BIOCONTROL IN STORE

Spatial and behavioural aspects of foraging by *Uscana lariophaga*, egg parasitoid of *Callosobruchus maculatus*, in stored cowpea

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Biocontrol in store: spatial and behavioural aspects of foraging by *Uscana lariophaga*, egg parasitoid of *Callosobruchus maculatus*, in stored cowpea

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Stellingen

– 1 –

In tegenstelling tot veel andere trichogrammatidae vertoont *Uscana lariophaga* gericht gastheerzoekgedrag.

Ormel, G.J., Gort, G. & Van Alebeek, F.A.N. (1995). *Bull. Entomol. Res.* 85: 113-123;
Van Huis, A., Schütte, C., Cools, M.H., Fanget, Ph., Van der Hoek, H. & Piquet, S.P. (1994). *Stored Prod. Prot., Proc. 6th Int. Working Conf. Stored-prod. Prot.*, pp. 1158-1164;
dit proefschrift

– 2 –

Het feit dat *Uscana lariophaga* tijdens superparasitering de sexratio van haar nakomelingen aanpast in de richting van het geslacht met de hoogste overlevingskans geeft aan dat deze superparasiteringen niet veroorzaakt worden door gebrekkige gastheerdiscriminatie.

Dit proefschrift

– 3 –

Het motto "de vijanden van onze vijanden zijn onze vrienden" gaat voor de natuurlijke vijanden van plaaginsecten niet op.

– 4 –

De empirische onderbouwing van enkele van de meest invloedrijke ecologische modellen deugt niet.

Hall, C.A.S. (1988). An assessment of several of the historically most influential theoretical models used in ecology and of the data provided in their support. *Ecol. Modell.* 43: 5-31.

– 5 –

Biologische bestrijding speelt zich in essentie af op het niveau van individuen en is niet "in essentie een populatiefenomeen, het gevolg van (...) de interactie van een natuurlijke vijand-populatie met een gastheerpopulatie".

Contra: Mills, N.J. & W.M. Getz. (1996). Modelling the biological control of insect pests: a review of host-parasitoid models. *Ecol. Modell.* 92: 121-143.

– 6 –

Het Westen en het (nabije) Oosten hebben elkaar nog heel wat te leren.

– 7 –

Alleen stellingen die betwijfeld kunnen worden maken contact met de werkelijkheid.

Contra: Descartes

Stellingen behorend bij het proefschrift van Clemens Stolk,
"Biocontrol in store: spatial and behavioural aspects of foraging by *Uscana lariophaga*, egg parasitoid of *Callosobruchus maculatus*, in stored cowpea"
Wageningen, 6 december 2002

Abstract

Cowpea (*Vigna unguiculata* Walpers), an important crop for West African subsistence farmers, is often infested in storage by the bruchid beetle *Callosobruchus maculatus* Fabricius. The indigenous egg parasitoid *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae) is responsible for substantial mortality of *C. maculatus* eggs and might therefore be used in a conservation strategy of biological control. This thesis focuses on foraging behaviour of *U. lariophaga* females in a spatial context. In stored cowpea, the bruchid oviposits in clusters. *Uscana lariophaga* is well adapted to such clusters, since it shows a strong arrestment response after an encounter with an unparasitized host. Previous investigations had already shown attraction of the parasitoid to host-related odours; it is now shown that directed search probably occurs at a short distance (4-6 beans) from the host patch. The probability that a host patch in stored cowpea is found decreases rapidly with increasing distance between the host patch and the site of release of the parasitoid. The 'critical distance' within which the host patch is found by the parasitoid increases if more searching time is allowed. If an experienced parasitoid arrives in a host patch and encounters parasitized hosts, it is likely to superparasitize, but it will stop superparasitizing as soon as an unparasitized host has been encountered. Superparasitism by experienced females is not due to failure in host discrimination, as appears from the fact that females adapt the sex ratio of their offspring during superparasitism. If no or few hosts are available, the parasitoid lives shorter than when many hosts are available. This reduced longevity at low host densities may be due to an increased walking activity at low host densities. Finally, the potential of a simulation model for a better understanding of *U. lariophaga* foraging behaviour is shown, and consequences of behaviour for the prospects of biological control are discussed.

Prefatory note

In this thesis I report on studies of the behaviour of *Uscana lariophaga*, an egg parasitoid of the stored product pest *Callosobruchus maculatus*. A few years ago, I asked a number of scientists for advice on this research project during a brainstorm session. One of the participants in that session suggested that I could watch or follow *U. lariophaga* inside bean stocks by means of an endoscope, as used in medicine. Although this idea was never put into practice, it illustrates the difficulties of studying the behaviour of a tiny parasitoid inside a stock of beans. I hope that this thesis will nevertheless contribute to a better insight (in the metaphorical, not the endoscopical sense) in the behaviour of *U. lariophaga* in stored cowpea.

The title of this thesis starts with the words "Biocontrol in store". One of the meanings of "in store" is described by Webster's International Dictionary as "in accumulation, in readiness, in preparation", and the American Heritage Dictionary describes it as "forthcoming". It is my wish that this work will ultimately lead to a better fulfillment of the promise of biological control in stored product.

Clemens Stolk
Wageningen, August 2002

Please note that, in Chapters 3-6, the following representation of statistical p-values is used:

real p-value	**presentation in text**
$p \geq 0.01$	exact value is given
$0.001 \leq p < 0.01$	$p < 0.01$
$0.0001 \leq p < 0.001$	$p < 0.001$
$p < 0.0001$	$p << 0.001$

Contents

CHAPTER 1

General Introduction

...When the winter came the grasshopper had no food and found itself dying of hunger, while it saw the ants distributing every day corn and grain from the stores they had collected in the summer.
– from 'The Ant and the Grasshopper', attributed to Aesop (6th century BC)

Food storage

Although some animals, such as certain species of ants, squirrels, and woodpeckers, store food (Levinson & Levinson, 1998), storage of food in large quantities is mainly a human practice. The need to store food in substantial amounts arose when man started practicing agriculture (Munro, 1966; Stein, 1986). Periods between harvests had to be overcome by food storage. Large scale grain storage in sophisticated granaries was probably first practiced by the ancient Egyptians (Levinson & Levinson, 1994a,b). Today, the quantity of dry food products that is stored annually probably exceeds two billion metric tons globally. Of these, cereals such as rice, wheat and maize are the most important ones. In industrialized countries, cereals and pulses are mostly stored in large silos. Developing countries, on the other hand, typically have a large population of subsistence farmers who practice small-scale on-farm storage of cereals and pulses (Compton *et al.*, 1993; Gahukar, 1994). Subsistence farmers often use traditional storage structures, such as clay or straw granaries or underground pits (Giles, 1964; Hindmarsh *et al.*, 1978).

Storage pests

When humans store food for their own (and their cattle's) nutrition, they inadvertently also provide a luxurious environment for rodents, insects, mites, and fungi. These organisms infest and exploit the stored resource unless protective measures are taken. As for insect pests of storage, which are the topic of this Introduction, they or their ancestors originally probably occurred in field crops, semidried fruits, nests of gregarious insects, and nests of spiders, birds and small mammals (Levinson & Levinson, 1994a). Some of these insects are now mainly or almost exclusively associated with stored products. About 40 insect species from about 10 coleopteran families and two lepidopteran families are frequently encountered as

Table 1. Some common insect pests of stored products (Munro, 1966; Evans, 1987; Gahukar, 1994; Allotey, 1991).

Scientific name	English name	Order and Family
Acanthoscelides obtectus	dried bean beetle	Coleoptera: Bruchidae
Ahasverus advena	foreign grain beetle	Coleoptera: Silvanidae
Alphitobius diaperinus	black fungus beetle	Coleoptera: Tenebrionidae
Callosobruchus chinensis	azuki bean beetle	Coleoptera: Bruchidae
Callosobruchus maculatus	cowpea beetle	Coleoptera: Bruchidae
Corcyra cephalonica	rice moth	Lepidoptera: Pyralidae
Cryptolestes ferrugineus	rust red grain beetle	Coleoptera: Cucujida
Cryptolestes pusillus	flat grain beetle	Coleoptera: Cucujidae
Ephestia cautella	tropical warehouse moth	Lepidoptera: Pyralidae
Ephestia elutella	warehouse moth	Lepidoptera: Pyralidae
Ephestia kuehniella	Mediterranean flour moth	Lepidoptera: Pyralidae
Lasioderma serricorne	cigarette beetle	Coleoptera: Anobiidae
Latheticus oryzae	long-headed flour beetle	Coleoptera: Tenebrionidae
Oryzaephilus surinamensis	saw-toothed grain beetle	Coleoptera: Silvanidae
Plodia interpunctella	Indian meal moth	Lepidoptera: Pyralidae
Prostephanus truncatus	larger grain borer	Coleoptera: Bostrichidae
Ptinus tectus	Australian spider beetle	Coleoptera: Ptinidae
Rhizopertha dominica	lesser grain borer	Coleoptera: Bostrichidae
Sitophilus granarius	grain weevil	Coleoptera: Curculionidae
Sitophilus oryzae	rice weevil	Coleoptera: Curculionidae
Sitotroga cerealella	Angoumois grain moth	Lepidoptera: Gelechiidae
Tenebroides mauritanicus	Cadelle	Coleoptera: Trogossitidae
Tribolium castaneum	rust red flour beetle	Coleoptera: Tenebrionidae
Tribolium confusum	confused flour beetle	Coleoptera: Tenebrionidae
Trogoderma granarium	Khapra beetle	Coleoptera: Dermestidae
Zabrotes subfasciatus	Mexican bean beetle	Coleoptera: Bruchidae

pests of stored products, with a few beetle species responsible for most of the damage (Table 1). Damage due to stored-product pests is much higher in developing countries than in industrialized countries. One reason for this is that most stored-product pests have an optimum temperature for development above 30°C (Evans, 1987). In addition, effective protection methods are often lacking in developing countries due to poor infrastructure and lack of financial means (Taylor & Webley, 1979).

