeds, host clusters consisted of small polyamine gauze bags, filled with 65 cowpea seeds carrying fresh *C. maculatus* eggs (20 h old). In containers filled with pods, host clusters consisted of 5 cowpea pods, marked with a felt tip pen at one end for easy recovery. The total volume of a seed cluster was estimated by wrapping 65 seeds into thin plastic foil and immersing them in a known volume of water. The measured volume includes the air spaces between seeds. The total volume of a 65 seed cluster was 20 cm^3. The average volume of a 5 pod cluster was 71 cm^3 (air spaces included), and was estimated by measuring the total volume of 300 pods in an experimental container.

The exact number of eggs per cluster was variable, and was determined at the end of the experiment. In each replicate, host densities aimed at were 4, 8, 16, 32, and 64 *C. maculatus* eggs per cluster, although in reality the whole range of 1-85 eggs per cluster was covered. Upon recovery from the experimental units, host clusters were incubated in glass tubes until, 4-5 days later, parasitized eggs coloured black and were counted. One full combination of treatments was carried out twice a week, until 40 replicates were obtained.

Because of the amount of cowpea seeds and pods involved, seeds and pods were re-used throughout the experiment. In between replicates, seeds and pods were kept in a clean oven at 45°C for 48 h. As a result of this, we expect to have removed possible volatiles left by bruchid egg clusters or walking parasitoids. Due to frequent handling in the course of the experiment, the fraction of split and broken pods increased from about 5% to approximately 50%. Before each experiment, loose seeds were removed from containers filled with pods.

Analysis

Percentages of hosts clusters found (i.e. with one or more egg(s) parasitized) were calculated over different host cluster sizes. Clusters without any parasitism can be considered as clusters that were not visited (not found) by a parasitoid. We analyzed the functional response data for visited clusters only (i.e. clusters with at least one egg parasitized) (for a discussion, see Chapter 6). Clusters without parasitism are included in Figure 8.2 and Table 8.2, but have been ignored in the regressions. Linear and non-linear regression were applied, as models of a Holling Type I and Type II functional response respectively (Chapter 6). Rogers (1972) random parasitoid equation for host depletion over time was chosen as an appropriate model. The attack rate (a) and the handling time (Th) from Rogers random parasitoid equation were calculated by least squares non-linear regression using Statgraphics software (see also Chapter 6). Equality of slopes of linear regression lines was tested with an ANOVA followed by comparison of regression coefficients, and plateau levels were compared with a GT2-test (Sokal & Rohlf, 1981).

Results

Dispersal studies

Wasps came out faster in the light than in the dark, from columns filled with pods (Fig. 8.1). In total darkness, up to 7 h after release, the number of wasps recovered was the same for columns filled with seeds and pods. Using accumulated numbers of wasps arriving (13 replicates), significant differences between columns were found (Kruskal-Wallis one-way ANOVA, KW=19.3, df=1,231, $p<0.001$). Significantly more wasps arrived from the column in the light filled with pods than from both columns in darkness filled with seeds and pods (Kruskal-Wallis multiple comparison, $\alpha < 0.05$). There was no significant difference in total arrivals between pods and seeds in darkness.

The maximum net displacement rate is the speed of the first wasps arriving on top of the 20 cm column. Displacement rates can only be (under)estimated based on the hourly observations. The first wasps were observed one hour after release, giving an estimated net displacement rate of $5.56*10^{-5}$ m/s, both for seed and pod columns. This is equivalent to 4.8 m/day or approximately 0.14 bodylength/s. Real distances covered will be larger because of the tortuous walking path within the cowpea stock, and actual walking speed within the seed and pod mass will also be higher. Based on the first 50% of the wasps recovered (i.e. the most active parasitoids), we can calculate median net displacement rates also. With median arrival times of 6, 8 and 9 h in pods in light, pods in darkness and seeds in darkness respectively (Fig. 8.1), median net displacement rates are 0.93, 0.69 and $0.62 * 10^{-5}$ *m/s* respectively.

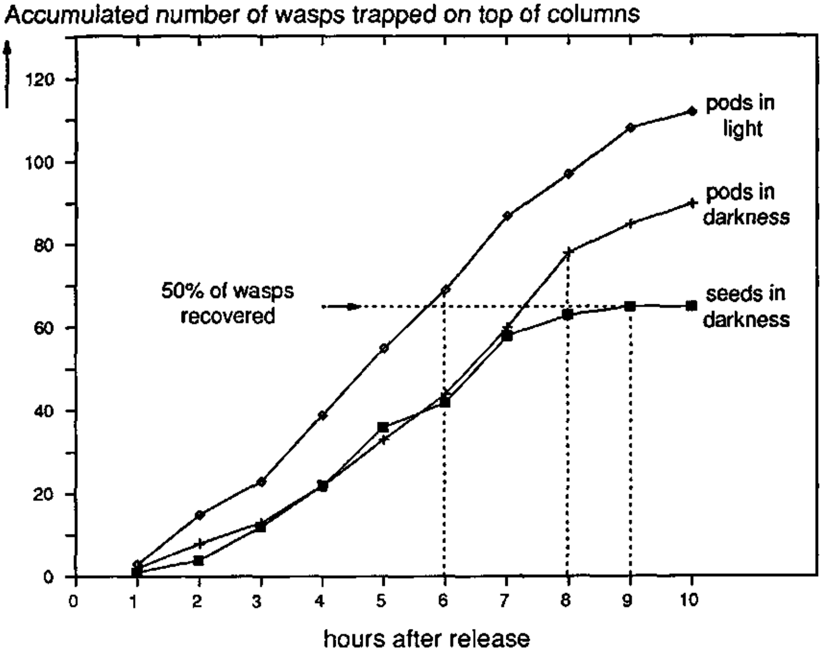

Figure 8.1 Cumulative number of *Uscana lariophaga* wasps arriving at top of a column filled with cowpea seeds or pods (in light or in darkness) in the first ten hours after release. Total of 13 replicates (of ten wasps each) per treatment. Vertical lines indicate median arrival times.

Functional response experiments

Host clusters with higher numbers of eggs tend to have a higher chance of being found by *U. lariophaga* females than smaller clusters (Table 8.1). The overall percentage of clusters found was the same for the pod and seed habitat ($X^2 = 1.0$, $p > 0.25$). Although wasps were released directly under the host cluster, about 40% of the host clusters did not show any trace of parasitism (Table 8.1).

Table 8.1 The percentage of host clusters showing egg parasitism by *U. lariophaga* in a seed and in a pod habitat, at various host egg densities per cluster.

No. host eggs	seed habitat		pod habitat	
per cluster	% paras.	n	% paras.	n
1 - 5	37.8	45	55.6	27
6 - 9	50.0	32	48.3	29
10 - 20	72.5	40	55.6	45
21 - 35	53.7	41	64.1	39
36 - 70	70.3	37	76.0	50
71 - 100	--	--	63.6	11
total	56.4 a	195	61.5 a	201

a: Overall percentages parasitism are not significantly different (X^2=1.0, p>0.25)

The functional response of *U. lariophaga* females in visited clusters can be described by a Type II Rogers' curve (Fig. 8.2, Table 8.2, line C). Below 40 eggs per cluster, a Type I linear regression can also be fitted, as a tangent of the Rogers curve (Fig. 8.2, line A). The regression coefficient of this line is the average level of parasitism in clusters of less than 40 host eggs (Table 8.2, line A). The parasitization level over this range is significantly higher in seed clusters (44%) than that in pod clusters (32%) (ANOVA, F=12.8, df=1,160, p<0.001).

Table 8.2 Variables and correlation coefficients for the regression lines and Rogers (1972) random parasitoid equation curves fitted on the functional response data given in Figure 8.2.

line	seed habitat	pod habitat	Description of lines and curves
A	$Y = 0.44 * X + 1.50$ $R^2 = 55.4\%$ $p < 0.001$ $n = 86$ $(x < 40)$	$Y = 0.32 * X + 0.76$ $R^2 = 40.9\%$ $p < 0.001$ $n = 79$ $(x < 40)$	linear regression over low densities (Type I)
B	$y = 21.7$ (± 8.0) $n = 24$ $(x \geq 40)$	$y = 18.8$ (± 8.3) $n = 44$ $(x \geq 40)$	plateau level, mean over high densities (Type I)
C	$a = 0.27$ (± 0.06) Th = 0.07 h (± 0.02) $R^2 = 66.3\%$ F = 357, df = 109	$a = 0.16$ (± 0.04) Th = 0.08 h (± 0.03) $R^2 = 55.3\%$ F = 254, df = 122	Rogers random parasite equation (Type II), non-linear least squares regression
zero's	n = 85	n = 77	excluded from analysis

y = number of hosts parasitized; x = number of hosts offered; R^2 = coefficient of deter-mination; p = chance of regression coefficient being equal to 0; n = number of data points; a = attack rate in cm^2/s; Th = handling time in h; F = F-value from the ANOVA; df = degrees of freedom.

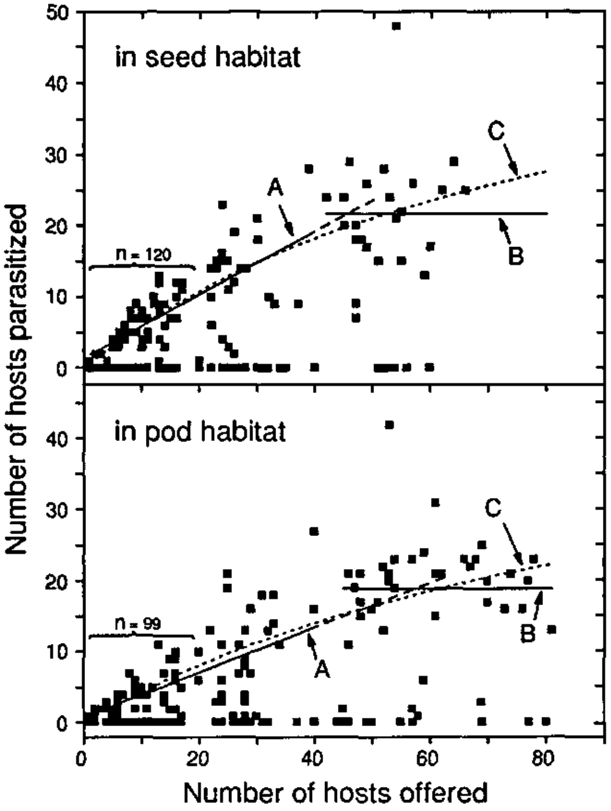

Figure 8.2 Functional response of *U. lariophaga* to different egg densities of *C. maculatus* in a seed habitat and in a pod habitat. Regression lines (A and B) assume a Type I functional response; curves C were fitted with Rogers (1972) random parasite equation (a Type II response). Details of regression lines, curves and parameters are given in Table 8.2.

Based on *U. lariophaga* limited egg load (Chapter 6), a plateau level was calculated over host densities above 40 eggs per cluster (line B, Fig. 8.2 and Table 8.2). Plateau levels were the same in the seed and pod habitat (Table 8.2). At densities above 40 eggs per cluster, the Rogers curves continue to increase, which is not realistic in view of *U. lariophaga* limited egg supply.

Discussion

Dispersal rates

Vertical dispersion of natural enemies of stored product pests, in order to locate loci of infestations in bulk masses, is a prerequisite for successful biological control in storage systems. Movements of pest through stored wheat was investigated extensively by Surtees (e.g. 1964, 1965), and dispersal also was a topic in several aeration and modified atmosphere studies (e.g. Navarro *et al.*, 1981; Armitage *et al.*, 1983; Adler, 1992). However, if net displacement rates were measured in any of these studies, it usually was over relatively long time intervals.

The predatory bug *Xylocoris flavipes* (Reuter) (Hemiptera, Anthocoridae) successfully penetrated a 2.6 m column with wheat within 2 months after release (Press *et al.*, 1979). The larval parasitoids *Anisopteromalus calandrae* and *Choetospila elegans* (Hymenoptera: Pteromalidae) penetrated a 2.2 m column with wheat and located hosts within 7 days after release (Press, 1992). *Anisopteromalus calandrae* moved predominantly upwards and, if released on top, remained near the wheat surface (Press, 1988). The egg parasitoids *Trichogramma pretiosum* Riley and *T. evanescens* Westwood (Hym.: Trichogrammatidae), when released on top of a wheat mass, parasitized lepidopteran eggs up to 20 and 55 cm depth respectively in 6 days (Brower, 1990; Schöller *et al.*, 1994). *Eupelmus vuilleti* (Craw) (Hymenoptera; Eupelmidae), a larval parasitoid of bruchids in stored cowpea, predominantly moved upwards in an uninfested cowpea column. This parasitoid located hosts over 77 cm within 48 h after release, and did so more efficiently in the upward direction than downwards (Cortesero, 1994).

The time intervals in the studies cited above do not allow for precise estimates of displacement rates through bulk masses. Based on the maximum displacement rates in our experiments (20 cm/h), colonizing *U. lariophaga* could cover distances of several meters in one day. This is more than the internal diameter of traditional West African cowpea granaries.

Dispersal and habitat structure

Few studies investigated the effect of habitat structure on dispersal and foraging behaviour of insect pests and their natural enemies; some examples can be found in Boethel & Eikenbarry (1986). Many reports aim at a description of spatial patterns of parasitism in one type of habitat (for a review see: Walde & Murdoch, 1988), but few compare different habitats and their effect on spatial distributions.

At the herbivore level, Jones (1977) demonstrated that the spatial pattern of host plants influenced the oviposition pattern of *Pieris rapae* butterflies. The patchiness of the habitat and the number of host plants in the habitat may have an effect on prey and predators dispersal behaviour, and so can strongly influence the outcome of predator - prey population interactions (e.g. Kareiva, 1987; Nachman, 1987). In habitats where plants are touching or connected, and prey and predators can more easily disperse, overall population interactions become more stable (Sabelis *et al.*, 1983; Nachman, 1987). In *C. maculatus* and *C. chinensis*, the effect of body size and of legume seed size on the ability of the bruchids to penetrate a column of seeds was investigated (Watanabe, 1984, 1985, 1986). In columns with larger seeds (relative to bruchid body size) dispersion was more easy. Smaller bruchid individuals also dispersed more easily than large individuals (Watanabe, 1984, 1985, 1986).

For parasitoids, the spatial structure of the habitat also interacted with foraging behaviour in some studies. The height of host clusters within a habitat significantly influenced parasitization rates of *Trichogramma minutum* and *T. pretiosum* (Hymenoptera: Trichogrammatidae), and indications were found that habitat type (soybean fields versus natural vegetation) influenced parasitism by *T. minutum* (Thorpe, 1984). The spatial architecture of fruit trees appears to influence spatial patterns of parasitism in the leafminer parasitoid *Cirrospilus vittatus* (Hymenoptera, Eulophidae) (Casas, 1990, 1991).

In the present study, in total darkness, *U. lariophaga* movement in a pod habitat was not significantly different from movement in a seed habitat. Body size in *U. lariophaga* is so small (0.4 mm) relative to cowpea pod and seed size, that dispersal will not be hampered by body size constraints. In a pod habitat, the number of recovered wasps in the light was significantly higher than recovery in darkness. Thus, the effect of light on dispersal (positive phototaxis) is stronger than any possible effect of different habitat types. Probably, our number of replicates is insufficient to demonstrate significant differences in number of wasps arriving from pods and seeds in the dark. Arrival of wasps in darkness was the same for seed and pod columns until 7 h after release. From then onwards, it appears as if there is a difference between the two habitats (Fig. 8.1) (although numbers are too small to be significant). One can hypothesize that there are two groups of wasps with different behaviour. One group is not actively searching for hosts, and moves upwards to leave the column. This movement rate is apparently the same in a pod as in a seed habitat. A second group of wasps may be actively searching for hosts in the columns. They move more slowly, and arrive later at top of the column. For these wasps, foraging in seeds may be different from foraging in pods, as Fig. 8.1 suggests (see also below).

Habitat structure and functional response

The percentage of host clusters without parasitism (44% and 38% for seeds and pods respectively) is surprisingly high, in spite of the fact that wasps were released directly below the clusters. We have not been able to identify the causes for this result. The chance of clusters being parasitized appears to be related to the number of host eggs in a cluster, as was also found in Chapter 5.

If clusters without parasitism are left out of the analysis and linear regression is applied over clusters of less than 40 eggs, a significant difference is found between seed and pod clusters. Seed clusters have higher rates of parasitism (44%) than pod clusters (32%) (Table

8.2, line A). Rates of parasitism are substantial lower than results from earlier experiments (Chapter 6 and 7).

The fact that dispersal did not differ in a pod and a seed habitat but that parasitism within clusters with eggs does so, may result from area restricted search by *U. lariophaga* females after oviposition. In the dispersal studies, no hosts were present in the columns and parasitoids did not demonstrate area restricted search. In the functional response experiments, upon the first parasitization of a host in a cluster, the walking track will show more turns over larger turning angles, and as a result wasps will remain longer on one unit (pod or seed) compared with parasitoid behaviour before first host contact (Chapter 7). In the seed clusters, host eggs are on average more close together (seed cluster volume is smaller than pod cluster volume, for equal surface area), and seeds share many contact points facilitating walking from one seed to the next. Thus, the chance of finding a next host egg is relatively large. Contrary, in pods, eggs are more widely apart (larger cluster volume), and pods share relatively few contact points where wasps can change from one pod to the next pod. Thus, area restricted search may result in a lower chance to find eggs on other pods, and thus in wasps leaving a pod cluster early, when part of the available host eggs are still unexploited.

In summary, *U. lariophaga* can find host clusters equally well in a seed as in a pod habitat. Colonising females move at rates as high as 4.8 m per day. Since foraging *U. lariophaga* females are attracted to host clusters by volatile host odours from distances of at least 5-10 cm (van Huis *et al.*, 1994; Chapter 4), *U. lariophaga* could well cover the volume of stored cowpea in traditional granaries, and locate host clusters therein.

Within host clusters, *Uscana lariophaga* appears more efficient at finding eggs in seeds than in pod clusters (over host densities below 40 eggs per cluster). This is probably due to area restricted searching behaviour after oviposition. Since median host densities in West African cowpea granaries are often well above 40 eggs per 100 seeds (Chapter 2), in practice a reduced searching efficiency in pods will be of limited importance. At higher host densities, *U. lariophaga* becomes egg limited, and neither walking behaviour nor the habitat structure will significantly influence her foraging success.

Acknowledgements

The assistance of Kwaku Kyei Antwi in the experiments is gratefully acknowledged. Thanks are also due to Mariët Cools and Gerard Pesch for technical assistance and maintainance of the rearings. The research was financially supported by the European Community (project no. TS2 029 F ESF and TS3 CT 930201), partly under the administration of the Netherlands Foundation for Biological Research (BION) (code 436.992). The financial support to K.K.A. by the International Institute of Tropical Agriculture (IITA) is also gratefully acknowledged.

Chapter 8

References

Adler, C., 1992. Vertical dispersion of adult *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) in a wheat column flushed with modified atmospheres. J. Stored Prod. Res. 28: 201-209.

Armitage, D.M.; Day, P.G.; Lewis, G.J., 1983. The effect of aeration on the vertical distribution of grain beetles (Coleoptera) in wheat. Bull. ent. Res. 73: 233-237.

Boethel, D.J.; Eikenbarry, R.D. (eds), 1986. Interactions of Plant Resistance and Parasitoids and Predators of Insects. Ellis Horwood Limited, Chichester.

Brower, J.H., 1990. Host location ability of *Trichogramma pretiosum* Riley in inshell peanuts under laboratory conditions. J. Agric. Entomol. 7: 265-273.

Casas, J., 1990. Multidimensional host distribution and nonrandom parasitism: a case study and a stochastic model. Ecology 71: 1893-1903.

Casas, J., 1991. Density dependent parasitism and plant architecture. Insect Parasitoids; 4th European Workshop, Perugia, 3-5 April, 1991; REDIA 74(3) (Appendice): 217-222.

Caswell, G.H., 1973. The storage of cowpea. Samaru Agric. Newsl. 15: 73-75.

Caswell, G.H., 1974. The development and extension of nonchemical control techniques for stored cowpeas in Nigeria. Proc. 1st Int. Working Conf. Stored Product Entomol., Savannah, Georgia, pp. 63-67.

Cortesero, A.M., 1994. La récherche de l'hôte chez *Eupelmus vuilleti* (Crw.). Analyse des rélations tritrophique entre la plante (*Vigna unguiculata* Walp), l'hôte (*Bruchidius atrolineatus* Pic) et le parasitoide. Thesis, Université François Rabelais, Tours, 142 pp.

Dicke, M., 1996. Direct and indirect effects of plants on performance of beneficial organisms. In: J.R. Ruberson (ed), Handbook of Pest Management. Marcel Dekker Inc., New York, in press.

Germain, J.F.; Monge, J.P.; Huignard, J., 1987. Development of two bruchid populations (*Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (Fab.) infesting stored cowpea (*Vigna unguiculata* Walp) pods in Niger. J. Stored Prod. Res. 23: 157-162.

Huignard, J.; Leroi, B.; Alzouma, I.; Germain, J.F., 1985. Oviposition and development of *Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in *Vigna unguiculata* (Walp) cultures in Niger. Insect Sci. Applic. 6: 691-699.

Huis, A. van, 1991. Biological methods of bruchid control in the tropics: a review. Insect Sci. Applic. 12: 87-102.

Huis, A. van; Kaashoek, N.K.; Lammers, P.M., 1990. *Uscana lariophaga* (Hym.: Trichogrammatidae), egg parasitoid of two bruchid species of cowpea in West Africa. Proc. Exp. Appl. Entomol., Neth. Entomol. Soc. (N.E.V.), Amsterdam, 1: 101-106.

Huis, A. van; Kaashoek, N.K.; Maes, H.M., 1991. Biological control of bruchids (Col.: Bruchidae) in stored pulses by using egg parasitoids of the genus *Uscana* (Hym.: Trichogrammatidae): a review. In: F. Fleurat-Lessard & P. Ducom, (eds), Proc. 5th Int. Working Conf. Stored-product Prot., Bordeaux, Volume 1, pp. 99-107.

Huis, A. van; Schütte, C.; Cools, M.H.; Fanget, Ph.; Hoek, H. van der; Piquet, S.P., 1994. The role of semiochemicals in host location by *Uscana lariophaga*, egg parasitoid of *Callosobruchus maculatus*. In: E. Highley, E.J. Wright, H.J. Banks & B.R. Champ (eds), Stored Product Protection. Proc. 6th Int. Working Conf. Stored-product Prot., CAB International, Wallingford, Volume II, pp. 1158-1164.

Jones, R.E., 1977. Movement patterns and egg distribution in cabbage butterflies. J. Animal Ecol. 46: 195-212.

Kareiva, P., 1987. Habitat fragmentation and the stability of predator-prey interactions. Nature 326: 388-390.

Kitch, L.W.; Shade, R.E.; Murdock, L.L., 1991. Resistance to the cowpea weevil (*Callosobruchus maculatus*) larva in pods of cowpea (*Vigna unguiculata*). Entomol. exp. appl. 60: 183-192.

Margolies, D.C., 1993. Adaptation to spatial variation in habitat: spatial effects in agroecosystems. In: K.C. Kim & B.A. McPheron (eds), Evolution of Insect Pests. Patterns in Variation. John Wiley & Sons, New York, pp. 129-144.

Nachman, G., 1987. System analysis of acarine predator-prey interactions. II. The role of spatial processes in system stability. J. Animal Ecol. 56: 267-281.

Navarro, S.; Amos, T.G.; Williams, P., 1981. The effect of oxygen and carbon dioxide gradients on the vertical dispersion of grain insects in wheat. J. Stored Prod. Res. 17: 101-107.

Press, J.W., 1988. Movement of a weevil parasitoid, *Anisopteromalus calandrae* (Howard), within a column of wheat in relation to host location (Note). J. Agric. Entomol. 5: 205-208.

Press, J.W., 1992. Comparative penetration efficacy in wheat between the weevil parasitoids *Anisopteromalus calandrae* and *Choetospila elegans* (Hymenoptera: Pteromalidae). J. Entomol. Sci. 27: 154-157.

Press, J.W.; Flaherty, B.R.; Arbogast, R.T., 1979. Vertical dispersion and control efficacy of the predator *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) in farmers stock peanuts. J. Kansas Entomol. Soc. 52: 561-564.

Rogers, D. 1972. Random search and insect population models. J. Animal Ecol. 41: 369-383.

Sabelis, M.W.; Alebeek, F. van; Bal, A.; Bilsen, I. van; Heijningen, T. van; Kaiser, P.; Kramer, G.; Snellen, H.; Veenenbos, R.; Vogelezang, J., 1983. Experimental validation of a simulation model of the interaction between *Phytoseiulus persimilis* and *Tetranychus urticae* on cucumber. Bull. OILB/SROP 1983/VI/3: 207 - 229

Schöller, M.; Reichmut, C.; Hassan, S.A., 1994. Studies on biological control of *Ephestia kuehniella* Zeller (Lep.: Pyralidae) with *Trichogramma evanescens* Westwood (Hym.: Trichogrammatidae) - host-finding ability in wheat under laboratory conditions. In: E. Highley, E.J. Wright, H.J. Banks & B.R. Champ (eds), Stored Product Protection. Proc. 6th Int. Working Conf. Stored-product Prot. CAB International, Wallingford, Volume II: 1142-1146.

Sokal, R.R.; Rohlf, F.J., 1981. Biometry. The principles and practice of statistics in biological research (2nd ed). W.H. Freeman and company, New York, 860 pp.

Surtees, G., 1964. Laboratory studies on dispersion behaviour of adult beetles in grain. VI.- Three-dimensional analysis of dispersion of five species in a uniform bulk. Bull. ent. Res. 55: 161-171.

Surtees, G., 1965. Ecological significance and practical implications of behaviour patterns determining the spatial structure of insect populations in stored grain. Bull. ent. Res. 56: 201-213.

Thorpe, K.W., 1984. Effects of height and habitat type on egg parasitism by *Trichogramma minutum* and *T. pretiosum* (Hymenoptera: Trichogrammatidae). Agric. Ecosyst. Environ. 12: 117-126.

Walde S.J.; Murdoch, W.W., 1988. Spatial density dependence in parasitoids. Ann. Rev. Entomol. 33: 441-466.

Watanabe, N., 1984. Comparative studies on ecology of the adzuki bean weevil, *Callosobruchus chinensis* (L.) and the cowpea weevil, *C. maculatus* (F.) (Coleoptera: Bruchidae). I. Effect of seed size on creeping of females into the pile. Jap. J. appl. Entomol. Zool. 28: 223-228.

Watanabe, N., 1985. Comparative studies on ecology of the adzuki bean weevil, *Callosobruchus chinensis* (L.) and the cowpea weevil, *C. maculatus* (F.) (Coleoptera: Bruchidae). II. Relation between female body size and degree of creeping into the stored pile of seeds. Jap. J. appl. Entomol. Zool. 29: 107-112.

Watanabe, N., 1986. Comparative studies on ecology of the adzuki bean weevil, *Callosobruchus chinensis* (L.) and the cowpea weevil, *C. maculatus* (F.) (Coleoptera: Bruchidae). IV. Effect of adult body size on easiness of escaping from the bottom of piled seeds. Jap. J. appl. Entomol. Zool. 30: 64-66.

General discussion and conclusions

9

Abstract
In this chapter, the methodology of indirect experiments on foraging behaviour, supplemented with direct observations, is discussed. Potential conservation strategies, that will improve several aspects of the searching efficiency of *U. lariophaga*, could be the supply of honey to parasitoids in granaries, and the manipulation of the temperature in storage during cold periods. In preliminary field tests, the egg parasitoid seems capable of significantly suppressing bruchid populations in the first months of storage. Further progress in the biological control of bruchid pests in stored cowpea in West Africa will come from a combination of simulation modelling and testing parasitoids in experimental granaries. Simulation models may help to study the population dynamics of host and parasitoid, and to identify the most promising environmental manipulations that would act as a conservation strategy of biological control of *C. maculatus*. Validation of such models should be carrier out under farmers' storage conditions. The combination of models and field tests could demonstrate whether manipulation of the storage environment to enhance the impact of *U. lariophaga* is a sound conservation strategy. This will proof the capabilities and limitations of the parasitoid acting as a biological control agent of *C. maculatus* in the cowpea storage environment.

Chapter 9

Methodology

The stored cowpea environment is a man-made ecosystem, consisting of a closely packed substrate of produce in a dark three-dimensional space. Compared to plants, on which pests and natural enemies generally can be observed easily in their natural habitat, stored products systems pose severe limitations on observing the behaviour of pests and benificials. The research described in this thesis consists mainly of indirect experiments on foraging behaviour in a 'semi-natural' design: experimental containers filled with cowpea seeds, which acted as black boxes. Such a setup has the disadvantage that the behaviour of parasitoids can no longer be observed directly. Parameters such as walking speed, host acceptance, host rejection, patch time allocation, and host discrimination cannot be measured. The alternative would be to do experiments in a two-dimensional setup, allowing observation of the parasitoid's behaviour. However, the advantage of the three-dimensional, indirect design is that the outcome of parasitoid behaviour is likely to be close to what can be expected from parasitoid performance under storage conditions. Because of the positive phototaxis and negative geotaxis of *U. lariophaga*, I expect that behaviour of *U. lariophaga* in a two-dimensional design will be essentially different from behaviour in a three-dimensional design. In order to compensate some of the disadvantages of the 'black box' approach, experiments were supplemented with direct observations in a two-dimensional design, of the egg laying capacity, initial egg load, host handling time and walking behaviour of the parasitoid. Direct observations turned out to be essential for a correct interpretation of the findings in the indirect experiments.