Because of the amount of labour that has been invested into a crop once it has been harvested and stored, damage done to stored product results in a higher economic loss than a similar percentage of damage to a crop in the field. Unfortunately, it is hardly known how much damage or loss occurs due to stored-product insects. Several authors have mentioned worldwide losses of up to 10% and losses in developing countries as high as 30%, but reliable and precise data to substantiate such figures are hardly available (Adams, 1977, Boxall, 1991). Loss of stored

product is characterized by different aspects: loss of weight, volume, processing quality, taste, nutritional value, seed viability, and economic value. Qualitative losses are generally more important, in terms of economic value, than quantitative losses. Weight losses, which are often mentioned in literature, are difficult to interpret, since dust, frass and insect debris are often included in the weight and because weight is also affected by moisture content. In addition, many authors have ignored the fact that subsistence farmers gradually remove the stored product for consumption, which has often lead to an overestimation of the damage. If, for example, the stored product that is left in a granary at the end of a storage season shows 20% damage, this does not mean that 20% of the stored product is lost, since part of the product had already been consumed at an earlier stage. Those investigations that have used adequate and reliable methodologies usually point towards volumetric losses in storage in developing countries of up to about 5% (Adams, 1977; Boxall, 1991). A more elegant method for expressing post-harvest loss has been used by Compton *et al.* (1998): they allowed experienced Ghanaian maize traders to price maize samples showing different levels of damage. Using this method, damage could be rapidly assessed in a meaningful way, taking account of the market price. Similarly, an elegant way to express the importance of storage pests would be to quantify the amount of money and labour that is invested in protective and curative measures. Unfortunately, such data are almost not available. Ultimately, however, insect infestations of stored product may lead to losses that are difficult to quantify, such as loss of goodwill for a grain trader or impoverished health of consumers due to contamination of food with mycotoxins (Adams, 1977; Stein, 1986).

Stored-product protection

Several types of measures are used to protect stored products. Most practices are aimed at the prevention of insect infestations in stored product. Available protection methods include minimization of field infestation at harvest; the use of resistant crop varieties and adequate storage structures; physical control methods such as the use of diatomaceous earth, ventilation to keep temperature and relative humidity low, the use of high N_2 or CO_2 concentrations, and hermetic storage; chemical control; traditional methods; and biological control. Traditional methods are mainly based on chemical and physical mechanisms. The adjective 'traditional' points to the long history of these methods, the use of natural and locally available materials, and the often simple level of technology involved. Stored-product protection in industrialized countries relies heavily on chemical control methods

whereas subsistence farmers in developing countries often use various traditional methods. In the next two paragraphs I therefore explore these two types of control methods in more detail, and I will show why biological control may be a viable alternative in certain cases in both industrialized and developing countries.

Two types of chemical control substances – fumigants and protectants – can be distinguished, based on speed of action and persistence of the insecticides that are used (White & Leesch, 1996). Fumigants are gases that tend to kill all insects present within hours or days but do not give long-lasting protection, whereas protectants are mostly contact insecticides with long-lasting residual action. Fumigants are mainly used to eradicate an already established pest population; protectants are used to prevent infestations. Well-known fumigants are phosphine and methyl bromide; popular protectants are organophosphates such as malathion, chlorpyrifos-methyl and pirimiphos-methyl. An advantage of fumigants over protectants is that they leave virtually no residues in the stored product. Fumigants can, however, not be used in all situations, since they require an airtight storage structure. If the storage structure is not airtight, the fumigation may not be effective, resistance may develop in insects, and humans may be exposed to the toxic gas (White & Leesch, 1996). In addition, methyl bromide, a popular fumigant, causes serious damage to the earth's ozone layer. For this reason, the use of methyl bromide will be banned in industrialized countries for all but a few applications from 2005 onwards (Insects Ltd., 2000). This has been agreed upon by more than 160 countries under the Montreal Protocol of the Vienna Convention in 1997, and the ban will apply to all these countries by 2015. Protectants of course do leave residues, but there is a growing number of consumers in industrialized countries that do not want any pesticide residues in their food (Credland, 1999). These developments have stirred increased attention for biological control of stored-product pests (Schöller *et al.*, 1997; Cox & Wilkin, 1998; Arbogast, 1984). The parasitoids *Trichogramma evanescens* and *Habrobracon hebetor* are already used commercially against stored-product moths in certain segments of the stored-product market, such as organic bakeries, flour mills, and wholesale trade (Prozell & Schöller, 2000; Reichmuth, 2000). In 1998, 26 million *T. evanescens* individuals were sold each month in Germany for use against stored-product moths (Prozell & Schöller, 2000). Application of natural enemies in large grain silos is still in an experimental phase, showing varying levels of success (Schöller *et al.*, 1997; Hansen & Jensen, 2002; Cox & Wilkin, 1998).

Many traditional methods to control stored-product pests exist. Some are associated with the construction of the storage structure. Storage in well-closed underground pits, for instance, may hamper insect development due to oxygen depletion (Hindmarsh *et al.*, 1978), while granaries are often placed on poles or stones to min-

imize access of rodents (Udoh *et al.*, 1994). Relatively large quantities of inert material, such as sand or wood-ash, are often added to stored products as a protective measure (Van Huis, 1991). These materials have an abrasive effect and presumably hamper insect movement. Numerous plant species are used in traditional methods for their insectidal or insect repellent action (Boeke *et al.*, 2001). In addition to complete leaves and other plant parts, extracts, oils, ashes and powders of these plants are used. Small quantities of food are often stored above the kitchen fire, because heat and smoke seem to have protective action (Dabiré, 1985). In addition, there are often magic or religious practices aimed at preventing infestation by stored-product pests (Temudo & Barros, 1998; Temudo, 2000). It is important to note that stored-product protection is often a gender issue: religious and social traditions may dictate which family member is responsible for the stored product and who may enter the storage room or take rations out of the granary (*e.g.*, Temudo & Barros, 1998). Traditional methods of stored-product protection differ widely between regions, and even from one village to the next. Much knowledge of traditional methods is now getting lost because it is not passed on to younger generations (Koné, 1993; Compton *et al.*, 1993). Advantages of traditional methods are the use of locally available materials and the comparative safety for the environment. Disadvantages are the sometimes low efficacy and unsuitability for protection of large amounts of stored product.

Chemical control is often not affordable for subsistence farmers, whereas those chemicals that are available pose a health risk due to lack of appropriate facilities and lack of training (Atteh, 1984; Taylor & Webley, 1979). Fumigants, for instance, cannot be safely applied in traditional granaries because of leakage (Brice & Golob, 1999). Governments and cotton companies often provide subsidized, highly toxic insecticides – such as the organochlorine endosulfan – for use in cotton. Unfortunately, farmers also use these products on their food crops, and store them in *e.g.* soft drink bottles at home (Ton *et al.*, 2000; Udo, 1998). As a result, in the 1999/2000 growing season in northern Benin alone, at least 33 people died from accidental endosulfan poisoning, while another 36 people suffered serious ill health (Ton *et al.*, 2000). Ten of these poisoning incidents, of which four fatal, were a result of endosulfan use in stored food products. In addition to these direct risks for human health, there is a risk of environmental pollution and of farmers becoming dependent on chemicals.

Biological control can be a safe and viable method of stored-product protection in developing countries. Three modes of biological control are distinguished: (1) classical biological control by introduction of new natural enemies; (2) conservation biological control, emphasizing preservation and enhancement of natural enemies that are already present; and (3) inundative biological control, based on

mass-rearing of natural enemies followed by repeated releases. Inundative release is thought to be difficult to achieve for subsistence farming systems because of high cost and demands on infrastructure (Van Huis *et al.*, 1991a). Classical biological control, on the other hand, has already been applied in the case of *Prostephanus truncatus*, a destructive pest of stored maize and cassava which was accidentally introduced into East and West Africa in the 1980's. Following the release of *Teretriosoma nigriscens*, a predatory beetle, the damage caused by *P. truncatus* has decreased (Richter *et al.*, 1998). Similarly, a conservation strategy of biological control may be a viable method, because natural enemies often already occur in stored products in developing countries (Van Huis, 1991; Van Huis *et al.*, 1991a; Haines, 1984; Haines, 1999).

Summarizing, a niche for biological control of stored product pests exists in both industrialized countries and in developing countries. The constraints and conditions under which biological control can be successful are very different for industrialized and developing countries. This thesis deals with a storage system in developing countries for which biological control may be an option, namely traditional storage of cowpea in West Africa.

Traditional storage of cowpea in West Africa

West Africa is one of the least developed regions of the world. Twelve[1] out of the world's 49 Least Developed Countries – as measured by indices such as income, life expectancy, adult literacy, and economic indicators – are West African (UN, 2001). Niger, for example, has a gross national product of US$ 200 per capita per year; life expectancy at birth is 47 years for men and 51 years for women; infant mortality under 5 years is 280 per 1,000 live births; 21% of the men and only 7% of the women are literate; and foreign direct investments amount to only US$ 9 million per year while the country has an external debt of US$ 1,613 million. Eighty-eight percent of the labour force is employed in agriculture (UN, 2001).

Much of the agricultural activity in West Africa is devoted to subsistence farming. An important crop for such farmers is cowpea (*Vigna unguiculata* Walpers). Cowpea is a leguminous, annual herb, with up to nine recognized subspecies, many varieties and great morphological variability (Padulosi & Ng, 1997). All cultivated cowpeas belong to *V. unguiculata* subspecies *unguiculata*. The pods are up to 30 cm long and contain 2-18 seeds, each weighing 0.1-0.5 g (Nwokolo & Ilechuk-

[1] Thirteen if Chad is included.

wu, 1996; Van Alebeek, 1996a). The seeds are characterized by a black or dark 'eye' around the hilum. Most cowpea is grown in West Africa, which is probably also the region where it was first cultivated (Padulosi & Ng, 1997). In 2001, 72% of the world's three million tons of cowpea production was grown in Nigeria alone, while neighbouring Niger was responsible for 10% of the production (www.fao.org). Cowpea seeds are both an important protein source for low income families and a source of income if sold at the market (King *et al.*, 1985; Nwokolo, 1996). Cooked seeds are eaten both plain and in soups and stews, and cowpea flour is used as an ingredient for deep-fried balls and for steamed dishes (Dovlo *et al.*, 1976). Apart from the seeds, young pods and leaves are eaten, and leaves and stems are used as animal feed and as green manure (Duke, 1990). Cowpea is relatively tolerant to drought, and it can produce relatively well under nitrogen-poor conditions due to nitrogen-binding, symbiontic nodule bacteria that occur in the roots (Turk *et al.*, 1980; Summerfield *et al.*, 1974). It is often intercropped with *e.g.* millet, sorghum, maize or cassava (Mortimore *et al.*, 1997).