Some investigators of natural enemies of stored product pests have used two-dimensional experimental designs to evaluate their effectiveness [e.g. LeCato & Arbogast (1979) with *Xylocoris flavipes* (Hete.; Anthocoridae); Delobel (1989) with *Uscana caryedoni* (Hym.; Trichogrammatidae); Tuda & Shimada (1995) with *Heterospilus prosopidis* (Hym.; Braconidae)]. Others have experimented in more semi-natural, three-dimensional setups like the ones described in Chapter 4 - 8 of this thesis [e.g. Flinn (1991) with *Cephalonomia waterstoni* (Hym.: Bethylidae); Smith & Press (1992) and Smith (1994) with *Anisopteromalus calandrae* (Hym.: Pteromalidae); Parajulee *et al.* (1994) with *Lyctocoris campestris* (Hete.; Anthocoridae); and Schöller *et al.* (1994) with *Trichogramma evanescens* (Hym.: Trichogrammatidae)]. Without proper comparisons between these setups and the performance under natural conditions, one cannot say which methods should preferably be used.

It is not surprising that some of the methodologies used in stored-products research closely resemble experimental designs employed in soil biology. Soil (micro-) arthropods and nematodes also move through and search within a three-dimensional habitat of small particles. For example, the vertical dispersion of insect parasitic nematodes (of the genera *Heterorhabiditis, Steinernema,* and *Neoaplectana*) has often been investigated in columns filled with soil substrate (e.g. Moyle & Kaya, 1981; Georgis & Poinar, 1983a,b,c; Westerman, 1991; Hanula, 1993). Insect larvae are generally used as baits to trap the nematodes and to measure their displacements. This type of design is much alike the experiments described in Chapters 5, 6 and 8 of this thesis.

Several nematode species show a tendency to move upwards (Georgis & Poinar, 1983a,c) like many stored product parasitoids (see Chapter 5). Entomogenous nematodes are thought to be attracted by chemical host cues (like CO_2) and cover larger distances when hosts are present (e.g. Georgis & Poinar, 1983b; Westerman, 1991), much like *Uscana* does in response to *C. maculatus* egg odours (see Chapter 4). The effect of different substrate (silica sand, loam, clay) has been investigated (Georgis & Poinar, 1983a) in experiments resembling our investigations with cowpea pods versus seeds, described in Chapter 8 of this thesis.

For entomogenous nematodes, soil structure and soil humidity are very important factors in movement. This makes it difficult to predict nematode penetration rates in sand columns with an artificial soil structure and water content, and to extrapolate these rates to nematode penetration in naturally structured soil in the field (e.g. Hanula, 1993). In *U. lariophaga*, the habitat structure (cowpea seeds or pods) had little effect on dispersal and foraging behaviour (Chapter 8). Therefore, it will be easier to generalize our findings with *U. lariophaga* from the laboratory experiments to field storage conditions, than similar collected data using entomogenous nematodes.

A number of questions investigated in this thesis apply equally well to the study of other stored product natural enemies as well as to soil dwelling predatory mites, nematodes, and springtails. Therefore, common approaches and experimental designs should be sought for, increasing the chance to improve our general understanding of behavioral and ecological processes in those special types of habitats.

Evaluation criteria of natural enemies in a conservation strategy of biological control in stored products

The criteria to evaluate *U. lariophaga* as a candidate for a conservation strategy were reviewed in paragraph 3.1, and are summarized in Table 9.1. Not all of these criteria (Table 9.1) can be manipulated to the same extent. For example, criterion 1 (*Environmental risks*) and 4 (*Host selection and suitability*) are mainly descriptive characteristics of a beneficial species, and cannot easily be changed. On the other hand, manipulating the climate, the provision of food or changing the habitat structure in a cowpea granary will almost certainly influence *U. lariophaga*'s dispersal, and several aspects of its searching efficiency. In recent years, many criteria have already been investigated for *U. lariophaga* (see § 3.1 for a review). The research presented in this thesis has focused on the investigation of different aspects of criterion 3: *compatibility with the storage environment*, and criterion 7: *searching efficiency* of *U. lariophaga*.

Table 9.1 Criteria for the evaluation of *U. lariophaga* in a conservation strategy. See paragraph 3.1 for an extensive review.

Evaluation criterion	Studied by Van Huis *et al.*[1]	Discussed in this Thesis
1. Environmental risks	--	--
2. Tolerance of climatic extremes	+	--
3. Compatibility with storage environment		
- Migration from and into granaries	--	Chapter 2
- Effects of light conditions	+	Chapter 8
- Dispersal within storage structures	--	Chapter 5 and 8
4. Host selection and suitability	+	--
5. Seasonal synchronization	+	--
6. Reproductive capacity	+	--
7. Searching efficiency		
- Host habitat location	+	Chapter 4
- Host community location within the habitat	+	Chapter 5
- Dispersal, travel speed (see criterion 3)	--	Chapter 5 and 8
- Functional response	--	Chapter 6, 7 and 8
- Aggregation and walking behaviour	--	Chapter 6, 7 and 8
8. Compatibility with other control methods	--	Chapter 2

1: For references, see Table 3.1

Criterion 3: Compatibility with the storage environment

One of the questions is whether our parasitoid is adapted to the man-made storage environment. Searching in a wild habitat with widely spaced host plants and rare but locally clustered hosts within that habitat, will differ from searching in a densely cropped cowpea field with low host densities clustered at the plant and pod level. This, again, will differ markedly from searching in a closed, dark cowpea granary with clumped or evenly distributed host eggs, often in high densities. Selection forces on searching strategies in each of these habitats are likely to be different, and the resulting characteristics of the wasps under study depend on the origin of the population and on the genetic exchange between populations from different habitats.

Very little is known about the occurrence of *U. lariophaga* and other *Uscana spp.* in wild vegetation, especially during the dry season. How are these populations able to persist? Do they switch between different bruchids hosts in the course of the year? In the desert areas of California (USA), *U. semifummipennis* demonstrates diapause in extremely hot periods (R. Mitchell, personal communication). We do not know whether diapause occurs in *U. lariophaga*. Nor do we know from which hosts and what kind of habitat *U. lariophaga* originally comes, when colonizing cowpea fields in the rainy season. Nor do we know where they go, if they escape from cowpea stores or if the cowpea storage season comes to an end. Sofar, many of those questions could not be answered, because identification of *Uscana* field samples was not possible. However, new developments in molecular techniques may bring identification of *Uscana* samples within reach (e.g. van Kan *et al.*, 1996), allowing some of the questions on *U. lariophaga* field populations to be investigated.

If cowpea fields are always colonized by "wild" wasps from natural habitats, which subsequently enter the granary upon harvest, and if no remigration from stores back to fields occurs, a poor adaptation to the storage environment can be expected. If wasps, after a large number of generations within a granary, return to either the "wild" population or colonize new cowpea fields, and contribute significantly to the gene pool of the mixed population, then some of the adaptations to the storage environment may be preserved in the wasps.

For now, it seems that *U. lariophaga* possesses a number of characteristics which allow the wasp to survive over many generations in storage, and, under specific conditions, to reach very high population densities. How these traits have been selected for and are being preserved within the population of *U. lariophaga*, can perhaps be clarified when DNA finger printing techniques become available.

Criterion 7: searching efficiency

Most of the research presented in this thesis was focused on the investigation of different aspects of the searching efficiency of *U. lariophaga*. For a proper evaluation of the potentials of *U. lariophaga* in a conservation strategy, some questions need to be answered:

+ Which environmental manipulations can improve the searching efficiency of *U. lariophaga* ?
+ Can *U. lariophaga* contribute to a significant reduction in losses of stored cowpea due to bruchids ?
+ How does the impact of *U. lariophaga* on bruchid populations compare to that of the larval parasitoids occurring in the cowpea storage system?
+ Can *U. lariophaga* be combined with larval parasitoids, and do such combinations result in significantly lower seed losses ?

These questions are being discussed hereafter.

Which environmental manipulations can improve the searching efficiency of Uscana lariophaga *?*

The parasitoid mobility studies demonstrated that colonizing *U. lariophaga* can cover distances of several meters in one day. This would be sufficient to cover the internal volume of most traditional West African cowpea granaries. Storage of cowpea as seeds or as pods has little influence on *U. lariophaga* dispersal behaviour. However, light significantly stimulated the female parasitoids movements (positive phototaxis), possibly leading to emigration of wasps out of granaries. Traditional straw cowpea stores are more open to light than clay granaries, and this emigration out of stores may be more important in straw than in clay granaries. Whether this would significantly influence the parasitoids population dynamics and impact is not yet clear. Lammers & van Huis (1989) found significantly less parasitism in straw granaries compared to clay granaries. It would be interesting to study whether the presence of host eggs or egg odours could suppress this phototaxis, just as it seems to suppress the negative geotaxis (Chapter 5). This topic requires further investigations. In theory, open walls of granaries would also allow more immigration of parasitoids into cowpea stores. However, results of our sampling survey (Chapter 2) suggest that this factor is of little or no importance.

Within host clusters, the rate of parasitism by *U. lariophaga* appears to be limited by egg load and egg maturation rate. Searching time and host handling time are not limiting factors, not even at higher host densities. Initial egg load determines the plateau level of the functional response, i.e. the maximum number of hosts parasitized. This implies that all measures that increase the realized fecundity of *U. lariophaga* females will result in a larger number of hosts parasitized per female. This will potentially improve the biological control of bruchids with *Uscana*. One such a measure could be the provision of honey. Feeding with honey increases *U. lariophaga* life-time fecundity three-fold, from approximately 25 to 75 eggs per female (Van Huis *et al.*, 1991). Honey also increase the longevity from two days without food to about ten days with honey (Van Huis *et al.*, 1991). A longer lifespan will also allow *U. lariophaga* females to travel longer distances, increasing their chances of host encounters. The provision of natural food sources, e.g. as wild flowers for nectar, is a well recognized method in the conservation of natural enemies (e.g. Hagen, 1986; Dent, 1991; Dutcher, 1993). However, the provision of alternative, artificial food sources, such as honey, has rarely been tested in practice at the field level (Hagen *et al.*, 1970; Hagen, 1986). The provision of honey to foraging parasitoids within cowpea storage structures still faces many practical problems, like easy access to the parasitoids, drying out of the honey, dust accumulation, and bruchids also feeding on it (S. Sagnia, DFPV Niamey, personal communication; Wäckers, 1996).

Temperature is a major factor strongly influencing insect biology. Manipulation of storage temperature conditions, e.g. through shading or exposure of the granary to sunshine, improved aeration or hermetic storage, will influence the fecundity, development time and mortality of both bruchids and their parasitoids. The final outcome of such manipulations is not easy to predict. In general, temperatures in between the lower and upper lethal extremes, increase the intrinsic rate of increase in *U. lariophaga* relative to that of its host *C. maculatus* (Van Huis *et al.*, 1994).

I did not evaluate the effect of temperature on foraging behaviour (e.g. the functional response, walking speed, etc.). However, from what is generally known, we may expect that rates of parasitism and the upper plateau levels in the functional response will increase at higher temperatures below the lethal maximum (e.g. Flinn, 1991; Hong & Ryoo, 1991; Smith, 1994; Tuda & Shimada, 1995). This may be a result of increased walking speed, increased activity, shorter handling time, and, for *U. lariophaga* in particular, also because of a higher egg maturation rate in the ovaria. Thus, higher temperatures in storage, especially during the relatively cold period of December - February, may improve bruchid control.

Little is known of temperature effects on host location abilities of parasitoids. Direct sunlight will cause air convection currents in granaries, in the upward direction along the hot walls, and downward along the centre of the stored bulk. During the night, the direction of air currents will be reversed. Whether these air currents, carrying olfactory information from host eggs with them, assist or confuse *U. lariophaga* in host (community) location, is not yet clear and will be complex to investigate.

Can Uscana lariophaga *contribute to a significant reduction in losses of stored cowpea due to bruchids ?*

Within a host cluster, *U. lariophaga* demonstrates a Type II functional response. This implies that parasitism caused by individual parasitoids is inversely density dependent. Type II responses are considered to cause instability in parasitoid-host relationships and may be disadvantageous for biological control. However, *U. lariophaga* females also show a strong arrestment response,leading to aggregation in high density host clusters. Besides, clusters with more host eggs are more easily found by *U. lariophaga* than clusters with few eggs. At the population level within a multiple patch habitat, this aggregation behaviour may still lead to an overall density dependent parasitism of host patches (for a review, see: Walde & Murdoch, 1988).

The final proof, whether *U. lariophaga* is capable of significantly reducing losses from bruchids in stored cowpea, should come from field tests. Delobel (1989) did such tests with *U. caryedoni* Viggiani for the control of *Caryedon serratus* (Oliver) (Col.: Bruchidae) in stored groundnuts. He concluded that biological control of this bruchid with *U. caryedoni* has little potential, mainly because the parasitoid did not penetrate into groundnut stocks, and could not bridge the gap between successive, non-overlapping generations of its host in storage. Lammers & Van Huis (1989) monitored *U. lariophaga* populations in experimental cowpea granaries in Niger, and observed high rates of parasitism (60%) just after a peak in bruchid egg density. They could not conclude whether *U. lariophaga* parasitism just followed the increase and subsequent sharp decline in bruchid egg densities, or that the parasitoid significantly influenced the decline in host densities.

More insight stems from experimental work performed in 1991 in Niger (Sankung Sagnia, DFPV, Niger; Arnold van Huis & Conny Schütte, Dept. of Entomology, WAU, The Netherlands; unpublished data). Twenty experimental clay granaries were used, each filled with nine kilogram of clean cowpea seeds. In the control, about 25 *C. maculatus* (in different developmental stages) were introduced. Three treatments (with five replicates each) were compared: a low, medium, and high initial bruchid density, with respectively 25, 120 and 360 bruchids (in different developmental stages) inoculated, together with 100 *C. maculatus* eggs parasitized by *U. lariophaga*. Granaries were sampled every two weeks, and after three months the losses to seeds were evaluated. Results are summarized in Table 9.2. In the control granaries, bruchid populations in the five replicates grew exponentially. In the low density infestation, three out of five granaries had an exponential bruchid development, somewhat lagging behind the control. The medium density infestation had a much reduced population growth and increased rates of parasitism. The high initial bruchid density had stabilisation of bruchid populations in three out of five granaries from week 6 onwards, with the lowest egg densities and the highest rates of parasitism. After 12 weeks in storage, the percentage of seeds damaged at the high initial bruchid density was reduced by more than 80% compared to the control.