Except from areas where farmers have access to irrigation water from rivers or lakes, cowpea is grown only during the rainy season. The rainy season starts in May or June and may last until August or September (*e.g.* Van Huis *et al.*, 1990). There is no rainfall during the other months of the year. Storage of cowpea takes place during the dry season and up to the next harvest. The harvest typically amounts several hundred kilograms of cowpeas (Sagnia & Schütte, 1992). Directly after the harvest, cowpeas are usually stored as whole-pods. They are threshed and stored as seeds several weeks or months later, when they are needed for consumption or for trade. Subsistence farmers often store their cowpeas in traditional granaries, constructed of natural materials such as straw, wood, and clay. These granaries vary greatly between and even within regions (Sagnia & Schütte, 1992). Cowpea prices increase over the dry season, implying that farmers who manage to keep their cowpea in good condition until the end of the storage season can generate more income (Caswell, 1961, 1981; Sagnia & Schütte, 1992).

Storage pest: *Callosobruchus maculatus*

Unfortunately, cowpea is often infested by two or three species of bruchid beetles (Coleoptera: Bruchidae) (Jackai & Daoust, 1986; Singh *et al.*, 1990). These beetles oviposit on the ripening pods in the field. Hatching larvae penetrate the pod wall and enter a seed, where they develop up to pupation. The adult emerges through a 'window' in the seed. One of these bruchids, *Callosobruchus maculatus* Fabricius, is well adapted to storage (Credland, 1990); emerging females continue to oviposit

on pods or seeds during storage in the granary. At 30°C and without access to food, females live for about a week during which they lay about 75 eggs (Boeke, submitted). Development from egg to adult takes about three weeks. Two forms occur: the flying form, which lays few eggs and tends to disperse from storage, and the flightless form, which can lay up to 120 eggs in storage (Messina & Renwick, 1985b). The flying form is induced at high bruchid densities in storage. *Callosobruchus maculatus* is the only serious storage pest of cowpea; at the same time it is probably one of the most destructive pests of stored products. An estimated 20-40% of the stored cowpea seeds in Northern Nigeria are annually infested by this pest; in individual granaries loss can be complete (Caswell, 1981). Many farmers would like to sell cowpea at the end of the storage season, when prices are high; but because of the large risk of infestation they tend to sell it soon after the harvest, when prices are still low (Sagnia & Schütte, 1992; Van Alebeek, 1996b). At the end of the storage season it is hard to find undamaged cowpea at local markets, while the few cowpea stocks that are then still uninfested may have been treated with potentially unsafe pesticides.

Many traditional methods are aimed at prevention or control of *C. maculatus* in stored cowpea. Examples include the use of sand or wood-ash, and the use of plant materials with supposed insecticidal or insect repellant action. The efficacy of these methods, however, seems to be limited, and their use is hampered by other problems. Sand, for example, fills up the emergence holes left by bruchids in the seeds, and it is difficult to remove this sand before cooking (I. de Groot, personal communication). Neem oil, an effective natural insecticide, may spoil the taste of beans (Naik & Dumbre, 1985), while other natural insecticides might have chronic negative effects on human health (Schulten, 1991; Compton *et al.*, 1993).

An elegant control method that has recently been developed is 'hermetic storage', *i.e.*, storage in hermetically closed oil drums or in three layers of plastic bags ('triple bagging'). This kills all insects by suffocation, and it prevents reinfestation as long as the bag or drum stays closed (Murdock *et al.*, 1997; Van Huis, 1991). The method can also be combined with simple solarization techniques, which kill insects within hours (Kitch *et al.*, 1992). Since oil drums and plastic bags can be used for several years, hermetic storage is a relatively sustainable method. A disadvantage is that the protective action disappears when the drum or bag is opened (*e.g.* to take out rations for consumption) or when it is damaged. Damage may occur through corrosion in the case of oil drums, and by rodents in the case of plastic bags. Even emerging bruchids can gnaw through plastic bags, thus nullifying the suffocative effect. Another problem is that for subsistence farmers, especially in the arid Sahel region, the availability and cost of even simple materials such as an oil drum or solid plastic bags can be an obstacle (Hindmarsh *et al.*, 1978; Temudo,

2000). Prices of oil drums range from 5 to 15 US dollars (Murdock *et al.*, 1997; C. Stolk, personal observation). Strong plastic bags are cheaper – a typical harvest of 200 kg can be stored in bags worth about US$ 3 (Kitch & Ntoukam, 1991) – but they are often not available at local market places (A. Adandodon, personal communication).

Another option for the protection of stored cowpea is biological control. In West Africa, *C. maculatus* is attacked by a number of natural enemies both in the field and in storage. The most important ones are the larval parasitoids *Dinarmus basalis* (Rond) (Hymenoptera: Pteromalidae), *Eupelmus vuilleti* (Crawford) and *E. orientalis* (Crawford) (Hymenoptera: Eupelmidae), and the egg parasitoid *Uscana lariophaga* (Steffan) (Hymenoptera: Trichogrammatidae). These naturally occurring parasitoids are responsible for substantial mortality of *C. maculatus*. In a faunistic study by Monge *et al.* (1991), larval parasitoids represented about 50% of the total number of insects that emerged from harvested cowpea seeds over a 10-month period; and *U. lariophaga* has been identified as the most important mortality factor for *C. maculatus* eggs in the field (Sagnia, 1994). In a survey in Niger, egg parasitism by *U. lariophaga* was found in 69% of all granaries, with parasitization rates of up to 73% (Van Alebeek, 1996b). These parasitoids have therefore received considerable attention as potential biocontrol agents (Van Huis *et al.*, 1991a; Sanon *et al.*, 1998). An augmentative or conservation strategy, aimed at preserving and possibly multiplying those natural enemies that are already present, might be feasible for subsistence farmers. Mexican farmers, for instance, rear parasitoids at village level for the control of the coffee berry borer (Galvez, 1992). Other possibilities include: incorporating a food source in the storage system that is advantageous for the parasitoid but inaccessible to the bruchids (Van Huis *et al.*, 1991a); or mixing the beans with seeds on which the bruchids oviposit but in which their larvae cannot develop. The latter strategy would result in more hosts for the egg parasitoid while at the same time bruchid eggs are wasted on a trap crop. Recently, a good trap crop seemed to have been found in the widespread annual herb *Crotalaria retusa* L. (Leguminosae-Papilionoideae). In an olfactometer choice test, *C. maculatus* showed significant attraction to *C. retusa* seeds, and in a no-choice test it also oviposited on these seeds (Lenting, 2000). Yet, *C. maculatus* larvae could not develop on these seeds. Further experiments, however, have failed to confirm the protective action of *C. retusa* seeds, either alone or in combination with *U. lariophaga*, on stored cowpea seeds (Djomamou, 2001). In addition, seeds of *Crotalaria* spp., including C. *retusa*, are well known for their high toxicity in vertebrates (*e.g.*, Hooper & Scanlan, 1977).

In this thesis, attention is focused on *U. lariophaga*. This parasitoid has the advantage over larval parasitoids that it kills the host in the egg stage, before the

larvae have inflicted any damage. In the next section, the parasitoid will be further introduced, starting with a brief description of the genus.

Potential biocontrol agent: *Uscana lariophaga*

The genus *Uscana* Girault currently contains 25 described species from all continents (Lin, 1994; Fursov, 1995; Vigianni, 1996; Pajni & Sood, 1999; Pintureau *et al.*, 1999). All known hosts are eggs of bruchid or buprestid beetles. Five species have been described from Africa: *U. caryedoni* Viggiani from Congo-Brazzaville, *U. diogenae* (Risbec) from Senegal, *U. johnstoni* (Waterston) from the Sudan, *U. lariophaga* from Mali, and *U. terebrator* Vigianni from Cape Verde islands. *Uscana lariophaga* is know to occur also in Niger and Benin, and it probably also occurs in other West African countries. At least two species of *Uscana* occur in Ivory Coast (Rasplus, 1990). Based on molecular characterization of the ITS2 sequence of the rDNA gene complex of various *Uscana* samples collected in Benin, Van Heerwaarden (2000) considered it likely that at least three *Uscana* species occur in West Africa, one of which is *U. lariophaga*. One of the other two species might be *U. caryedoni*. Of these three species, however, only *U. lariophaga* can be easily reared on *C. maculatus* eggs (C. Stolk, personal observation).

Uscana lariophaga is a 0.4 mm long solitary endoparasitoid of eggs of among others *C. maculatus* and *Bruchidius atrolineatus* (Pic). At 30°C, parasitized host eggs turn black within about three days; 8-11 days after parasitization the adult wasp emerges through an emergence hole in the chorion (Van Huis *et al.*, 1994a). Males develop slightly faster than females (Van Huis & Appiah, 1995). Sex ratio is about 70% females under rearing conditions (Van Huis *et al.*, 1994a). Upon emergence, females have an egg load of about 25 eggs, and additional eggs mature at a rate of 0.7-0.9 eggs·h^{-1} (Van Huis *et al.*, 1991b; Van Alebeek *et al.*, 1996b). Average longevity is two days in the absence of food; if honey is provided, longevity is increased fivefold and lifetime fecundity threefold (Van Huis *et al.*, 1991a). *Uscana lariophaga* females are attracted to odours emanating from host eggs and cowpea seeds (Van Huis *et al.*, 1994b; Ormel *et al.*, 1995). In functional response experiments, Van Alebeek *et al.* (1996b) showed that females can parasitize 25 eggs in 4 h and 40 eggs in 24 h. The parasitoid can parasitize more hosts if the hosts are clustered or uniformly distributed than when they occur in a random pattern (Van Alebeek *et al.*, 1996a), and it disperses faster in a stock of cowpea pods than in a stock of cowpea seeds (Van Alebeek, 1996a). *Uscana lariophaga* has a strong negative geotaxic response (Van Alebeek & Van Huis, 1997).

In experimental cowpea stocks, a single *U. lariophaga* inoculation can suppress

C. maculatus populations during at least three months by up to 86% as compared to the control treatment (Lammers & Van Huis, 1989; Van Huis *et al.*, 1998, in press). At low initial bruchid densities, however, *U. lariophaga* achieves a lower level of control. Van Huis *et al.* (1998) suggested that it may be more difficult for *U. lariophaga* to locate hosts, and consequently to establish itself, when host densities are low. In this thesis, I therefore focus on aspects that are associated with low host densities. For example, at low host densities, spatial aspects might be more important, since host eggs will then probably occur in clusters rather than uniformly throughout a cowpea stock. This thesis is therefore oriented towards describing and analyzing spatial aspects of the foraging behaviour of *U. lariophaga* in stored cowpea.

In Chapter 2, I describe the three dimensional spatial pattern of egg clusters of the host, *C. maculatus*. Chapter 3 describes the searching behaviour of *U. lariophaga* at short time and spatial scales, and in Chapter 4 I analyze host finding success of *U. lariophaga* in cowpea stocks as a function of distance and time. Behavioural responses of *U. lariophaga* to finding eggs that are already parasitized, or to not finding any eggs at all, are described in Chapters 5 and 6, respectively. Finally, implications of *U. lariophaga* behaviour for the prospects of biological control are discussed in Chapter 7.

Acknowledgements

I thank Arnold van Huis, Wopke van der Werf and Joop van Lenteren for their comments on earlier versions of this chapter.

CHAPTER 2

C. Stolk, A. Stein, S.B. Slumpa, S.K. Tiase & A. van Huls

Exploring the foraging environment of *Uscana lariophaga*: spatial distribution of *Callosobruchus maculatus* eggs in stored cowpea

Abstract

Knowledge of the spatial distribution of stored product insects may reduce the dependency on chemicals for control of these insects. Biological control, for instance, could be improved based on such knowledge. In this chapter we describe the three-dimensional spatial oviposition pattern of *Callosobruchus maculatus* in stored cowpea. Individual *C. maculatus* females oviposited in clusters of 70±15 (SD) eggs. These clusters were variable in shape. In any cluster 90 to 95% of the eggs fitted into a volume of 19.1±3.5 cm^3. The egg density was highest at the center of a cluster and decreased towards the periphery. A statistically significant relationship existed between the number of eggs n in a cluster and the cluster volume, V (cm^3): $V = 11.5 + 0.11 \cdot n$. We also investigated the spatial egg distribution of beetles which emerged from egg clusters such as those produced by individual females. Their oviposition was not confined to one specific area but was scattered throughout the bean mass. No effect of the density of the 'parent' cluster on the spatial egg pattern could be detected. These results give insight into the foraging environment which the egg parasitoid *Uscana lariophaga*, a promising candidate for biological control of *C. maculatus*, is facing. We argue that the probability p of encountering at least one other bean with eggs after a parasitization is a function of the number n of beans that are visited: $p = 1-0.4 \cdot (0.37)^{(n-1)}$.

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Introduction

In recent years we have seen an increased attention for non-chemical methods of stored-product protection, including biological control of stored-product pests (Arbogast, 1984; Brower *et al.*, 1996; Schöller *et al.*, 1997; Adler, 1998; Cox & Wilkin, 1998; Schöller, 1998a, 1998b). Compared to alternative methods, chemical control requires little knowledge about the behaviour or biology of pest insects. A better understanding of the behaviour of pest insects is therefore often needed to become less dependent on chemical means of pest control (Compton *et al.*, 1993; Arbogast *et al.*, 1998; Credland, 1999). For example, release of a natural enemy on top of stored grain would be rather useless if the pest insect mainly occurs below a depth of stored grain that the natural enemy does not or cannot reach. Similarly, knowledge of behaviour of stored-product insects could help formulate better monitoring procedures, which in turn could help reduce pesticide use (Hagstrum *et al.*, 1985, 1988; Subramanyam & Hagstrum, 1996; Meikle *et al.*, 1998). In this chapter we describe the three-dimensional oviposition pattern of the stored-product beetle *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae) in stored cowpea as part of a research project which deals with the possibilities for biological control of this pest.

Callosobruchus maculatus causes considerable losses in stored beans (Adams, 1977; Caswell, 1981; Schulten, 1982). This is particularly a problem in cowpea stored by West-African subsistence farmers, for whom cowpea constitutes an important protein source (Nwokolo, 1996; van Alebeek, 1996). The egg parasitoid *Uscana lariophaga* Steffan (Hymenoptera: Trichogrammatidae) is studied as an interesting candidate for biological control of *C. maculatus* (Lammers & van Huis, 1989; van Huis *et al.*, 1990, 1991a, b; 1994a, 1998). Previous research on this system focussed on host-finding behaviour and functional response of this parasitoid (van Huis *et al.*, 1994b; Ormel *et al.*, 1995; van Alebeek *et al.*, 1996b; van Alebeek & van Huis, 1997). Van Alebeek *et al.* (1996a) showed that the spatial distribution of host eggs influences the effectiveness of the parasitoid. Until now, however, very little is known about the actual spatial distribution of *C. maculatus* eggs in stored cowpea.

Callosobruchus maculatus prefers to oviposit on seeds that have no eggs or fewer eggs than average (Messina & Renwick, 1985a, c; Messina & Mitchell, 1989; Credland & Wright, 1990). This results in a more or less uniform distribution of eggs when a relatively small number of seeds is offered (Messina & Mitchell, 1989). In bulk stored beans, however, a low density infestation of bruchids is likely to result in a highly aggregated distribution of eggs. Quantitative aspects of this distribution are unknown, as are the resulting distributions at higher densities. Thus, at present, we do not know what type of universe *U. lariophaga* is facing. The only avail-

able information concerning spatial oviposition patterns of *Callosobruchus* spp. in stored beans indicates that beetle movement is probably hampered by small intra-bean spaces (Gundurao & Majumder, 1964; Watanabe, 1984, 1985, 1986). Temperature preference of bruchids may play a role if temperature gradients occur in stored beans; however, *Acanthoscolides obtectus*, another bruchid that is associated with stored beans, does not show a clear preference for specific temperatures (Deal, 1941). The flying morph of *C. maculatus* is attracted to light (Keever & Cline, 1983), but for oviposition dark conditions are preferred (Iloba & Osuji, 1986; Khattak, 1991; see Mbata *et al.*, 1997 for *C. subinnotatus*). We could not find any reports concerning the geotaxic response of bruchids.

The objective of this study is to quantify the three-dimensional spatial distribution of *C. maculatus* eggs in stored cowpea. For that purpose we analyse two experiments using spatial statistical procedures. In the first experiment we characterise the spatial distribution of eggs in an egg cluster laid by individual *C. maculatus* females. In the second experiment we study the spatial egg distribution produced by the next generation of beetles (which emerges from such an egg cluster). In this experiment we use clusters of three different egg densities: low, medium, and high. We discuss consequences of the spatial distribution of *C. maculatus* eggs for *U. lariophaga*.

Materials and Methods

Cowpea (*Vigna unguiculata*) seeds of the variety 'Black Eyes' were used in the experiments and in the insect rearing. Before use, the beans were frozen for at least two days to exclude contamination by insects, and subsequently dried at 45°C for at least two days.

The *C. maculatus* culture originated from the Niamey region in Niger. Beetles were reared in petri dishes on cowpea seeds at L12:D12. The temperature was kept at 35±1°C during photophase and 25±1°C during scotophase. All experiments were carried out at 30°C.

Experiment 1: Three-dimensional oviposition pattern of individual females. In the first experiment, 1 l glass beakers (diameter 10 cm) were used as experimental units. The beakers were filled with cowpeas to a height of 6 cm. An open, 2 cm long gelatine capsule containing a freshly emerged *C. maculatus* female was placed at the centre of the seed surface, after which the beakers were filled to a height of 12 cm. The beakers were covered with tissue paper to prevent accidentally occurring beetles or parasitoids from entering. The females that were used in this experiment were

obtained from a batch of infested seeds from which all adult beetles had been sieved off 1 h earlier. Females were allowed to mate before they were used in the experiment.

After one week, the beakers were emptied layer by layer using a circular sampling device. The sampling device consisted of a piece of cardboard, just fitting into the beaker. On one side it was covered with double sided tape, on the other side a handle was connected. The sampling device was gently pushed into the beaker to minimize dislocation of beans from their original sites in the bean stock. Cowpea seeds would stick on the taped face of the device. In this way each beaker was broken down into 36 layers, each of which was about 3.3 mm thick.

The seeds were carefully inspected for eggs, and the position of each egg was recorded. For each egg, a set of coordinates (x, y, z) was determined based on the bean layer in which it was found and the position it had in that layer. During observations, a hand lens and a stereomicroscope were used because freshly laid eggs are not easily visible with bare eyes.

The experiment was replicated 19 times. A record of the number of beans which carried more than one egg was kept for 16 of the replicates.

Experiment 2: Spatial distribution of eggs deposited by females emerging from an egg cluster. When females emerge from a cluster of infested beans such as observed in experiment 1, they may oviposit around the emergence site, or, alternatively, move elsewhere before oviposition starts. In the latter case, there could be a preferred direction of moving, or a preferred location to oviposit. In this second experiment we investigated how females distribute their eggs in a stock of beans when they emerge from a cluster of infested beans in that stock.

The experimental unit consisted of 17.5 liter plastic containers (height 28 cm, top diameter 31 cm, bottom diameter 27 cm) which were filled with beans. Three wire frames with the capacity of holding a single layer of beans were used to obtain two-dimensional cross-sections of the three-dimensional bean mass (Figure 1). All frames were constructed of 6 mm thick plastic bars and were covered on both sides with metal gauze (mesh width 3.2 mm) which allowed beetles to freely move into and out of the frames. One to three small plastic blocks in each frame supported the metal gauze in order to keep the width fixed. The first frame was trapezium-shaped (with two parallel sides) and fitted vertically into the container, whereas the other two were semi-circles and fitted horizontally into the container, each on one side of the vertical frame.