Table 9.2 Assessment of egg densities, rates of egg parasitism and the percentage of damaged cowpea seeds, in 40 gram samples after 12 weeks of storage in experimental granaries inoculated with different densities of *C. maculatus* and with *U. lariophaga* in 1991 in Niger.

		after 12 weeks of storage		
# bruchids inoculated	parasitoid inoculation	number of eggs	% of eggs parasitized	% of seeds damaged
25	-- (control)	2000	--	23%
25	+ *Uscana*	1200	58%	10%
120	+ *Uscana*	850	78%	7%
360	+ *Uscana*	450	84%	4%

(Source: unpublished data provided by Sankung Sagnia, DFPV, Niger; Arnold van Huis & Conny Schütte, Department of Entomology, WAU, The Netherlands).

These experiments point towards similar difficulties for *U. lariophaga* as for *U. caryedoni* (Delobel, 1989). *Uscana lariophaga* has a much shorter developmental time (8-10 days) and longevity (3 days), compared to those of *C. maculatus* (three weeks and one week, respectively). For the survival of *U. lariophaga* populations in storage, overlapping bruchid generations with a continuous supply of fresh bruchid eggs is required. A period of 14 days without fresh host eggs would wipe out the *U. lariophaga* population in a granary. At the start of the storage season, when bruchid densities are still relatively low, freshly laid eggs may not be sufficiently available at all times to allow the establishment and increase of *U. lariophaga* populations. In three out of five replicates in the lowest bruchid density treatment, *U. lariophaga* did not become sufficiently established to exert control over the bruchid population. Therefore, field sampling of cowpea at the moment of harvest, to assess *C. maculatus* egg densities and distributions at the start of the storage season, should have a high priority. The problem of *U. lariophaga* establishment at the start of the storage season (which is an aspect of seasonal synchronisation, criterion 5 in Table 9.1) is especially relevant, because granaries function as ecological islands (see Chapter 2) and apparently are not easily (re)colonized by parasitoids from nearby granaries. The lack of synchronisation may be overcome by *U. lariophaga* inoculations later in the storage season, when the bruchid population is at a higher density and generations start to overlap. Increasing the longevity of *U. lariophaga* through honey as an additional food supply (Van Huis et al., 1991) could also be a conservation strategy.

How does the impact of Uscana lariophaga *on bruchid populations compare to that of the larval parasitoids occurring in the cowpea storage system?*

Ideally, an evaluation of the relative importance of several parasitoids in a conservation strategy of biological control should follow the same protocol of experiments for each species. The characteristics as investigated in *U. lariophaga* should be compared with available data on the larval parasitoids that have been published in the literature. Research on larval parasitoids was reviewed in paragraph 3.1, and unfortunately, the experiments with *U. lariophaga* have little in common with the work done on the larval parasitoids, making comparisons very difficult.

Data concerning the seasonal synchronisation (see also above) have been published and enable a comparison between species. The two larval parasitoids, *Dinarmus basalis* and *Eupelmus vuilleti*, have a developmental time of 12 to 18 days, and a longevity of approx. 20 - 30 days, which allows them to bridge gaps until the next bruchid generation (Van Alebeek, 1991). *Uscana lariophaga* has a developmental time of 8-10 days and a longevity of 2-3 days (Van Huis *et al.*, 1991). Thus, establishment of larval parasitoids in granaries is probably less critical than for egg parasitoids. This may explain the observation that *Uscana spp.* were present in 69 % of all samples taken in Niger in 1992, whereas larval parasitoids were found in 92 % of the samples (Chapter 2).

The importance of the different parasitoids for the reduction of cowpea losses has been evaluated in several field experiments. The relative importance of the larval parasitoids *D. basalis* and *E. vuilleti* was compared in 1992 in Niger (Monge *et al.*, 1995) and in 1994 in Togo (Glitho *et al.*, 1995). Results can be summarized as follows. Inoculation of stores with *D. basalis* can reduce bruchid populations by 70-97% compared to the control, while inoculations with *E. vuilleti* reduced bruchid populations by 32-36% only. In case both larval parasitoids were introduced together, *E. vuilleti* was the dominant parasitoid, while *D. basalis* became marginal in the system, and the bruchid population attained levels comparable to those in granaries with *E. vuilleti* alone. The reasons for this negative interaction are the ability of *E. vuilleti* females to kill eggs of *D. basalis*, and the facultative hyperparasitic behaviour of *E. vuilleti* on *D. basalis* larvae (see § 3.1 and Van Alebeek, 1991; Van Alebeek *et al.*, 1993; Leveque *et al.*, 1993). *Dinarmus basalis* is superior to *E. vuilleti* in suppressing the bruchid populations, and therefore a good candidate for a conservation strategy. However, the naturally occurring *E. vuilleti* cannot easily be excluded from the system, and significantly reduces the suppression of the bruchid population by *D. basalis*.

The impact of *U. lariophaga* relative to *D. basalis* was measured in experiments in December 1992 (Sankung Sagnia, DFPV, Niger; Pascal Dupont, Univ. of Niamey, Niger; Arnold van Huis & Marcus van Es, Dept. of Entomology, WAU, The Netherlands; unpublished data). Twenty experimental granaries were each filled with seven kilogram of cowpea seeds, and divided over four treatments. All treatments were inoculated with approximately 400 *C. maculatus* (in different developmental stages, to ensure overlapping generations). One treatment (+ *Uscana*) received 100 *C. maculatus* eggs parasitized by *U. lariophaga*, another treatment (+ *Dinarmus*) received 100 *C. maculatus* larvae parasitized by *D. basalis*, and the last treatment (+ *Uscana* + *Dinarmus*) received 100 stages of each parasitoid species at inoculation. The control received *C. maculatus* only. Parasitoid populations introduced consisted of mixed ages. Granaries were sampled every two weeks for three months. Results are summarized in Table 9.3.

The inoculation with many more bruchids in 1992 compared to 1991 (400 versus 25 respectively) resulted in much higher egg densities and damaged seeds in the control (compare Table 9.2 and 9.3). The treatment with *U. lariophaga* gave less reduction of the bruchid population and damaged seeds, with the same rate of egg parasitism, compared to those observed in 1991. The treatment with *D. basalis* had much less seed losses compared to the control, and significantly reduced the bruchid population in terms of egg density (to 15% of the control).

Table 9.3 Estimated egg densities, rates of *U. lariophaga* egg parasitism, the number of bruchid and parasitoid emergence holes, the percentage emergence holes from *D. basalis*, and the percentage of damaged cowpea seeds, in 40 gram samples after 12 weeks of storage in experimental granaries inoculated with *C. maculatus* and the parasitoids in 1992 in Niger (?? = data lacking).

parasitoid inoculation	number of eggs	% of eggs parasitized	number of emergence holes	% Dinarmus emergence holes	% of seeds damaged
-- (control)	6500	--	??	--	88%
+ *Uscana*	3000	56%	??	--	50%
+ *Dinarmus*	950	--	98	15%	31%
+ *Uscana* + *Dinarmus*	1300	9%	95	16%	36%

(Source: unpublished data provided by Sankung Sagnia, DFPV, Niger; Pascal Dupont, University of Niamey, Niger; Arnold van Huis and Marcus van Es, Department of Entomology, WAU, The Netherlands).

Can Uscana lariophaga *be combined with larval parasitoids, and do such combinations result in significantly lower seed losses ?*

Multiple introductions and coexistence of several parasitoids has been the subject of an ongoing debate in discussions of biological control theory (e.g. Huffaker *et al.*, 1976; May & Hassell, 1981; Hassell & May, 1986). The general conclusion is that it is very unlikely that new, additional natural enemies negatively influence the control already exerted by existing parasitoid species in the system. However, in the case of *D. basalis* and *E. vuilleti*, reasonable levels of bruchid control by the former species are disrupted by the presence of the latter species (see above). Coexistence of *U. lariophaga* with *D. basalis* was investigated in 1992 in Niger, in the set of experiments summarized in Table 9.3. In the combination of *U. lariophaga* and *D. basalis*, egg parasitism was very poor (9%), and for all other parameters this treatment gave about the same results as the treatment with *D. basalis* alone. The impact of *U. lariophaga* is greatly reduced by *D. basalis* populations. Probably, the larval parasitoid changes the bruchid host population from overlapping into separated generations, thus causing synchronisation problems for *U. lariophaga.*

Thus, it seems that, if parasitoid species are inoculated as single species in stores, both *D. basalis* and *U. lariophaga* significantly suppress the bruchid population, but the former does so more effectively than the latter. *Uscana lariophaga* in its turn is more efficient than *E. vuilleti*. Combinations of *D. basalis* with *E. vuilleti*, or *D. basalis* with *U. lariophaga* result in poorer or the same suppression of bruchid populations as when *D. basalis* is the only parasitoid. The combination of *E. vuilleti* with *U. lariophaga* and the combination of all three parasitoids have not been tested yet. The outcome such combinations is not yet clear, but reduction of *D. basalis* by *E. vuilleti* may make room for a better performance of *U. lariophaga*. Conservation strategies should focus on *D. basalis* and *U. lariophaga*. Both species are capable of protecting cowpea in storage for at least 3 months after harvest. Since it is difficult to exclude naturally occurring parasitoids from the system, it is not likely that the detrimental effects of additional parasitoid species can be prevented.

The research presented in this thesis has helped to fill the gap in our knowledge of searching behaviour in *U. lariophaga*. Looking back at the important characteristics of a natural enemy for conservation biological control (Table 9.1), most of the evaluation criteria have now been studied in the laboratory. In order to integrate this knowledge, and to be able to make a final evaluation of the potential use of *U. lariophaga* in biological control, two roads are open and should ideally be followed concurrently: the construction of explanatory population simulation models, and testing the performance of *U. lariophaga* if inoculated into cowpea granaries.

Population models would allow us to estimate the relative importance of different characteristics of parasitoids and to study the impact of environmental manipulations at the population level of the bruchid host and the parasitoid. Simulation models have the disadvantage that their construction may take many years and that their prediction accuracy is often poor. The advantage of models is that one can simulate different scenario's and environmental conditions, and obtain answers and insights in a relatively short time. This in contrast to expensive, long running field experiments in which only very few variables can be tested one by one. A good illustration of this approach is given by Van Roermund (1995). Some of the following major issues raised above can be explored by use of simulation models:

+ the effect of providing honey in storage;
+ the manipulation of temperatures during the cold period in December - February;
+ the minimum levels of bruchid densities required for establishment of *U. lariophaga*;
+ the interaction between *U. lariophaga* and *D. basalis*; and
+ the possible occurrence of dis-synchronisation between bruchid and parasitoid populations.

Simulation models can also help in narrowing down the choice of critical environmental conditions that should be tested in field experiments. Especially under research conditions in West Africa, such an approach may help to make optimal use of the often limited scientific infra-structure, materials and resources.

At the same time, the proof is in the eating of the pudding. Inoculations of *U. lariophaga* in experimental and farmer's cowpea granaries should be carried out in order to demonstrate the feasibility and limitations of *U. lariophaga*'s control capabilities under selected, manipulated environmental conditions. The development of *U. lariophaga* rearings under small farmers' or farmers community conditions will be an essential step to proceed (see e.g. Bhatnagar, 1987). The development of guidelines for sound farmer's conservation practices will be another major objective, taking into account the huge diversity in storage methods and techniques, traditions, ethnic background, language, and resource availability as observed in West Africa. I do hope that the work presented here will contribute a significant step on the road towards achievement of these goals.

Acknowledgements

In writing this chapter, I was much encouraged by discussions with Joop van Lenteren, Marcel Dicke, Arnold van Huis, Herman van Roermund, Pascal Dupont, Sankung Sagnia, and Conny Schütte. Thanks are also due to several of those persons and Marcus van Es, for the sharing of unpublished data on their field experiments in Niger.

References

Alebeek, F.A.N. van, 1991. Interspecific host discrimination by two solitary ectoparasitoids of immature stages of Bruchidae. Meded. Fac. Landbouww. Rijksuniv. Gent 56/3b: 1011-1020.

Alebeek, F.A.N. van; Rojas-Rousse, D.; Leveque, L., 1993. Interspecific competition between *Eupelmus vuilleti* and *Dinarmus basalis*, two solitary ectoparasitoids of Bruchidae larvae and pupae. Entomol. exp. appl. 69: 21-31.

Bhatnagar, V.S., 1987. Conservation and encouragement of natural enemies of insect pests in dryland subsistence farming: problems, progress and prospects in the Sahelian zone. Insect Sci. Applic. 8: 791-795.

Delobel, A., 1989. *Uscana caryedoni* (Hym.: Trichogrammatidae): possibilites d'utilisation en lutte biologique contre la bruche de l'arachide, *Caryedon serratus* (Col.: Bruchidae). Entomophaga 34(3): 351-363.

Dent, D., 1991. Insect Pest Management. CAB International, Wallingford.

Dutcher, J.D., 1993. Recent examples of conservation of arthropod natural enemies in agriculture. In: R.D. Lumsden & J.L. Vaughn (eds.), Pest Management: Biologically Based Technologies, Proc. Beltsville Symp. 18, Agric. Res. Service, USDA, Beltsville, pp. 101-108.

Flinn P.W., 1991. Temperature-dependent functional response of the parasitoid *Cephalonomia waterstoni* (Gahan) (Hymenoptera: Bethylidae) attacking rusty grain beetle larvae (Coleoptera: Cucujidae). Environ. Entomol. 20(3): 872-876.

Georgis, R.; Poinar Jr., G.O., 1983a. Effect of soil texture on the distribution and infestivity of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae). J. Nematol. 15: 308-311.

Georgis, R.; Poinar Jr., G.O., 1983b. Effect of soil texture on the distribution and infestivity of *Neoaplectana glaseri* (Nematoda: Steinernematidae). J. Nematol. 15: 329-332.

Georgis, R.; Poinar Jr., G.O., 1983c. Vertical migration of *Heterorhabdidtis bacteriophora* and *H. heliothidis* (Nematoda: Heterorhabditidae) in sandy loam soil. J. Nematol. 15: 652-654.

Glitho, I.A; Tchamoou, K.S.; Tchokossi, E., 1995. Essai de lutte biologique contre *Callosobruchus maculatus* Fab., coléoptère bruchidae dépredateur du niébé (*Vigna unguiculata* Walp.). Paper presented at 15th WASA Conference, 18-22 September 1995, University of Cape Coast, Ghana, 11 pp.

Hagen, K.S., 1986. Ecosystem analysis: plant cultivars (HPR), entomophagous species and food supplements. In: D.J. Boethel & R.D. Eikenbary (eds), Interactions of Plant Resistance and Parasitoids and Predators of Insects. Ellis Horwood, Chichester, pp. 151-197.