At the centre of the trapezium-shaped frame, three to nine beans were replaced with infested beans, the so-called inoculum beans. Similarly, two to eight inoculum beans were placed in the semi-circular frames. The number of inoculum beans was

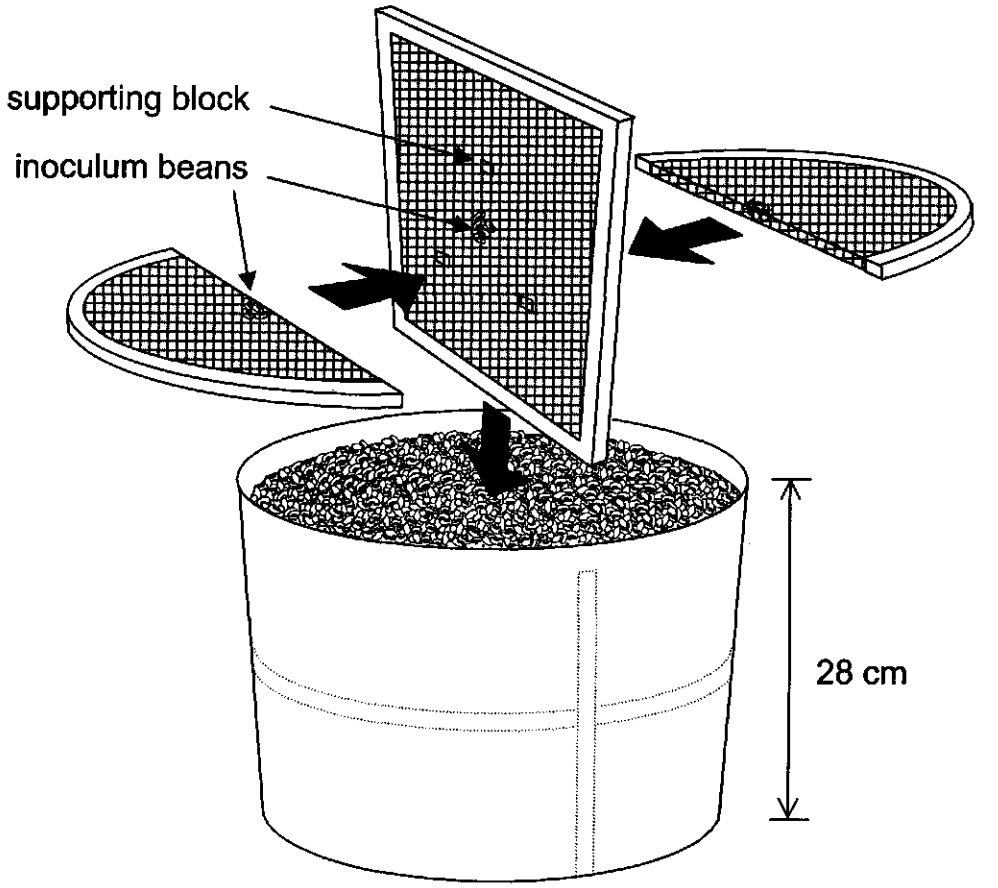

Figure 1. Schematic representation of the experimental setup used in experiment 2. Three frames, each covered with metal gauze and filled up with a single layer of beans, were placed in a container. The rest of the space in the container was filled up with beans. Arrows indicate the position of inoculum beans and supporting blocks.

varied because we aimed at three different beetle densities (see next paragraph). The frames were mounted into the container and the container was filled up with beans, such that a cluster of infested beans was created at the centre of the bean mass (Figure 1). Finally, the container was covered with wire netting (1 mm mesh width) and a plastic lid of which about 20% of the surface area had been perforated to allow ventilation.

The inoculum beans had been prepared by allowing one individual female, coupled with a male, to oviposit on 15-30 seeds for a period of two days, starting 20-21 days before the start of the experiment. Thus the beetles emerging from a

cluster of inoculum beans all shared the same mother. We aimed at clusters of three different densities by selecting inoculum beans carrying the appropriate number of egg shells. The intended densities were: low density (five emerging females per container); intermediate density (ten emerging females per container); and high density (20 emerging females per container). We assumed a sex ratio of 50% females (e.g., Howe & Currie, 1964). The inoculum beans carried on average 1.8 ± 0.9 (SD) egg shells. All egg shells that were present on the inoculum beans before the experiment started were marked so that they could be distinguished from eggs that might be deposited on the inoculum beans during the experiment. In total, 7-25 beans were used in each container as inoculum beans.

The containers were incubated in the dark. After ten days they were placed in a freezer for at least three days to stop oviposition and development of already deposited eggs. The containers were subsequently emptied and the frames carefully removed. Adult beetles were sieved from the seeds which had been outside the frames. These beetles were sexed and counted to check the number of females in the container.

Each individual bean in the frame was examined for presence of eggs. For each bean which carried one or more eggs, we recorded the number of hatched and non-hatched eggs, and a pair of coordinates (x, y) in cm. Hatched eggs mostly have a white egg shell whereas non-hatched eggs are either transparent or have the larva still inside the egg. During observations a stereomicroscope was used. Adult beetles that were found in the frames were sexed and added to the number of beetles that walready found for the rest of the container. Coordinates (x_i, y_i) were also measured for the inoculum beans. The number of emergence holes in the inoculum beans was counted and compared to the number of marked egg shells. The experiment was replicated three times.

Data analysis. We calculated cumulative probability functions of eggs and egg densities as follows (see Bailey & Gatrell, 1995). Assume a point s in space, with

s = the centre $(\bar{x}, \bar{y}, \bar{z})$ of an egg cluster in experiment 1, or

s = the average inoculum point $(\bar{x}_i, \bar{y}_i)$ for a cross-section in experiment 2.

The distance of s to the j-th egg is denoted by h_j, and the distance of s to the closest wall of the beaker or wire-frame by h_w. The cumulative count function $\hat{G}^*(h)$ of eggs for any $0 < h < h_w$ equals

$$\hat{G}^*(h) = \#(h_j < h) \qquad (1)$$

where # means "the number of". From this, we obtain the cumulative probability function $\hat{G}(h)$:

$$\hat{G}(h) = \frac{\hat{G}^*(h)}{n} \tag{2}$$

where n is the total number of eggs. The egg density function $\hat{g}^*(h)$ for $\frac{1}{2}\Delta h \leq h < h_w - \frac{1}{2}\Delta h$ is defined as

$$\hat{g}(h) = \frac{\#(h - \frac{1}{2}\Delta h \leq h_j < h + \frac{1}{2}\Delta h)}{V} \tag{3}$$

where Δh is a small distance (0.25 and 1 cm in experiment 1 and 2, respectively) and V equals the volume or surface area of the sphere-shaped or circular shell in which the egg density is determined, according to:

$$\begin{cases} V = \frac{4}{3}\pi \ (h + \frac{1}{2}h)^3 - (h - \frac{1}{2}h)^3 \} & \text{for experiment 1} \\ V = \pi \ (h + \frac{1}{2}h)^2 - \ (h - \frac{1}{2}h)^2 \} & \text{for experiment 2} \end{cases} \tag{4}$$

Both $\hat{G}(h)$ and $\hat{g}(h)$ were calculated at intervals of Δh.

In addition, we calculated frequency distributions of eggs in the three orthogonal directions x, y, and z for experiment 1, and in the vertical direction for experiment 2. We also calculated the linear distance of each egg to its nearest neighbour and to subsequent neighbours. Normality was tested by inspecting Q-Q plots and P-P plots, and by the formal Kolmogorov-Smirnov normality test with Lilliefors significance correction.

For experiment 1, the volume of each egg cluster was expressed as the volume within which 90-95% of the eggs in that cluster occurred. These volumes, or 'three-dimensional contour diagrams', were found using kernel estimation (Bailey & Gatrell, 1995). We subdivided the space in the beaker into small cells, and calculated the egg intensity λ for each cell s using the intensity function

$$\hat{\lambda}_\tau(s) = \sum_{h_j \leq \tau} \frac{9}{4\pi\tau^3}\left(1 - \frac{h_j^2}{\tau^2}\right) \tag{5}$$

Here h_j is the distance between the current cell s and the egg s_j, and the summation is only over values of h_j which do not exceed the 'bandwidth' τ (adapted from Bailey & Gatrell, 1995; see Bowman & Azzalini, 1997). We used a fixed value of τ of 1.0 cm. The grid cells with an intensity higher than a certain threshold value were counted as part of the egg cluster. For each replicate, the threshold intensity was chosen such that 90-95% of the eggs in the cluster fitted into this volume. If no vol-

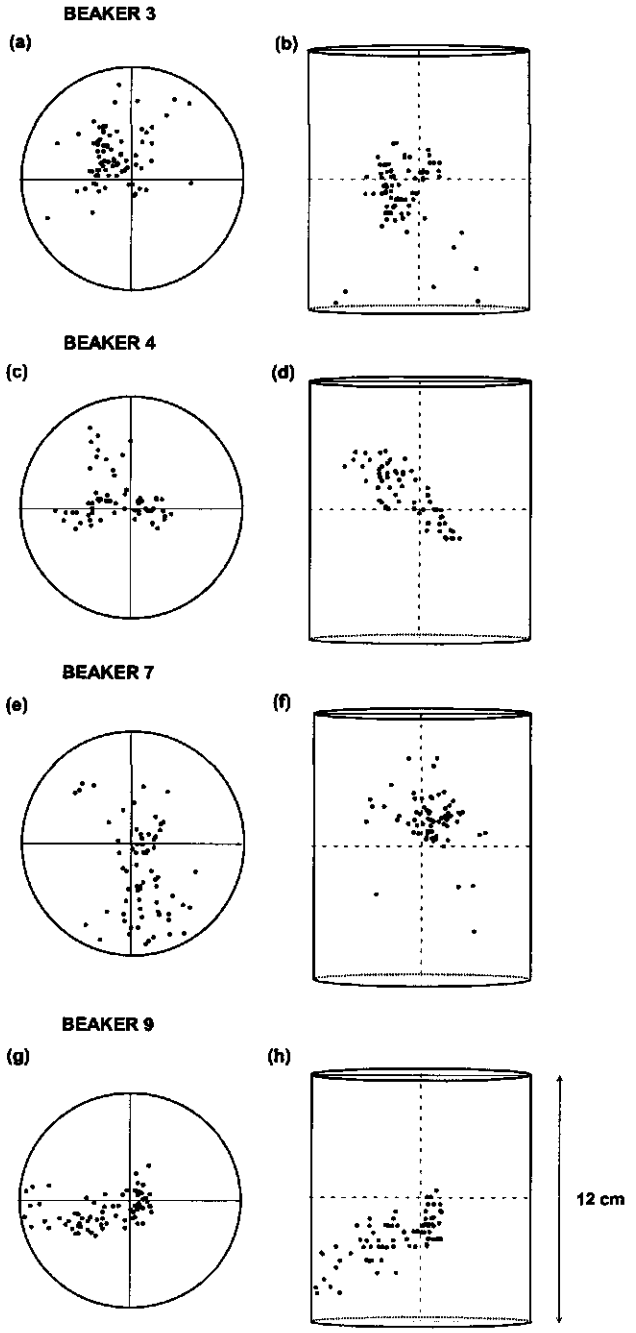

Figure 2. Graphical representations of four of the egg clusters that were observed in experiment 1. (**a**), (**c**), (**e**) and (**g**): view from top of beaker; (**b**), (**d**), (**f**) and (**h**): side view of slightly turned beaker.

ume incorporating 90-95% of the eggs could be determined, we took the average of the volumes that incorporated 83-88% and 95-100% of the eggs, respectively.

The number of seeds present in a glass beaker filled with 200 ml of cowpea was counted in order to be able to express egg densities as eggs·seed^{-1}. To express nearest-neighbour distances in terms of beans, we measured the size of 171 seeds in three orthogonal directions, and we counted the number of neighbouring seeds for 35 beans in a bean mass. This bean mass was mixed with a little water and then frozen so that it could be broken down seed by seed.

The spatial configuration of eggs in experiment 1 was visually inspected using ArcView GIS 3.2 with the extensions Spatial Analyst 1.1 and 3D Analyst 1.0. For experiment 2, graphical representations of the horizontal (circular) cross-sections were obtained by fitting the two semicircular frames together and inserting data from a small strip of the trapezium-shaped frame. We counted the number of beans present in three trapezium-shaped frames and in six semicircular frames in order to be able to express egg densities as eggs·seed^{-1}.

Statistical tests were carried out using SPSS 7.5. Kernel estimation was implemented through a Pascal program.

Results

Experiment 1. The average number of eggs found in a beaker was 69.6 ± 15.4 (SD). On average, 11.6 ± 7.4 (SD) seeds per beaker carried more than one egg. Some representative examples of egg clusters are shown in Figure 2.

On average, 95% percent of all eggs occurred within 3.75 cm from s (Figure 3a). The egg density was highest at the centre of an egg cluster (about 0.6 egg/seed) and declined towards the edges of an egg cluster (Figure 3b). Some clusters were much more dense than others (data not shown).

Individual egg clusters were highly variable in shape (Figure 2). Averaged over all the beakers, however, the eggs seem to follow a normal distribution in the three orthogonal directions x, y, and z around the centre of a cluster (Figure 4). Nevertheless, the normality test for all data pooled showed that the distribution deviates significantly from the normal distribution in all three directions ($P<0.05$). These empirical distributions are characterized by the following standard deviations: σ_x = 1.20 cm, σ_y = 1.19 cm, and σ_z = 1.18 cm.

The distribution of nearest to 6th nearest neighbour distances is given in Figure 5. In 14 out of the 19 egg clusters a significant ($P<0.05$) positive relationship was found between the distance $d_1(s)$ of an egg to its nearest neighbour and its distance h to the centre of the egg cluster, indicating that these clusters were more 'loose'

Table 1. Details of the inoculum beans and the resulting beetle densities in the different treatments, as well as the number of eggs that were found in the cross-sections after ten days of incubation. Egg densities were calculated using the surface area of the wire-frames and the number of beans in such wire-frames. For the horizontal cross-sections, the numbers of eggs that were found in the two semicircular cross-sections were put together. Averages ± standard deviation are given.

Treatment	# inoculum beans	# egg shells	# emerged females	# emerged adults (total)	# eggs in vert. frame	# eggs in horiz. frame	Egg density			
							in vertical frame		in horizontal frame	
							10^{-2} eggs·cm^{-2}	10^{-2}eggs·bean^{-1}	10^{-2} eggs·cm^{-2}	10^{-2} eggs·bean^{-1}
Low density	8.0 ± 1.0	10	8.7 ± 2.3	14.7 ± 4.9	46.3 ± 19.6	12.3 ± 16.4	7.0 ± 3.0	3.0 ± 1.2	2.3 ± 3.0	1.2 ± 1.5
Medium density	12.3 ± 1.2	20	13.7 ± 1.2	25.3 ± 4.0	41.0 ± 15.1	19.3 ± 13.0	6.2 ± 2.3	2.6 ± 1.0	3.6 ± 2.4	1.8 ± 1.2
High density	18.3 ± 6.1	40	20.0 ± 4.4	43.7 ± 4.7	119.7 ± 99.0[1)]	53.0 ± 25.1[1)]	18.1 ± 15.0[1)]	7.6 ± 6.3[1)]	9.8 ± 4.6[1)]	5.0 ± 2.4[1)]

[1)] Replicate 3 of the high density treatment was an outlier (234 eggs in the vertical frame, 125 in the combined horizontal frames). Without this replicate, 62.5 ± 2.1 eggs were found in the vertical frame and 41.0 ± 19.8 in the combined horizontal frames. The corresponding egg densities are $9.4 \cdot 10^{-2}$ eggs·cm^{-2} (4.0 eggs·bean^{-1}) for the vertical frame and $7.6 \cdot 10^{-2}$ eggs·cm^{-2} (3.9 eggs·bean^{-1}) for the horizontal frame.

towards their edges. For all data pooled, the equation describes the data reasonably well, although R^2=0.207 ($P<0.0001$; all units in cm).

The average volume of an egg cluster, expressed as the volume within which 90-95% of the eggs occurred, was 19.1±3.5 (SD) cm^3. This corresponds to about 5% of the volume of the beaker. There was a significant relationship between the number n of eggs in a cluster and the cluster volume V ($V = 11.5 + 0.11 \cdot n$; R^2=0.245; $P < 0.05$).

The 200 ml glass beaker contained 822 seeds, implying that on average 4.1 seeds fit into one cm^3. This figure was used to estimate egg densities in terms of eggs per bean. The seeds measured 9.0 ± 0.9, 5.3 ± 0.6, and 6.3 ± 0.6 mm in three orthogonal directions, respectively (average ± SD). Each seed was surrounded by 9.5 ± 0.3 (average ± SE) neighbouring seeds.

Experiment 2. For each treatment, one example of the spatial distribution of the eggs in the cross-sections is shown in Figure 6. The egg density (eggs·cm^{-2} or eggs·$bean^{-1}$) was consistently higher in the vertical wire-frames than in the horizontal wire-frames (Table 1). One replicate of the high density treatment had an exceptionally high number of eggs in the cross-sections. This replicate was omitted from analysis because we suspected that a contamination might have been involved. In addition, a regression analysis followed by reliability analysis showed that this replicate was not very reliable (both the studentized residual and Cook's distance for this replicate exceeded critical values). Note that the beetle densities that we aimed at with the inoculum beans in the different treatments were not always precisely obtained (Table 1).

Large variability occurred in spatial egg patterns in the cross-sections. Some cross-sections seemed to show clusters of eggs while others had the eggs distributed throughout the cross-section in a random fashion. On average, 50% of the eggs occurred within 7-10 cm from the average inoculum point s (Figure 7). This was true for both the horizontal and the vertical cross-sections. Hatched and non-hatched eggs did not differ significantly in their relative distribution functions (data not shown). Average egg densities $\hat{g}(h)$ as a function of distance from s varied between 0 and 0.1 eggs·$bean^{-1}$ for hatched eggs and between 0 and 0.05 eggs·$bean^{-1}$ for non-hatched eggs (Figure 8). These egg densities did not show a consistent pattern. There was no relationship between the average or median distance h from s at which eggs were laid, and the density treatment. There was also no consistent relationship between beetle density and vertical egg distribution (data not shown). The number of beans in the trapezium-shaped frame and in the semicircular frame was 1567 ± 171 and 531 ± 15, respectively (average ± SD). These figures were used to express egg densities as eggs per bean.

Discussion

When a natural enemy is evaluated for use in biological control, the first evaluation is mostly based on characteristics such as the functional response or the intrinsic rate of population increase r_m. These characteristics are often measured in experimental setups in which space, or the spatial distribution of hosts or prey, hardly play a part. The spatial distribution of pest insects may however have important consequences for the performance of the natural enemy. Its foraging

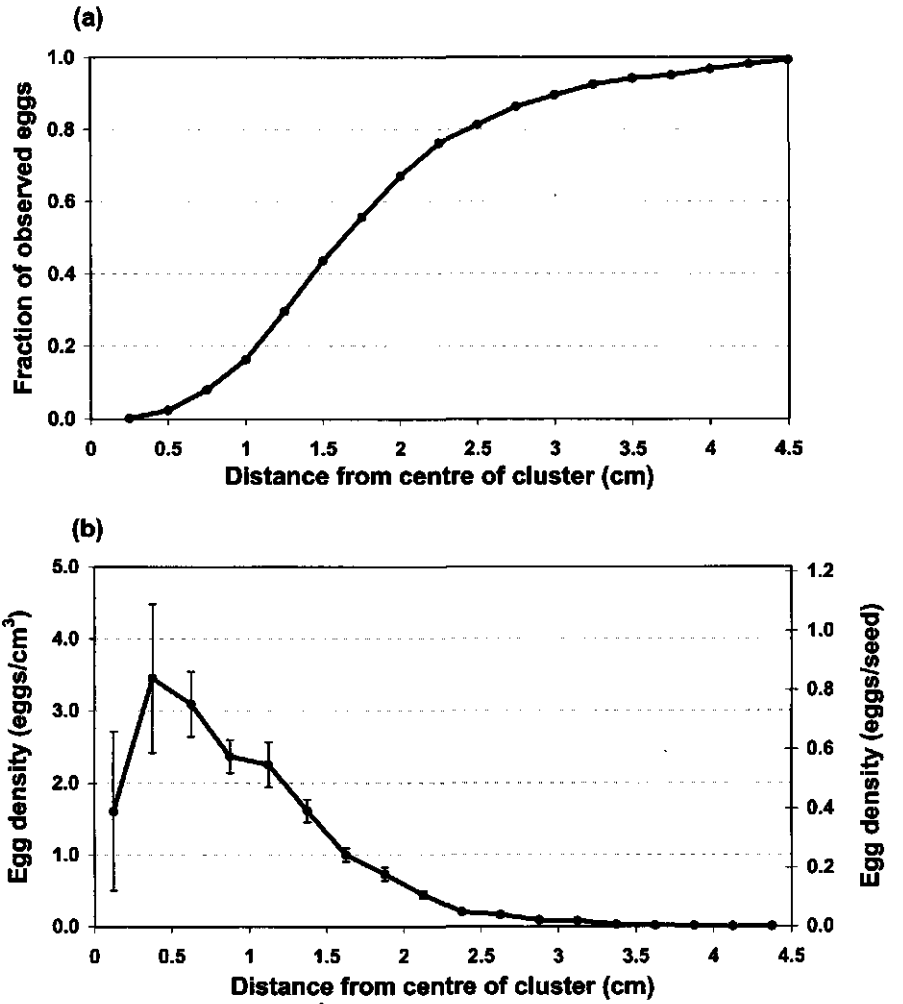

Figure 3. (a) The cumulative fraction $\hat{G}(h)$ of eggs and (b) the egg density $\hat{g}(h)$, both as a function of the distance h from the centre s of the egg cluster. The average for 19 beakers is shown; error bars indicate standard errors.