Hagen, K.S.; Sawall Jr., E.F.; Tassen, R.L., 1970. The use of food sprays to increase effectiveness of entomophagous insects. Proc. Tall Timbers Conf. Ecol. Animal Control Habitat Manage. 2: 59-81.

Hanula, J.L., 1993. Vertical distribution of black vine weevil (Coleoptera: Curculionidae) immatures and infection by entomogenous nematodes in soil columns and field soil. J. Econ. Entomol. 86: 340-347.

Hassell, M.P.; May, R.M., 1986. Generalist and specialist natural enemies in insect predator-prey interactions. J. Animal Ecol. 55: 923-940.

Hong, Y.S.; Ryoo, M.I., 1991. Effect of temperature on the functional and numerical responses of *Lariophagus distinguendus* (Hymenoptera: Pteromalidae) to various densities of the host, *Sitophilus oryzae* (Coleoptera: Curculionidae). J. Econ. Entomol. 84(3): 837-840.

Huffaker, C.B.; Simmonds, F.J.; Liang, J.E., 1976. The theoretical and empirical basis of biological control. In: C.B. Huffaker & P.S. Messenger, Theory and Practice in Biological Control. Academic Press, New York, pp. 41-78.

Huis, A. van; Arendse, P.W.; Schilthuizen, M.; Wiegers, P.P.; Heering, H.; Hulshof, M.; Kaashoek, N.K., 1994. *Uscana lariophaga*, egg parasitoid of bruchid beetle storage pests of cowpea in West Africa: the effect of temperature and humidity. Entomol. exp. appl. 70: 41-53.

Huis, A. van; Kaashoek, N.K.; Maes, H.M., 1991. Biological control of bruchids (Col.: Bruchidae) in stored pulses by using egg parasitoids of the genus *Uscana* (Hym.: Trichogrammatidae): a review. In: F. Fleurat-Lessard & P. Ducom (eds.), Proc. 5th Int. Working Conf. Stored-product Prot., Bordeaux, volume 1, pp. 99-108.

Kan, F.J.P.M. van; Silva, I.M.M.S.; Schilthuizen, M.; Pinto, J.D.; Stouthamer, R., 1996. Use of DNA-based methods for the identification of minute wasps of the genus *Trichogramma*. Proc. Exper. Appl. Entomol., N.E.V. Amsterdam 7: 233-237.

Lammers, P.M.; Huis, A. van, 1989. *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae), egg parasitoid of the stored insect pests *Callosobruchus maculatus* (F.) and *Bruchidius atrolineatus* Pic. (Col.: Bruchidae): population studies in the field and in storage in Niger. Proc. Int. Conf. Int. Pest Manage. 3: 1013-1022.

LeCato, G.L.; Arbogast, R.T., 1979. Functional response of *Xylocoris flavipes* to augoumois grain moth and influence of predation on regulation of laboratory populations. J. Econ. Entomol. 72: 847-849.

Leveque, L.; Monge, J.P.; Rojas-Rousse, D.; Alebeek, F. van; Huignard, J., 1993. Analysis of multiparasitism by *Eupelmus vuilleti* (Craw) (Eupelmidae) and *Dinarmus basalis* (Rond) (Pteromalidae) in the presence of one of their common hosts, *Bruchidius atrolineatus* (Pic) (Coleoptera Bruchidae). Oecologia 94: 272-277.

May, R.M.; Hassell, M.P., 1981. The dynamics of multiparasitoid-host interactions. Am. Naturalist 117: 234-261.

Monge, J.P.; Dupont, P.; Idi, A.; Huignard, J., 1995. The consequences of interspecific competition between *Dinarmus basalis* (Rond) (Hymenoptera: Pteromalidae) and *Eupelmus vuilleti* (CRW) (Hymenoptera: Eupelmidae) on the development of their host populations. Acta Oecol. 16: 19-30.

Moyle, P.L.; Kaya, H.K., 1981. Dispersal and infectivity of the entomogenous nematode, *Neoaplectana carpocapsae* Weiser (Rhabditida: Steinernematidae), in sand. J. Nematol. 13: 295-299.

Parajulee, M.N.; Phillips, T.W.; Hogg, D.B., 1994. Functional response of *Lyctocoris campestris* (F.) adults: effects of predator sex, prey species and experimental habitat. Biol. Control 4: 80-87.

Roermund, H.W.J. van, 1995. Understanding biological control of greenhouse whitefly with the parasitoid *Encarsia formosa*. From individual behaviour to population dynamics. Doctoral Thesis, Wageningen Agricultural University, Wageningen.

Schöller, M.; Reichmut, C.; Hassan, S.A., 1994. Studies on biological control of *Ephestia kuehniella* Zeller (Lep.: Pyralidae) with *Trichogramma evanescens* Westwood (Hym.: Trichogrammatidae) - host-finding ability in wheat under laboratory conditions. In: E. Highley, E.J. Wright, H.J. Banks & B.R. Champ (eds), Stored Product Protection. Proc. 6th Int. Working Conf. Stored-product Prot., CAB International, Wallingford, Volume II, pp. 1142-1146.

Smith, L., 1994. Temperature influences functional response of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) parasitizing maize weevil larvae in shelled corn. Ann. Entomol. Soc. Am. 87(6): 849-935.

Smith, L.; Press, J.W., 1992. Functional response of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae): influence of host numbers versus host density. J. Entomol. Sci. 27(4): 375-382.

Tuda, M.; Shimada, M., 1995. Developmental schedules and persistence of experimental host-parasitoid systems at two different temperatures. Oecologia 103: 283-291.

Wäckers, F.L., 1996. Can food provision increase the efficacy of biological control agents in storage systems? Abstract, Proc. 20th Int. Congr. Entomol., Firenze, August 25-31, 1996, pp. 551.

Walde, S.J.; Murdoch, W.W., 1988. Spatial density dependence in parasitoids. Ann. Rev. Entomol. 33: 441-466.

Westerman, P.R., 1991. Comparison of migration and host searching ability of various *Heterorhabditis* species and isolates in sand columns. IOBC/WPRS Bull. 14: 32-35.

Summary

Cowpea is an important protein source for small scale farmers' families in West Africa. During storage in the dry season, huge losses occur due to seed beetles of the family Bruchidae. Biological control of bruchid pests with the egg parasitoid *Uscana lariophaga* is a possible option to reduce cowpea losses in storage. This option has been explored in the work described here, focussing on the searching behaviour of the parasitoid *U. lariophaga.*

In **Chapter 1**, an overview is given of cowpea production in West Africa, and the losses caused by insect pests in the field and in storage. Very low initial field infestations (1%-2%) of seeds attacked by the bruchid pest *Callosobruchus maculatus* can result in total loss of the harvest after 6 months of storage. Biological control of this bruchid pest in a conservation strategy is an option compatible with subsistence farming conditions in West Africa. Conservation is a strategy whereby environmental manipulations aim to increase the impact of native natural enemies on indigenous pest populations.

In West Africa, several parasitoids of bruchid pests in cowpea occur. One of these is the egg parasitoid *Uscana lariophaga.* A sampling survey demonstrated their presence in cowpea stores in Niger (**Chapter 2**), but usually at relatively low levels. However, some incidental observations of very high levels (60-80%) of egg- and larval parasitism indicate the potential for natural control of bruchid pests by parasitoids. If we can identify the factors that realize this potential, and manipulate these factors, then we may be able to achieve better control of bruchids in cowpea storage.

In a conservation strategy, one has to choose which characteristics of a natural enemy are important to study. Subsequently, one has to select which environmental factors should be manipulated to improve the impact of the natural enemy on the pest population. In **Chapter 3**, I review which characteristics of a parasitoid should be studied in a conservation strategy (see Table 3.1) for stored products. At the same time, I review the work that has already been done in this direction for to the egg parasitoid *Uscana lariophaga.* From this review, I concluded that searching efficiency was the main characteristic that required further investigation. The searching efficiency of a parasitoid involves several characteristics. Parasitoid foraging behaviour can be regarded as a hierarchical series of subsequent steps of decreasing scale. In each step, the parasitoid 'zooms in' her searching behaviour and concentrates on a smaller, specific part of the habitat (see Chapter 3 and Figure 3.1):

+ host habitat location, i.e. searching for habitats that are suitable for potential hosts; this behaviour includes the response to odours from the host and/or the host's food plant;
+ dispersal and orientation of the parasitoid within the habitat of the host;
+ the location of host communities or clusters within the habitat; often guided by host odours.

Once the parasitoid arrives within a host cluster, important characteristics are:

- host location, including walking behaviour;
- the response to different host densities (i.e. functional response), and
- the response to different distributions of the host.

The presentation of research on searching efficiency of *U. lariophaga* in this thesis follows this hierarchical approach, and the steps are discussed in the subsequent chapters of this thesis.

In **Chapter 4**, it was demonstrated that *U. lariophaga* responds to the odour of dry, uninfested cowpea seeds. This response could be used in host habitat location, allowing *U. lariophaga* females to locate cowpea fields and stores. The odour from freshly laid *C. maculatus* eggs also attracted *U. lariophaga* females. More wasps arrived at the odour source and did so more quickly in the presence of *C. maculatus* eggs than in the presence of uninfested cowpea seeds. Egg odour can thus be used by *U. lariophaga* females in the location of host communities and clusters within a habitat, and in the location of hosts within those communities. An oviposition experience did not change the response of *U. lariophaga* to odours, as may be expected from small, short-lived egg parasitoids.

Once the parasitoid has arrived in the host habitat, i.e. the cowpea granary filled with seeds or pods, it must orient itself and disperse within this habitat in order to locate host communities. Would *U. lariophaga* be able to locate from a distance discrete clusters of hosts occurring within the habitat? Knowing that *U. lariophaga* is attracted by *C. maculatus* egg odour, it is likely that this odour also plays a role in the location of host clusters within the habitat. Results of spatial orientation in the habitat and host cluster location are presented in **Chapter 5**. *Uscana lariophaga*, if released within a mass of cowpea seeds, predominantly moves upwards, i.e. demonstrates a negative geotaxis. This phenomenon has been demonstrated in several other parasitoids of stored product pests, although the reason for this behaviour is not yet understood. However, it also seems that, after having encountered host eggs, this negative geotaxis in *U. lariophaga* becomes less important and host clusters below the release point are also visited and parasitized.

Clusters with more *C. maculatus* eggs are more frequently parasitized than clusters with few host eggs. This is irrespective of the volume over which eggs in a cluster are dispersed, and irrespective of the host density (number of eggs per seeds) within a cluster. It is hypothesized that egg odour concentration and/or the volume of the odour gradient around a host cluster, related to the number of eggs in that cluster, determine the chance that *U. lariophaga* finds a host cluster. In olfaction experiments, some indications were found to support this hypothesis. However, persistent contaminations of the experimental setup with adsorbed host odours prevented a further test of the hypothesis.

After host clusters have been located by the parasitoid, the behaviour within clusters becomes important. In **Chapter 6**, the number of host eggs parasitized by individual *U. lariophaga* females in clusters with different numbers of eggs (i.e. the functional response) is described. However, there is much confusion and contradiction over the methodology to test this aspect and to analyse the results. The experimental setup and the method of analysis both strongly influence the outcome and conclusions of functional response experiments.

In **Chapter 6**, I describe the methodology of indirect observations which we developed for the specific situation of a stored product pest in a dark three-dimensional environment. These indirect observations were supplemented with direct measurements of parameters that could explain the outcome of the indirect experiments, e.g. the parasitoid's egg laying capacity, initial egg load, and handling time of hosts. The response of wasps was analysed in visited clusters only. The process before this step, i.e. the actual location of the clusters, was already discussed in Chapter 5.

Uscana lariophaga demonstrates a functional response which can be described as a Holling Type II response. Parasitoids are strongly arrested within a host cluster, resulting in rates of parasitism close to 100% in clusters with few eggs. In larger host clusters, a plateau level is attained in the maximum number of hosts that can be parasitized in the given amount of foraging time, although considerable variation in this maximum occurs. Traditionally, this plateau level of a Type II functional response is thought to result from the time required to handle each host during parasitism. At higher densities, the accumulated handling time leaves no time to locate additional hosts in the total amount of foraging time that is available. However, direct observations proved that handling times in *U. lariophaga* are so short that they cannot limit the number of parasitized hosts at the level observed. By testing the parasitoid's egg laying capacity and counting the load of mature eggs in the ovaria of dissected females we demonstrated that the initial egg load and the egg maturation rate limit the number of hosts *U. lariophaga* can parasitize in a given amount of time.

The functional response is influenced by many factors, which makes it difficult to compare results from different experimental setups. However, one can study the effect of one variable in an otherwise tightly controlled experimental design. One such variable is the distribution of hosts within a host cluster. The bruchid pest *C. maculatus* occurs in a number of geographical strains that demonstrate marked differences in biology and behaviour, one of which is the egg dispersion pattern. Patterns of egg distribution may affect the chance that *U. lariophaga* locates eggs.

Therefore, we tested the influence of different egg distribution patterns on the functional response of *U. lariophaga*. Results of these experiments are given in **Chapter 7**. The number of eggs and seeds in a host cluster were manipulated in such a way, that three different egg distribution patterns were obtained: an even egg distribution (one egg per seed), a clumped or aggregated egg distribution (with increasing numbers of eggs per seed at higher densities), and a distribution with one egg per seed, but in which those seeds with eggs were mixed with various numbers of clean seeds, creating a distribution which, at lower densities, resembles a random distribution.

Again, as in Chapter 6, a strong arrestment was found in low density host clusters. At densities below 20 eggs per cluster, rates of parasitism in the even and clumped distributions were 89% and 75% respectively. In the random egg distribution, *U. lariophaga* females found significantly fewer eggs. Arrestment may occur if area restricted search occurs after oviposition. This is a change in the walking behaviour of a parasitoid after contacting a host. The walking speed declines and the number of turns and the turn angles increase. This results in a more intensive search on a small area close to the host. Such behaviour is adaptive when hosts occur in clumped distributions, as is the case with *C. maculatus* eggs in the field and in experimental storage conditions. It would also explain lower rates of parasitism as observed in the random distribution. Observations of individual tracks confirm the hypothesis that *U. lariophaga* does show area restricted search after oviposition, explaining the results from the functional response experiments under different host distributions.

Cowpea is traditionally stored either in the pods or as threshed seeds. Storage in intact pods provides substantial protection against *C. maculatus* attack, since the pod wall acts as a chemical and physical barrier against the first instar larva drilling towards the seeds. As soon as pods become damaged, exposing the seeds to *C. maculatus*, the bruchid females will predominantly oviposit on the seeds. Cowpea stored in pods is a habitat substantially different from cowpea stored as seeds, in terms of intergranular space and number of contact points between seeds or pods. One can imagine that the parasitoids' searching behaviour is different in both habitats, and as a result, biological control may differ. In **Chapter 8**, the effect of different habitats, cowpea seeds or pods, on walking behaviour and the functional response of *U. lariophaga* is investigated.

In columns filled with pods, more wasps arrived at the top if light from above was present compared to columns in the dark. Thus, *U. lariophaga* demonstrated positive phototaxis. In darkness, approximately the same number of wasps emerged from columns filled with seeds as from columns with pods. Habitat type did not significantly influence dispersal rates. Median net displacement rates through columns were in the range of 0.5 - 0.8 m per day, which seems sufficient to cover the internal volume of traditional cowpea stores in *U. lariophaga*'s life time. When comparing the functional response to egg clusters in a seed and pod environment with relatively few (< 40) host eggs, the seed habitat had significantly higher rates of parasitism (44%) than the pod habitat (32%). Thus, dispersal did not differ in a pod and a seed habitat but the rate of parasitism within egg clusters did. This may result from area restricted search by *U. lariophaga* females after oviposition. In the dispersal studies, no hosts were present in the columns and parasitoids did not demonstrate area restricted search. In the functional response experiments, area restricted search did occur. The smaller cluster volume and more contact points in a seed habitat resulted in more eggs being parasitized in seeds than in pods. In the pod habitat, wasps left an egg cluster earlier, with part of the host eggs still being unexploited.

In the general discussion, **Chapter 9**, the methodology of indirect experiments on foraging behaviour supplemented with direct observations is discussed. Potential conservation strategies, that will improve several aspects of the searching efficiency of *U. lariophaga*, could be the supply of honey to parasitoids in granaries, and the manipulation of the temperature in storage during cold periods. In preliminary field tests, the egg parasitoid seems capable of significantly suppressing bruchid populations in the first months of storage.

Further progress in the biological control of bruchid pests in stored cowpea in West Africa will come from a combination of simulation modelling and testing parasitoids in experimental granaries. Simulation models may help to study the population dynamics of host and parasitoid, and to identify the most promising environmental manipulations that would act as a conservation strategy of biological control of *C. maculatus*. Validation of such models should be carrier out under farmers' storage conditions. The combination of models and field tests could demonstrate whether manipulation of the storage environment to enhance the impact of *U. lariophaga* is a sound conservation strategy. This will proof the capabilities and limitations of the parasitoid acting as a biological control agent of *C. maculatus* in the cowpea storage environment.

Samenvatting

Het zoekgedrag van de eiparasiet *Uscana lariophaga:* bijdrage aan de biologische bestrijding van zaadkeverplagen in opgeslagen cowpea-bonen in West-Afrika

Cowpea (in het Nederlands ook wel 'ogenboon' genoemd) is een bonengewas dat veel wordt verbouwd in de traditionele, kleinschalige landbouw in de Sahelzone van West-Afrika. Het is een belangrijke voedsel- en eiwitbron voor boerenfamilies die leven van de zelfvoorzienende landbouw. De bonen worden in de droge tijd langdurig bewaard in opslagplaatsen. Tijdens die opslag kunnen zaadkevers uit de familie der *Bruchidae* grote schade aanrichten. Zij leggen hun eieren op de bonen, waarna de larven zich een weg naar binnen eten en de bonen uithollen.

In de zelfvoorzienende landbouw hebben boer(inn)en weinig financiële en technische middelen om deze zaadkevers te bestrijden. Eén van de strategieën die wel binnen bereik liggen, is het vergroten van de rol van inheemse sluipwespen in de opslagplaatsen, om zo de sterfte onder de zaadkevers te verhogen. Het versterken van de rol van reeds in de natuur voorkomende sluipwespen is een vorm van biologische bestrijding die in vaktaal "conservation" wordt genoemd. Deze vorm van bestrijding zou de schade in opslag op een duurzame wijze kunnen beperken.

In dit proefschrift wordt de mogelijkheid van biologische bestrijding van zaadkeverplagen in opgeslagen cowpea-bonen met behulp van de sluipwesp *Uscana lariophaga* nader onderzocht. Het onderzoek spitst zich daarbij toe op het gedrag van de sluipwesp bij het zoeken naar haar gastheren.

Hoofdstuk 1 begint met een inleiding over de productie van cowpea in West-Afrika, en de schade die in de cowpea-bonen aangericht kan worden door insecten in het veld en in de opslagplaatsen. Wanneer bij de oogst slechts 1% à 2% van de bonen eieren of larven bevatten van de zaadkever *Callosobruchus maculatus*, dan kan na zes maanden opslag de gehele oogst door de nakomelingen van deze kevers verloren gaan. Na een overzicht van de verschillende methoden voor de bestrijding van deze insectenplaag, volgt een antwoord op de vraag waarom biologische bestrijding een goede keus zou zijn. Ook worden de van nature reeds aanwezige sluipwespsoorten besproken die daarvoor in aanmerking zouden komen. Eén van die soorten is de sluipwesp *Uscana lariophaga*, een parasiet die haar eitjes legt in de eieren van de zaadkever *C. maculatus*. Daardoor ontwikkelt zich uit het keverei geen nieuwe keverlarve, maar komt er een nieuwe sluipwesp uit het eitje. In dit proces wordt de zaadkever de gastheer van deze parasiet genoemd.

De omslag van het proefschrift toont op de achtergrond een monster van cowpea-bonen (met eieren en boorgangen van zaadkevers) uit een opslaghutje in het dorp Gonou in Niger (februari 1993). De kleine foto op de voorzijde van de omslag laat een vrouwtje van de sluipwesp *Uscana lariophaga* zien (lengte 0,4 mm), terwijl zij een ei van een zaadkever parasiteert. De foto's op de achterzijde tonen een traditioneel opslaghutje voor cowpea in Niger (boven), een vrouwtje van de zaadkever *Callosobruchus maculatus* op cowpea-bonen (midden) en een proefveld met cowpea-bonen in Niamey, Niger (onderaan).

Door cowpea-monsters te nemen uit opslagplaatsen en van markten in Niger, en door die te onderzoeken op de aanwezigheid van zaadkevers en sluipwespen (**hoofdstuk 2**) kon worden aangetoond dat sluipwespen met grote regelmaat in opgeslagen cowpea voorkomen. Maar hun aantallen in opslag zijn meestal zo klein dat zij de schade door zaadkevers niet voldoende kunnen beperken. Hoopgevend zijn enkele incidentele waarnemingen waarbij sluipwespen 60% tot 80% van de kevereieren en -larven in een opslaghutje hadden geparasiteerd. Deze voorbeelden laten zien dat een natuurlijke biologische bestrijding met behulp van inheemse sluipwespen, onder bepaalde omstandigheden, mogelijk is.

Wanneer het lukt om de factoren vast te stellen die de ontwikkeling van sluipwespen en de parasitering van de gastheer bevorderen en wanneer die factoren ook in andere opslagplaatsen in de goede richting kunnen worden beïnvloed, dan moet het mogelijk zijn de schade door zaadkevers aanzienlijk te verminderen en zo de bestrijding succesvol maken.

In **hoofdstuk 3** wordt een overzicht gegeven van de noodzakelijke eigenschappen van *Uscana lariophaga* voor een effectieve biologische bestrijding van zaadkevers in opslagplaatsen. Daarnaast wordt een samenvattend overzicht geschetst van het onderzoek dat tot dusver aan *Uscana lariophaga* is gedaan. Daaruit volgt de conclusie dat met name het zoekgedrag van deze sluipwespsoort nader onderzoek verdient.

Het zoekgedrag van sluipwespen kan overzichtelijk worden ingedeeld in een reeks opeenvolgende stappen op een steeds kleinere schaal. Die hiërarchie van stappen vormt de leidraad voor de structuur van dit proefschrift (zie Paragraaf 3.2 en Figuur 3.1). Deze verschillende stappen komen achtereenvolgens in de hoofdstukken 4 tot en met 8 van dit proefschrift aan bod.

+ Het opsporen door de sluipwespen van een geschikte leefomgeving ("habitat") waar gastheren zouden kunnen voorkomen. Vaak maken sluipwespen daarbij gebruik van geuren die door hun gastheer zelf of door de voedselplant van de gastheer worden afgegeven (hoofdstuk 4).
+ In de habitat wordt vervolgens gezocht naar groepen gastheren ("clusters"), waarbij opnieuw geuren van de gastheer of van zijn voedselplant een rol spelen. De vraag rijst

hoe de sluipwesp zich verplaatst binnen de habitat en hoe groot de kans is dat clusters worden opgespoord (hoofdstuk 5).

- \+ Binnen een cluster wordt weer gezocht naar de aanwezige gastheren. De reactie van een sluipwesp-vrouwtje op de dichtheid van de gastheren is een belangrijke onderzoeksvraag. Dit laatste aspect wordt "functional response" genoemd (hoofdstuk 6).
- \+ De ruimtelijke verdeling van gastheren binnen een cluster kan van invloed zijn op het succes waarmee gastheren worden gevonden en geparasiteerd. Het loopgedrag van de parasiet kan daarbij een belangrijke rol spelen (hoofdstuk 7).
- \+ De ruimtelijke structuur van de habitat waarbinnen de sluipwesp zoekt, kan eveneens het succes van een sluipwesp beïnvloeden. De parasiet moet zich door de opgeslagen massa bonen kunnen bewegen en in staat zijn het inwendige volume van opslagplaatsen te doorzoeken (hoofdstuk 8).

In **hoofdstuk 4** wordt beschreven hoe *Uscana lariophaga* door de geur van droge, onbeschadigde cowpea-bonen wordt aangetrokken. Hierdoor is ze in staat om de habitats waar haar gastheer *Callosobruchus maculatus* mogelijkerwijs voorkomt (cowpea-velden en -opslagplaatsen) op te sporen. De sluipwesp reageert nog veel sterker op de geur van versgelegde *C. maculatus*-eieren. Die geur vormt een betrouwbaar signaal dat de geschikte gastheer aanwezig is. Hierdoor is *Uscana lariophaga* ook in staat om binnen een geschikte habitat (clusters van) gastheereieren op te sporen. Wespen die reeds ervaring opdeden met die geur, door het leggen van een ei in het gastheerei, reageerden niet beter op de eigeur.

Voor een goede bestrijding van plagen binnen opslagplaatsen moet een sluipwesp in staat zijn om zich te verplaatsen naar alle mogelijke plekken waar gastheren voorkomen. Daarom is in **hoofdstuk 5** gekeken naar de ruimtelijke oriëntatie en de verplaatsing van *Uscana lariophaga* in experimentele opslagcontainers vol cowpea-bonen. Wespen die in een voorraad cowpea worden losgelaten, hebben een duidelijke voorkeur om zich omhoog te bewegen (negatieve geotaxis). De aanwezigheid van gastheereieren doet deze voorkeur verdwijnen en wespen kunnen dan ook dieper gelegen gastheereieren opsporen.

Clusters met veel gastheereieren worden vaker gevonden dan clusters met weinig eieren. Het volume waarover die eieren verspreid zijn en de dichtheid van eieren per boon lijken daarbij geen rol te spelen. Er zijn aanwijzingen dat de kans op het vinden van een gastheercluster door de sluipwesp bepaald wordt door de concentratie van de eigeur, die weer afhankelijk is van het aantal aanwezige gastheereieren.

Hoofdstuk 6 speelt zich af op het niveau waar de sluipwesp reeds in een cluster met gastheereieren is gearriveerd. Hoe individuele vrouwtjes van de sluipwesp reageren wanneer er verschillende aantallen gastheren in een cluster aanwezig zijn, wordt de "functional response" van een sluipwesp genoemd. Als *Uscana lariophaga* 24 uur kan zoeken, dan worden bijna alle eieren in clusters van minder dan veertig eieren gevonden en geparasiteerd.

Bij grotere clusters bereikt het aantal geparasiteerde eitjes een plafond dat stabiel blijft. Dit noemt men een Holling Type II functionele respons. Is de zoektijd slechts vier uur, dan is het patroon hetzelfde maar wordt het plafond in het aantal geparasiteerde eieren lager, rond de twintig eieren. De tijd die *Uscana lariophaga* nodig heeft voor het opsporen en parasiteren van gastheereieren, is geen beperkende factor voor het totaal aantal eieren dat een vrouwtje kan parasiteren. Het aantal rijpe eieren dat een vrouwtje in haar ovaria draagt en de hoeveelheid die erbij kan rijpen, bepalen hoeveel gastheereieren *Uscana lariophaga* in een bepaalde tijd kan parasiteren. Directe metingen maken deze interpretatie van de indirecte experimenten mogelijk. In clusters met veel eieren blijft een *Uscana lariophaga* vrouwtje doorzoeken, totdat ze (bijna) alle eieren heeft geparasiteerd of haar eivoorraad op is. Deze eigenschap, "arrestment" genoemd, is bij *Uscana lariophaga* sterk ontwikkeld.

De kever *Callosobruchus maculatus* komt in Afrika voor in een aantal geografische rassen ("strains") die verschillende biologische eigenschappen hebben. Zo zijn er keverrassen die hun eieren heel gelijkmatig verdelen over de aanwezige cowpea-bonen, terwijl andere rassen hun eieren meer op goed geluk of juist in groepjes op bonen leggen. Daarom is in **hoofdstuk 7** gekeken of die verdeling van eieren binnen clusters invloed heeft op de functionele respons van *Uscana lariophaga*. De sluipwesp parasiteert minder gastheereieren wanneer er in een cluster maar weinig eieren aanwezig zijn en wanneer die eieren willekeurig over de bonen zijn verdeeld. Wanneer eieren gelijkmatig of samengeklonterd op bonen gelegd zijn, dan kan *Uscana lariophaga* meer eieren vinden. Dit is een gevolg van haar zoekgedrag, dat verandert na een eilegervaring. Kort nadat *Uscana lariophaga* een ei heeft gelegd, loopt ze met veel meer bochten en bezoekt ze, mede daardoor, veel minder bonen in de omgeving dan enige minuten later. Dit gedrag zorgt ervoor dat de sluipwesp meer gastheereieren vindt als die in groepjes voorkomen. Wanneer eieren willekeurig en wijd verspreid voorkomen, dan zoekt de wesp lang op plaatsen waar geen eieren aanwezig zijn. Met dit gedrag lijkt *Uscana lariophaga* zich te hebben aangepast aan de verdeling van kevereieren zoals die in het veld wordt gevonden.

In West-Afrika wordt cowpea soms bewaard in de peulen, en soms als zaden die uit de peul zijn verwijderd. In opslag hebben deze twee vormen van bewaren een heel verschillende ruimtelijke structuur. In opslag raken de peulen van cowpea elkaar maar op een paar plaatsen en zijn er veel luchtholtes tussen de peulen. Zaden liggen veel compacter op elkaar, met veel contactpunten tussen de zaden. Deze ruimtelijke structuur is mogelijk van invloed op het zoekgedrag van *Uscana lariophaga*. In **hoofdstuk 8** is daarom bekeken hoe de sluipwesp zich verplaatst door een massa van zaden of peulen en hoe de functionele respons van *Uscana lariophaga* is in die twee omstandigheden. De structuur van de opslag, als peulen of zaden, heeft geen effect op de snelheid waarmee *Uscana lariophaga* zich door de opslag verplaatst.