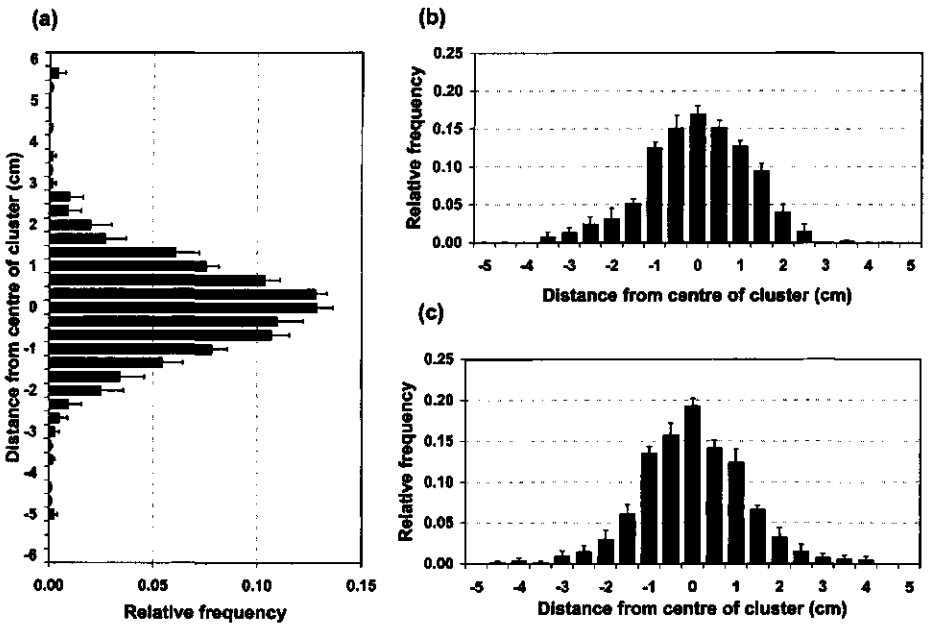

Figure 4. Frequency distribution of eggs relative to the centre s of the egg cluster (a) in the vertical direction, (b) in the horizontal direction x, and (c) in the horizontal direction y. The average over all 19 egg clusters is shown; error bars indicate standard deviation. Standard deviations were calculated from arcsin square root-transformed fractions and subsequently backtransformed.

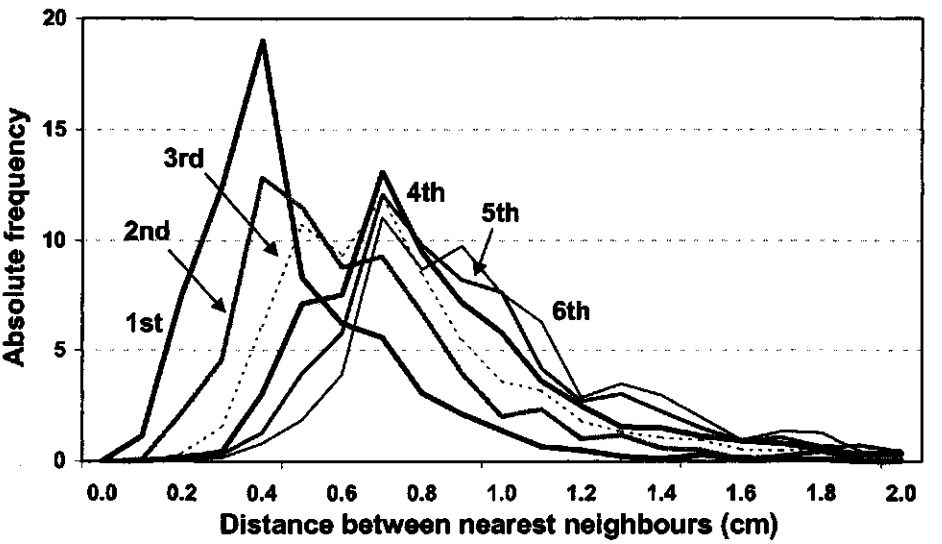

Figure 5. Distribution of the distances for each egg to its nearest neighbour and to subsequent neighbours, up to the sixth nearest neighbour. The average over all 19 egg clusters is shown.

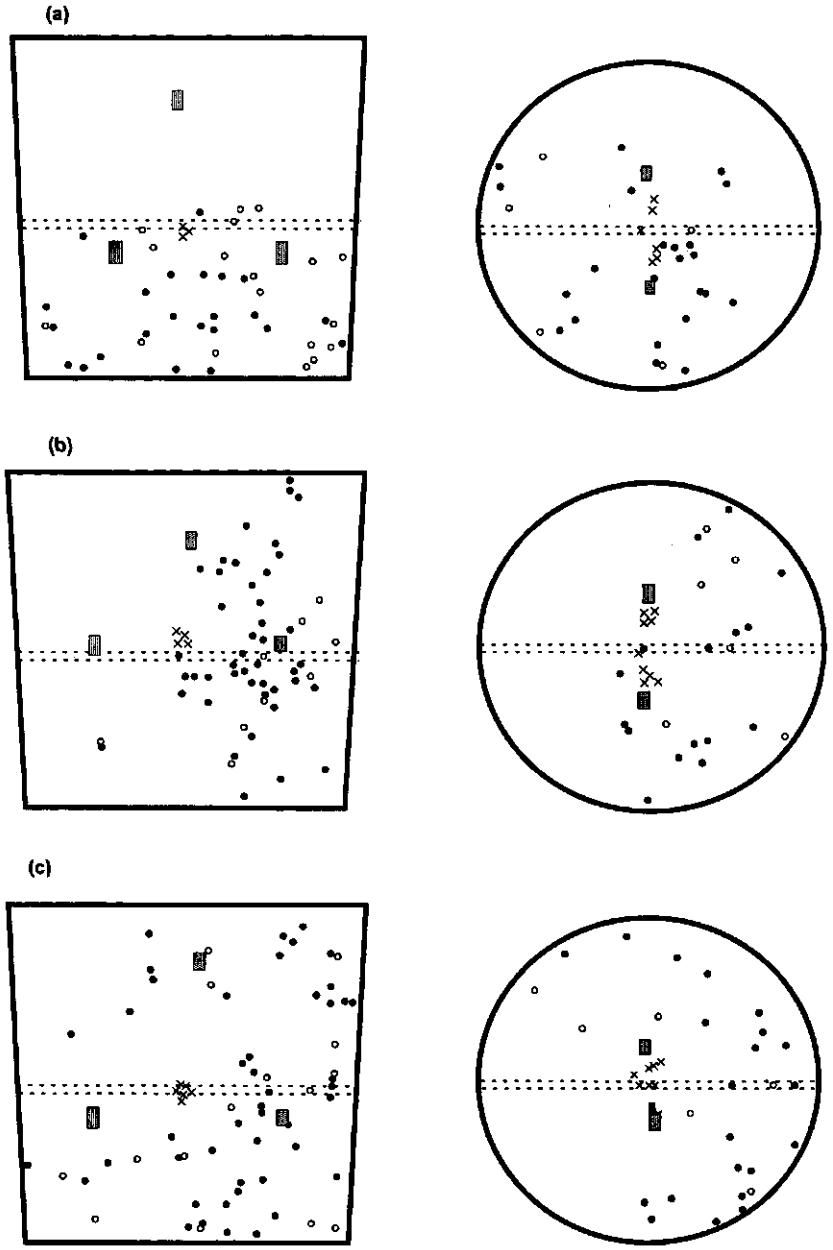

Figure 6. Graphical representations of the vertical (trapezium-shaped) and horizontal (circular) cross-sections taken from three containers in experiment 2. (**a**) Low density; (**b**) medium density; (**c**) high density. Filled circles (●) indicate hatched eggs, open circles (○) indicate non-hatched eggs, and crosses (×) indicate inoculum beans. Supporting blocks are shown with grey rectangles, and dashed lines indicate where the semicircular cross-sections and the trapezium-shaped cross-section came together (see Figure 1).

behaviour may be adapted to a certain type of distribution. This also applies to natural enemies of stored-product pests. This is the first detailed study into the spatial distribution of bruchid oviposition in stored product.

Many reports exist on the dispersal and spatial distribution of other stored-product insects, such as *Sitophilus granarius*, *Cryptolestes ferrugineus*, and *Tribolium castaneum* (Howe, 1951; Sharangapani & Pingale, 1955; Agrawal *et al.*, 1958; Surtees, 1965 and references therein; Prevett, 1964; Arbogast & Mullen, 1978, 1987).