Wanneer er licht van bovenaf aanwezig is, dan verplaatsen wespen zich aantoonbaar sneller door een kolom met peulen dan wanneer er geen licht aanwezig is (positieve phototaxis genoemd). De wespen blijken in staat om zich in een opslagplaats over enkele meters per dag te verplaatsen. Daarmee zijn ze in principe in staat om overal binnen de traditionele opslaghutjes gastheerclusters op te sporen.

De functionele respons in peulen is anders dan in zaden. Bij lage aantallen gastheereieren worden in clusters van zaden meer eieren geparasiteerd dan in clusters van peulen. Dit kan alweer het gevolg zijn van het loopgedrag van een wesp na een eerste eileg. In zaden, met veel onderlinge contactpunten, is het gemakkelijker om over te stappen en eieren op andere zaden te vinden. Peulen hebben veel minder contactpunten. Wespen die lang rondom de plaats van een eerdere eilegervaring blijven zoeken, zullen minder gemakkelijk de eieren op een andere peul bereiken.

In de algemene discussie (**hoofdstuk 9**) wordt de methodische aanpak van het onderzoek besproken. Op basis van de resultaten van dit onderzoek, worden daarna de mogelijkheden besproken voor biologische bestrijding van de zaadkevers met behulp van *Uscana lariophaga*. De volgende praktische vragen worden beantwoord.

+ Welke factoren kan men beïnvloeden om de sterfte van *Callosobruchus maculatus*-eieren door *Uscana lariophaga* te vergroten?
+ Is *Uscana lariophaga* in staat om de door zaadkevers veroorzaakte schade in opgeslagen cowpea belangrijk te verminderen?
+ Hoe verhoudt de sterfte onder zaadkevers, veroorzaakt door *Uscana lariophaga*, zich tot de sterfte die andere parasieten veroorzaken?
+ Kunnen eiparasieten en larvale parasieten in opgeslagen cowpea worden gecombineerd en leveren zulke combinaties een betere bestrijding van de zaadkevers op?

Tenslotte wordt geschetst hoe vervolgonderzoek aan *Uscana lariophaga* zou moeten bestaan uit een combinatie van verschillende benaderingen. Enerzijds zouden in West-Afrika proeven moeten worden gedaan waarbij *Uscana lariophaga* wordt losgelaten in experimentele opslagplaatsen van cowpea. Anderzijds zouden computermodellen kunnen helpen bij het kiezen van de factoren die in opslag gemanipuleerd moeten worden om de bestrijding te verbeteren.

Résumé

Le comportement de recherche de parasitoïde oophage *Uscana lariophaga:* une contribution à la lutte biologique contre les coléoptères Bruchidae déprédateurs des stocks de niébé en Afrique de l'Ouest

Le niébé (*Vigna unguiculata*) est une source de protéine importante parmi les familles de petits agriculteurs en Afrique de l'Ouest. Des pertes très importantes pendant le stockage du niébé à la saison sèche sont dues à l'action des ravageurs de graines coléoptères de la famille Bruchidae. La lutte biologique avec l'utilisation du parasitoïde oophage *Uscana lariophaga* serait une bonne option afin de réduire les pertes pendant le stockage. Dans cette étude on a développé l'utilisation de l'*Uscana lariophaga*, surtout le comportement de recherche de l'hôte par le parasitoïde.

Dans le **chapitre 1**, on trouve une vision générale sur la production de niébé en Afrique de l'Ouest et sur les pertes dues à l'action des insectes au niveau des champs et du stockage. Infestations initiales de l'ordre de 1% à 2% des graines infestées par le ravageur *Callosobruchus maculatus*, peuvent aboutir à la perte totale de la récolte après 6 mois de stockage. La lutte biologique de ces bruches avec une stratégie de conservation est une option compatible aux conditions de l'agriculture de subsistance en Afrique de l'Ouest. La stratégie de conservation a comme but la manipulation de l'environnement afin d'augmenter l'action des ennemis naturels dans les populations des ravageurs.

En Afrique de l'Ouest plusieurs parasitoïdes des bruches du niébé sont présents, et parmi eux le parasitoïde des oeufs *U. lariophaga*. Un échantillonnage a démontré que ces parasitoïdes sont présents généralement dans les stocks de niébé à Niger (**chapitre 2**), mais souvent à des niveaux très bas. Pourtant, observations occasionnelles montrent un taux de parasitisme d'oeufs et de larves très élevé (60%-80%), ce qui met en évidence la capacité de contrôle des ravageurs par les parasitoïdes. Avec l'identification des facteurs qui réalisent cette capacité de contrôle des parasitoïdes et la possibilité de manipuler ces facteurs, on pourrait être capable de bien contrôler les dégâts dus à ces ravageurs.

Dans la stratégie de conservation il faut choisir parmi les nombreuses caractéristiques de l'ennemi naturel, quelles sont importantes à étudier. Après, il faut sélectionner quels facteurs environnementaux peuvent être manipuler afin d'augmenter l'action des ennemis naturels dans la population ravageuse. Dans le **chapitre 3**, je révise quelles caractéristiques du parasitoïde, en relation avec la stratégie de conservation, peuvent être étudiées (Tableau 3.1).

Je révise aussi ce qui a été déjà fait sur l'oophage *U. lariophaga* dans le cadre de ma recherche. A partir de cela, j'ai pu conclure que l'efficacité de recherche était l'aspect qui avait plus besoin d'être étudié. L'efficacité de recherche implique plusieurs aspects. Le comportement de recherche d'un parasitoïde peut être décrit comme une série hiérarchique de phases consécutives en niveaux décroissantes d'échelle (Figure 3.1):

- localisation de l'habitat de l'hôte, en réponse aux odeurs de l'hôte et/ou de la plante de l'hôte;
- localisation des communautés ou rassemblements d'hôtes dans l'habitat.

Une fois que le parasitoïde arrive dans un rassemblement d'hôtes, les caractéristiques les plus importantes sont:

- localisation de l'hôte, avec le comportement de marche;
- la réponse aux densités différentes de l'hôte (i.e. la réponse fonctionnelle); et
- la réponse aux distributions différentes de l'hôte.

Dans cette thèse, la présentation des études sur l'efficacité de recherche d'*Uscana lariophaga* suit cet aspect hiérarchique et les différentes phases sont examinées dans les chapitres consécutives.

Dans le **chapitre 4**, il a été démontré que *U. lariophaga* répond à l'odeur des graines saines et sèches de niébé. Cette réponse peut être utilisée pour la localisation de l'habitat de l'hôte, ce qui permet aux femelles d'*Uscana lariophaga* de localiser les champs de niébé et aussi les stocks. L'odeur des oeufs récemment pondus par *C. maculatus* est aussi attractive aux femelles oophages. Un plus grand nombre d'individus arrivent à la source de l'odeur et plus vite, quand les graines de niébé portent des oeufs de *C. maculatus*. Donc, l'odeur des oeufs doit être perçue par les femelles d'*Uscana lariophaga* pour localiser les communautés d'hôtes et ces rassemblements dans l'habitat, et finalement pour localiser l'hôte à l'intérieur de cettes communautés. L'expérience d'oviposition n'a pas modifié la réponse du parasitoïde aux odeurs, ce qui s'accorde avec les modèles de petits parasitoïdes de vie très courte.

Une fois que le parasitoïde est arrivé dans l'habitat de l'hôte, i.e. le grenier de niébé rempli des graines ou des gousses, il doit s'orienter et se disperser dans l'habitat afin de trouver les communautés d'hôtes. Est-ce-que *U. lariophaga* est-elle capable de localiser à distance les petits rassemblements d'hôtes qui sont à l'habitat? Comme *U. lariophaga* est attirée par l'odeur des oeufs du *C. maculatus*, alors on peut soupçonner que cette odeur joue un rôle dans la localisation des rassemblements d'hôtes dans l'habitat. Les résultats sur l'orientation spatiale dans l'habitat de l'hôte et la localisation des rassemblements d'hôtes sont présentés dans le **chapitre 5**.

Quand *U. lariophaga* est lâchée dans les graines de niébé, elle s'oriente surtout vers la partie supérieure, ce qui montre un géotropisme négatif. Ce phénomène a été démontré chez plusieurs parasitoïdes des ravageurs de produits stockés, mais la raison de ce comportement n'a pas encore été élucidée. Cependant, il semble, qu'après le parasitoïde avoir rencontré des oeufs de l'hôte, le géotropisme négatif devient moins important et les rassemblements d'hôtes situés en bas du point de lâchage sont aussi visités par les parasitoïdes. Les rassemblements avec plus des oeufs de *C. maculatus* sont plus parasités que les rassemblements avec peu d'oeufs (**chapitre 5**). Ceci n'est pas lié ni avec le volume de la dispersion des oeufs, ni avec la densité d'hôtes dans le rassemblement (nombre des oeufs par graine). L'hypothèse c'est que la concentration de l'odeur des oeufs et/ou le volume de la sphère d'odeur autour du rassemblement, rapportés au nombre d'oeufs dans ce rassemblement, détermine la probabilité avec laquelle *U. lariophaga* trouve l'hôte. Cette hypothèse peut être supportée par quelques indices mises en évidence par expériences d'olfaction. Néanmoins, les contaminations du protocole expérimental avec l'adsorption des odeurs de l'hôte ne permettent pas de contrôler davantage cette hypothèse.

À la suite de la localisation d'un rassemblement d'hôtes, le comportement à l'intérieure du rassemblement devient important. Dans le **chapitre 6**, le nombre d'oeufs parasités par une seule femelle d'*Uscana lariophaga* dans des rassemblements avec différentes quantités d'oeufs est mesuré (i.e. la réponse fonctionnelle). Cependant, il reste encore beaucoup de doutes sur cette méthodologie pour examiner cet aspect et analyser les résultats. Le protocole expérimental et la méthode d'analyse jouent un rôle prépondérant sur les conséquences et conclusions des expériences sur la réponse fonctionnelle.

Dans le **chapitre 6**, on décrit la méthodologie d'observations indirectes, qui a été développée pour cette situation particulière des ravageurs des produits stockés dans un environnement sombre et tridimensionnel. En plus de cettes observations indirectes, quelques mesures directes ont été effectuées sur des paramètres qui pourraient expliquer les conséquences de l'expérimentation indirecte, telles quelles la capacité de ponte, la charge ovarienne et le temps de manipulation de l'hôte. La réponse des individus a été analysée seulement sur les rassemblements visités. La phase antérieure, i.e. la localisation des rassemblements, a déjà été présentée dans le chapitre 5.

Uscana lariophaga a une réponse fonctionnelle qui peut être décrite comme étant une réponse "Holling Type II". Les parasitoïdes restent dans les rassemblements d'hôtes, ce qui cause un taux de parasitisme proche de 100% quand le nombre d'oeufs est peu élevé. Dans les rassemblements plus nombreux, un plateau maximal est atteint correlé au maximum d'oeufs qui peuvent être parasité par l'oophage dans le temps de recherche donné. Il y a une variabilité considérable de ce taux maximal. En général, ce plateau, dans le cas d'une réponse fonctionnelle du type II, doit résulter du temps nécessaire pour manipuler l'hôte pendant le parasitisme. Dans le cas de densités élevées, le temps cumulé de manipulation ne laisse pas de temps disponible pour localiser davantage des hôtes. Cependant, les observations directes montrent que le temps de manipulation de l'hôte chez *U. lariophaga* est tellement court qu'il ne devrait pas limiter le nombre d'oeufs parasités au niveau observé. Chez *U. lariophaga* c'est la charge ovarienne initiale et le taux de maturation des oeufs qui limitent le nombre d'oeufs qu'elle peut parasiter dans un certain délai. Ceci a été démontré par des testes sur la capacité de ponte et la quantité d'oeufs mûrs dans les ovaires.

La réponse fonctionnelle est influencée par plusieurs facteurs, lesquels rendent difficile la comparaison des résultats de différents protocoles expérimentaux. Néanmoins, on peut étudier l'effet d'une seule variable avec un protocole expérimental autrement contrôlé. Une de ces variables c'est la distribution de l'hôte dans un rassemblement. Les ravageurs *C. maculatus* sont présents en Afrique dans un nombre de lignées géographiques qui ont de différences aux niveaux biologique et comportemental, parmi elles la dispersion des oeufs. La façon de distribuer les oeufs peut influencer la probabilité d'*Uscana lariophaga* de trouver les oeufs.

Donc, nous avons examiné l'influence de différentes façons de distribution des oeufs sur la réponse fonctionnelle chez *U. lariophaga*. Les résultats sont présentés dans le **chapitre 7**. Le nombre d'oeufs et de graines dans un rassemblement d'hôte ont été manipulés de telle façon que trois types de distribution d'oeufs ont été obtenus: une distribution égale avec un seul oeuf par graine, une distribution agrégée avec plusieurs oeufs par graine avec l'augmentation du nombre d'oeufs à densités élevées, et une distribution qu'à densités basses ressemble une distribution plus ou moins au hasard, avec un oeuf par graine, mais avec ces graines infestées nous avons mélangé plusieurs graines saines.

Encore, comme dans le chapitre 6, on trouve une forte tendance de rester dans les rassemblements à densité de l'hôte basses. À densité au dessous de vingt oeufs par rassemblement, les taux de parasitisme dans la distribution égale et la distribution agrégée ont été 89% et 75% respectivement. Dans la distribution d'oeufs au hasard, les femelles d'*Uscana lariophaga* ont trouvé significativement moins d'oeufs. La tendance de rester peut apparaître s'il y a une restriction de la surface de recherche après la ponte. La vitesse de la marche chute et le nombre de courbes et les angles de courbes augmentent. Le résultat de cette comportement est une recherche intensive sur une petite surface limitée proche de l'hôte. Les observations de parcours individuels confirment l'hypothèse qu'*U. lariophaga* a un comportement de recherche sur une surface restrictive après la ponte. Ce comportement est adaptatif quand les hôtes ont une distribution agrégée d'oeufs, comme chez *C. maculatus* aux champs du niébé et en conditions expérimentales de stockage. Il peut aussi expliquer le taux bas de parasitisme observé dans la distribution au hasard.

Le niébé est traditionnellement stocké soit en gousses soit en graines. Le stockage en gousse donne une bonne protection contre l'action de *C. maculatus*, parce que la paroi de la gousse agit comme une barrière chimique et physique contre le premier stade larvaire qui perce la paroi et les graines. Une fois que les gousses deviennent endommagées, exposant les graines au *C. maculatus*, la femelle bruche pondra de préférence sur les graines. Le niébé stocké en gousse c'est un habitat très différent du niébé stocké en graines. On peut imaginer que le comportement de recherche de parasitoïde est aussi différent dans les deux habitats, et donc, la lutte biologique doit être aussi différente. Dans le **chapitre 8**, l'effet des habitats différents, graines de niébé ou gousses, sur le comportement de marche et la réponse fonctionnelle a été abordé.

En colonnes remplies avec des gousses, un nombre plus grand de parasitoïdes arrivent jusqu'au sommet, s'il y a une lumière en haut, en comparaison à une colonne dans l'obscurité. Donc, *U. lariophaga* a une phototaxie positive. Dans l'obscurité, à peu près le même nombre d'oophages émergent des colonnes remplies des graines ou gousses. Le type d'habitat n'a pas eu d'influence significative sur le taux de dispersion. Les mediennes nettes des taux de déplacements entre les colonnes ont été entre 0.5 - 0.8 mètre par jour, ce qui paraît suffisamment pour couvrir le volume intern de greniers de niébé traditionnels pendant la durée de vie d'*Uscana lariophaga*. Quand on compare la réponse fonctionnelle au rassemblement d'oeufs dans l'environnement des graines ou gousses avec relativement peu d'oeufs de l'hôte (< 40), l'habitat des graines a un taux de parasitisme significativement plus élevé (44%) que l'habitat des gousses (32%). Alors, la dispersion n'a pas différé entre les deux habitats - graines et gousses-, mais le taux du parasitisme est différent. Ça doit être lié à la recherche sur une surface restrictive des femelles d'*Uscana lariophaga* après la ponte. Dans les études sur la dispersion, les hôtes n'ont pas été présents dans les colonnes et les parasitoïdes n'ont pas présenté une recherche de surface restrictive. Dans les expériences de la réponse fonctionnelle, cette recherche restrictive a apparu. Le rassemblement de volume plus petit et avec plus de point de contact, dans l'habitat des graines, a résulté en plus d'oeufs parasités en graines qu'en gousses. Dans l'habitat des gousses, les parasitoïdes ont laissé le rassemblements plus tôt, avec une partie des oeufs encore à exploiter.

Dans la discussion générale, en **chapitre 9**, la méthodologie d'expériences indirectes du comportement de recherche alliée avec l'observation directe est discutée. La stratégie de conservation, qui améliorerait plusieurs aspects de l'efficacité de recherche chez *U. lariophaga*, pourrait être la nourriture avec du miel pour les parasitoïdes en greniers, et aussi la manipulation de la température pendant les périodes froides. En testes préliminaires sur le terrain, les parasitoïdes oophages semblent être capables de basser significativement les populations de bruches dans les premiers mois de stockage.

Résumé

Un progrès additionnel dans la lutte biologique des bruches ravageuses dans le niébé stocké en Afrique de l'Ouest viendra de la combinaison de la modélisation simulée et le teste des parasitoïdes en greniers expérimentaux. Les modèles de simulation peuvent aider à étudier la dynamique des populations de l'hôte et du parasitoïde, et à identifier les manipulations environnementales plus fructueuses qui pourraient agir comme une stratégie de conservation dans la lutte biologique de *C. maculatus*. La validité de ces modèles permettrait sa mise en place dans les conditions de stockage des agriculteurs. La combinaison des modèles et des testes sur le terrain pourrait montrer si la manipulation de l'environnement de stockage afin d'augmenter l'action d'*Uscana lariophaga* est une stratégie de conservation judicieuse. Ça montrera les capacités et les limitations de l'action de parasitoïde dans la lutte biologique de *C. maculatus* dans l'environnement de stockage du niébé.

List of publications

Some chapters in this Thesis are or will be published (in a slightly different form) as:

Chapter:	**Publication:**
2	Alebeek, F.A.N. van, 1996. Natural suppression of bruchid pests in stored cowpea (*Vigna unguiculata* (L) Walp) in West Africa. International Journal of Pest Management 42: 55-60.
4	Ormel, G.J.; Gort, G.; Alebeek, F.A.N. van, 1995. Analyzing host-location in *Uscana lariophaga* Steffan (Hymenoptera; Trichogrammatidae), an egg parasitoid of bruchids (Coleoptera; Bruchidae), using Cox's proportional hazards model. Bulletin of Entomological Research, 85: 113-123.
5	Alebeek, F.A.N. van; Huis, A. van, 1997. Host location in stored cowpea by the egg parasitoid *Uscana lariophaga* (Hym., Trichogrammatidae). Zeitschrift für angewandte Entomologie, *accepted.*
6	Alebeek, F.A.N. van; Koning, C.M.; Korte, E.A.P. de; Huis, A. van, 1996. Egg limited functional response of *Uscana lariophaga*, egg parasitoid of bruchid beetle pests in stored cowpea. Entomologia Experimentalis et Applicata, *in press.*
7	Alebeek, F.A.N. van; Bezemer, T.M.; Huis, A. van; Lenteren, J.C. van, 1996. The functional response of *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae) under different egg distributions of its host *Callosobruchus maculatus* L. (Col.: Bruchidae). Entomologia Experimentalis et Applicata, *in press.*
8	Alebeek, F.A.N. van; Antwi, Kwaku Kyei; Huis, A. van, Lenteren, J.C. van. Dispersal and functional response of *Uscana lariophaga* in two different habitats: stored cowpea pods and seeds. Submitted to Journal of Stored Products Research.

Additional publications related to the research described in this thesis:

Alebeek, F.A.N. van; Groot, N., 1997. The arrestment response in *Uscana lariophaga* Steffan (Hym., Trichogrammatidae). Proceedings of the section Experimental & Applied Entomology of the Netherlands Entomological Society (N.E.V.) 8: (accepted).

Alebeek, F.A.N. van, 1994. A survey of Bruchid pests and their parasitoids in stored cowpea in Niger. Proceedings of the section Experimental & Applied Entomology of the Netherlands Entomological Society (N.E.V.) 5: 145-150.

Alebeek, F.A.N. van, 1994. The functional response of *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae) under different egg distributions of its host *Callosobruchus maculatus* L. (Col.: Bruchidae). In: E. Highley, E.J. Wright, H.J. Banks & B.R. Champ (eds.), Stored Product Protection. Proceedings of the 6th International Working Conference on Stored-product Protection, CAB International, Wallingford, II: 1152-1157.

Alebeek, F.A.N. van, 1993. *Callosobruchus maculatus* egg distributions in cowpea stock, and the functional response of the egg parasitoid *Uscana lariophaga* in relation to these patterns. Abstract. In: Proceedings of the European Bruchid Conference, Egham, United Kingdom.

Alebeek, F.A.N. van; Rojas-Rousse, D.; Huignard, J., 1993. Competition between females of *Eupelmus vuilleti* (Cwf.) (Hym.; Eupelmidae) and *Dinarmus basalis* (Rond.) (Hym.; Pteromalidae), two solitary ectoparasitoids of immature stages of *Callosobruchus maculatus* (Fab.) (Col.; Bruchidae). Entomologia experimentalis et applicata 69: 21-31.

Leveque, L.; Monge, J.P.; Rojas-Rousse, D.; Alebeek, F. van; Huignard, J., 1993. Analysis of multiparasitism by *Eupelmus vuilleti* (Craw) (Eupelmidae) and *Dinarmus basalis* (Rond) (Pteromalidae) in the presence of one of their common hosts, *Bruchidius atrolineatus* (Pic) (Coleoptera Bruchidae). Oecologia 94: 272-277.

Alebeek, F.A.N. van, 1992. Natural enemies of bruchids for biological control: a review. Abstract. In: Proceedings of the XIX International Congress of Entomology, Beijing, China, pp. 585.

Alebeek, F.A.N. van, 1991. Interspecific host discrimination by two solitary ectoparasitoids of immature stages of Bruchidae. Mededelingen van de Faculteit der Landbouwwetenschappen van de Rijksuniviversiteit Gent, 56/3B: 1011-1020.

Other publications:

Sar, T. van der; Alebeek, F.A.N. van, 1995. Een tuin vol beestjes. Vlinders 12(2): 4-7 (in Dutch).

Alebeek, F.A.N. van; Sar, T. van der, 1993. Vlinderen vanuit je tuinstoel. Vlinders 8(3): 11-13 (in Dutch).

Sar, T. van der; Alebeek, F.A.N. van, 1993. Hoe een kleine nachtpauwoog zich dik eet. Vlinders 8(4): 4-6 (in Dutch).

Alebeek, F.A.N. van; Lenteren, J.C. van, 1992. Integrated Pest Management for Protected Vegetable Cultivation in the Near East. FAO Plant Production and Protection Paper no. 114, 150 pp.

Alebeek, F.A.N. van, 1992. Pest Management. A Directory of information sources. Book review. Bulletin of Entomological Research 82: 142-143.

Alebeek, F.A.N. van; Sar, T. van der, 1991. Zoekt u soms iets ? Over rupsen verzamelen en uitkweken. Vlinders 6(3): 26-29 (in Dutch).

Alebeek, F.A.N. van, 1989. A Catalogue of Integrated Pest Management training and extension materials, for IPM projects in (sub)tropical regions. Department of Entomology, Wageningen Agricultural University and Technical Centre for Agricultural and Rural Cooperation (CTA), Wageningen. 305 pp.

Pak, G.A.; Heiningen, T.G. van; Alebeek, F.A.N. van; Hassan, S.A.; Lenteren, J.C. van, 1989. Experimental inundative releases of different strains of the egg parasite *Trichogramma* in Brussels sprouts. Netherlands Journal of Plant Pathology 95: 129-142.

Pak, G.A.; Noldus, L.P.J.J.; Alebeek, F.A.N. van; Lenteren, J.C. van, 1988. The use of *Trichogramma* egg parasites in the inundative biological control of lepidopterous pests of cabbage in The Netherlands. Ecological Bulletins 39: 111-113.

Alebeek, F.A.N. van; Minkenberg, O.P.J.M., 1987. Voorlichting over biologische bestrijding van plagen. Bedrijfsontwikkeling 18 (9): 295 - 298 (in Dutch).

Minkenberg, O.P.J.M.; Alebeek, F.A.N. van, 1987. Mineervliegen natuurlijk, biologisch of chemisch bestrijden? Groenten en Fruit 22 Mei, 28 - 31 (in Dutch).

Alebeek, F.A.N. van; Pak, G.A.; Hassan, S.A.; Lenteren, J.C. van, 1986. Experimental releases of *Trichogramma spp.* against Lepidopteran pests in a cabbage field in the Netherlands in 1985. Mededelingen van de Faculteit der Landbouwwetenschappen van de Rijksuniviversiteit Gent 51 (3a): 1017 - 1028

Sabelis, M.W.; Alebeek, F. van; Bal, A.; Bilzen, J. van; Heiningen, T. van; Kaizer, P.; Kramer, G.; Snellen, H.; Veenenbos, R.; Vogelezang, J., 1983. Experimental validation of a simulation model of the interaction between *Phytoseiulus persimilis* and *Tetranychus urticae* on cucumber. Bulletin OILB/SROP 1983/VI/3: 207 - 229

Curriculum vitae

Franciscus Adrianus Nicolaas van Alebeek was born in Utrecht, The Netherlands, on February 1, 1959. At a young age, in his elders' garden, he became interested in butterflies and other insects, and this curiosity has never left him since.

In 1977, after completing his primary and secondary education, he started his study in Crop Protection at the Wageningen Agricultural University. His major for the MSc. was in Entomology, and minors in Extension Science and Animal Ecology. During his MSc., he focused on biological control of insect and mite pests in agriculture by using parasitoids and predators. In addition, he obtained his degree for teaching Biology in secondary education. His practical internship was spent at the Institute for Plant Protection, on supervised and integrated control of insect pests in field vegetables.

In 1983 and 1984 he assisted in the 'International Course on Plant Protection' of the International Agricultural Centre (IAC) in Wageningen. In 1985 and 1986 he worked as a research assistant in the Department of Entomology of the Wageningen Agricultural University, on experimental releases of *Trichogramma* egg parasitoids against lepidopterous pests in cabbage.

He obtained his MSc degree in 1986 and worked during the subsequent two years as a research entomologist in the Department of Entomology of the Wageningen Agricultural University, on an inventory of training and extension materials on Integrated Pest Management (IPM) in (sub)tropical countries, which resulted in the book "Integrated Pest Management. A Catalogue of IPM training and extension materials".

In 1988 and 1989, he worked as a guest research entomologist at the 'Institut de Biocénotique Expérimentale des Agrosystèmes' of the University of Tours, France. His work concerned the interspecific competition between two ectoparasitoids of bruchid larvae in stored cowpea in West Africa.

In 1989 he returned to the Department of Entomology in Wageningen, to prepare a FAO workshop on IPM for protected vegetable cultivation, resulting in the FAO Plant Production and Protection Paper no. 114: "Integrated Pest Management for Protected Vegetable Cultivation in the Near East".

From 1990 till 1996, he was research entomologist in the international project "Biological control of Bruchids in West Africa", funded by the European Union. During the first phase (1990-1993), he was employed by the Netherlands Foundation for Biological Research (BION). During the final phase (1994-1996) of his PhD. research, he was employed by the Wageningen Agricultural University. As part of this research, he visited Niger and Burkina Faso, and participated in several international congresses and workshops. In 1996 he assisted in the 'International Course on Rural Extension' of the IAC in Wageningen.

Alongside his work, he relished a happy life with his wife, children, and friends, and enjoyed the study of butterflies and other insects in his surroundings.

A playful mind is a joy forever.

For all children between 4 and 84, and all adults with a curious attitude, here is some fun. Place this book, face down, on your left hand. Then take all the pages between your right thumb and forefinger, bend them upwards, and let all the pages quickly slip between your fingers from right to left (from the front page until the last page). You will see a little story on biological control of bruchids with *Uscana lariophaga*: bruchid eggs are being laid, parasitized, turn black, and finally produce new wasps. You could consider this as a visual representation of a population dynamic process, or even as a simple graphic simulation model. With my sincere apologies to left-handed people.

Voor grote en kleine kinderen tussen 4 en 84 jaar en alle andere nieuwsgierigen: een grapje. Leg dit boek met de achterkant naar boven op je linkerhand. Neem vervolgens alle bladzijdes tussen je rechter duim en voorvinger, buig ze omhoog en laat de bladzijdes dan snel tussen je vingers doorglippen, van voor naar achteren. Je ziet op die manier de biologische bestrijding van zaadkevers met behulp van de sluipwesp *Uscana lariophaga* in volle actie: kevereieren worden gelegd, geparasiteerd, kleuren donker, en uiteindelijk komen daar weer nieuwe sluipwespen uit. Dit is een vereenvoudigde weergave van de "populatie-dynamica", de wisselwerking tussen kevers en sluipwespen in de opslag. Je kunt het ook zien als een grafische simulatie. Met welgemeende excuses aan linkshandige personen.