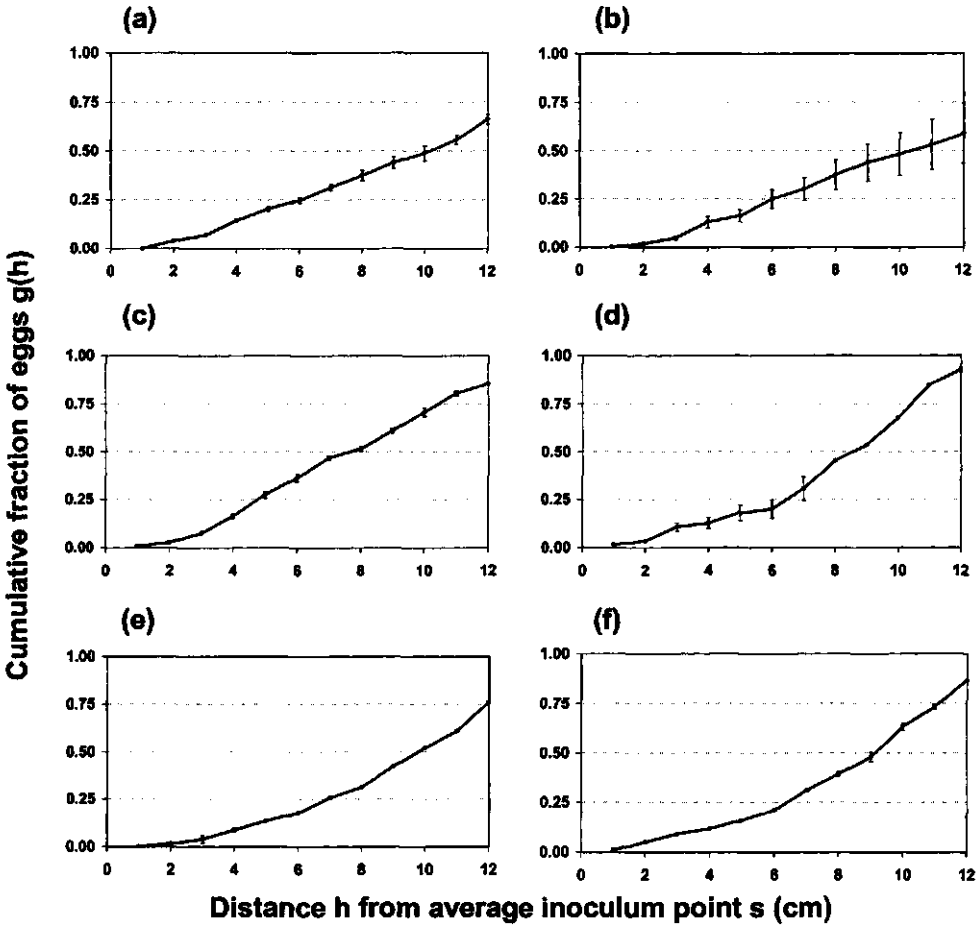

Figure 7. Cumulative probability functions $\hat{G}(h)$ of eggs as a function of distance h from the average inoculum point s for (a) and (b) low density, (c) and (d) medium density, (e) and (f) high density. (a), (c), and (d) show the data for the vertical cross-sections; (b), (d), and (f) show the data for the horizontal cross-sections. The outlier replicate in the high density treatment is omitted. Error bars indicate the standard error. Standard errors were calculated from arcsin square root-transformed fractions and subsequently backtransformed.

Although these reports are often contradictory, the following two trends can be extracted: (1) Beetles preferably penetrate deep into the grain, but after some time (e.g., two weeks) they move again upwards. Moving upwards is stimulated by unfavourable conditions and by disturbance, caused by frequent encounters between adult beetles. (2) Movement of stored product beetles is random, but

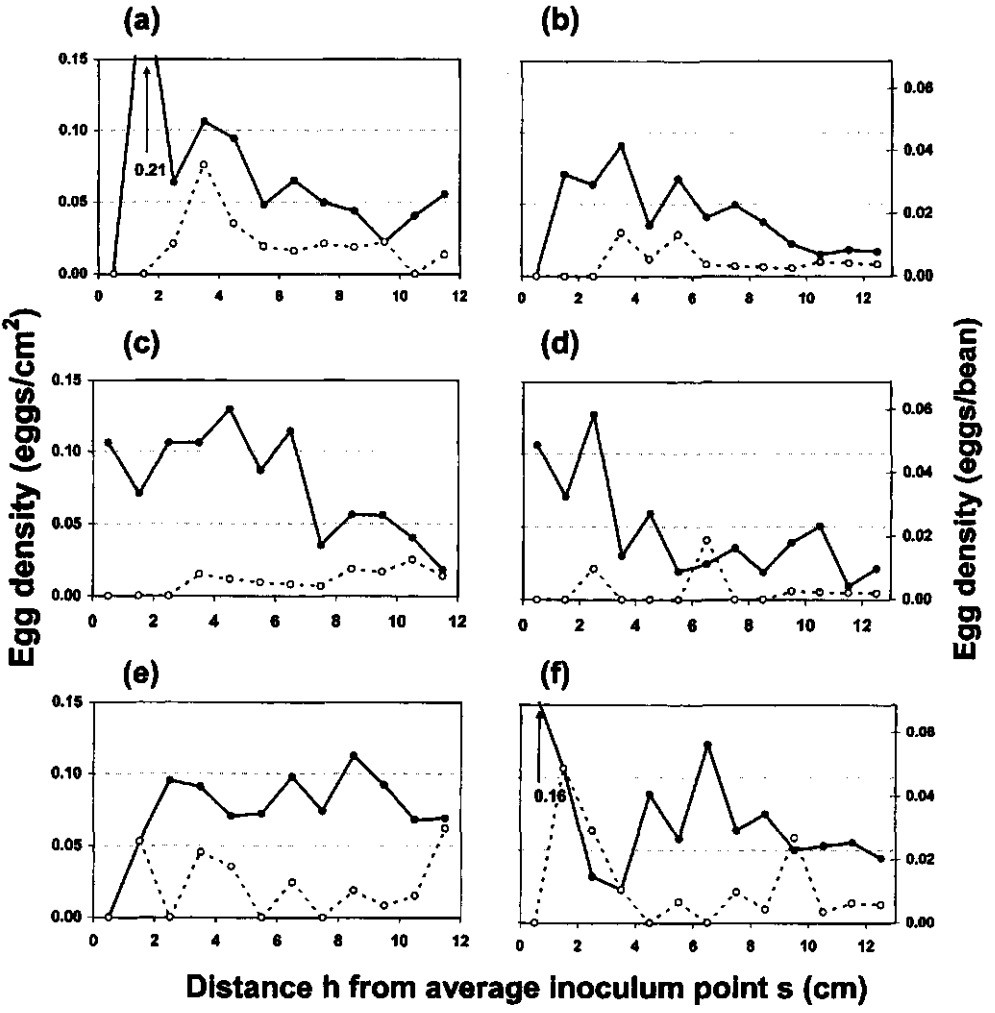

Figure 8. Egg density $\hat{g}(h)$, expressed as eggs·cm^{-2} and as eggs·bean^{-1}, as a function of distance h from the average inoculum point s for (**a**) and (**b**) low density, (**c**) and (**d**) medium density, (**e**) and (**f**) high density. (**a**), (**c**), and (**d**) show the data for the vertical cross-sections; (**b**), (**d**), and (**f**) show the data for the horizontal cross-sections. The egg density per bean is based on the fact that on average 2.2 beans occurred per cm^2 wire-frame. The outlier replicate in the high density treatment is omitted. •••••○•••••non-hatched eggs, ——●——hatched eggs.

reduced movement (or an increased turning rate) under certain environmental conditions leads to (a) accumulation of individuals and (b) less mutual disturbance, and therefore less dispersal to, e.g., the surface of the grain. In addition, some species tend to accumulate in specific places such as in corners or near the walls of grain bins (Smith, 1978; Hagstrum *et al.*, 1985; Arbogast *et al.*, 1998). Beetles that were investigated in these studies, however, have biologies that are different from that of *C. maculatus*. Most notably, adults of these beetles are all long-lived, and they feed on the stored product themselves, whereas the adult life of *C. maculatus* is limited to a few days, during which they do not feed on the beans. These results may therefore not be directly extrapolated to *C. maculatus*.

The results of our experiments indicate that individual *C. maculatus* females produce distinct clusters of eggs. Oviposition by females that in turn emerge from such clusters is not confined to a small area or volume in the bean mass. It is not clear whether these females also oviposit in clusters. It is possible that such three-dimensional clusters occurred in experiment 2, but that we did not recognize them as such because several clusters merged and/or because the cross-sections just grazed most of the clusters. We did not detect any effect of initial beetle density on the spatial egg distribution or egg pattern.

In the same experiment, it appeared that the egg density was consistently higher in the vertical wire-frames than in the horizontal wire-frames. This may indicate that dispersal of the beetles was predominantly in the vertical direction (both up- and downwards). The total numbers of eggs that are expected in the wire-frames if the beetles oviposited homogeneously throughout the container (both inside and outside the wire-frames) can be calculated based on the volume of the wire-frames relative to the volume of the containers, on the number of females in each container, and on their average fecundity (about 75 eggs per female at 30°C). These expected numbers of eggs appear to be not significantly different from the observed numbers of eggs ($P>0.05$, data not shown). This implies that beetles did not have a preference for oviposition inside or outside the wire-frames, suggesting that the wire-frames themselves did not constitute an important barrier for beetle movement.

It is possible that the natural dispersal of ovipositing beetles has been overestimated by our experimental setup in experiment 2. Beetles may have walked over the wire-frames, resulting in dispersal over larger distances than if they would have walked over beans only. Another possible source for overestimation is the fact that all beetles in each container in this experiment were siblings. If females would prefer to mate with non-siblings, then they may have searched for non-siblings and consequently dispersed over longer distances than if non-siblings would also have been present in the containers. Such behaviour might be selected for because it avoids inbreeding depression (Fellowes, 1998; Wilmsen Thornhill, 1993).

The data presented in this chapter give insight into the foraging environment which the egg parasitoid *U. lariophaga* faces when searching for *C. maculatus* eggs in stored cowpea. It is known that *U. lariophaga* shows area-restricted search after a parasitization (van Alebeek & Groot, 1997). This results in a higher searching efficiency in an environment in which host eggs are clustered or uniformly distributed than in an environment with randomly distributed eggs (van Alebeek *et al.*, 1996a). The results of experiment 1 show that *C. maculatus* eggs in stored cowpea indeed occur in clusters. On the other hand, on cowpea pods in the field, *C. maculatus* eggs occur in much lower densities and show a lower degree of clustering. For example, Huignard *et al.* (1985) found that, throughout the growing season, 11-48% of all pods in a cowpea field carried *C. maculatus* eggs. The average egg density on these pods with eggs was always about two eggs per pod. *Uscana lariophaga* occurs on eggs in the field as well, and this 'field' distribution of hosts may present a selective counter-force in this respect.

Nearest neighbour distances, expressed as the linear distance from one egg to the other, do not represent travelling distances for the parasitoid accurately. Parasitoids walk over the surface of beans, not through them. However, we can estimate the number of beans that *U. lariophaga* has to pass to get from one host egg to the next by combining nearest neighbour distances with bean sizes and the num-

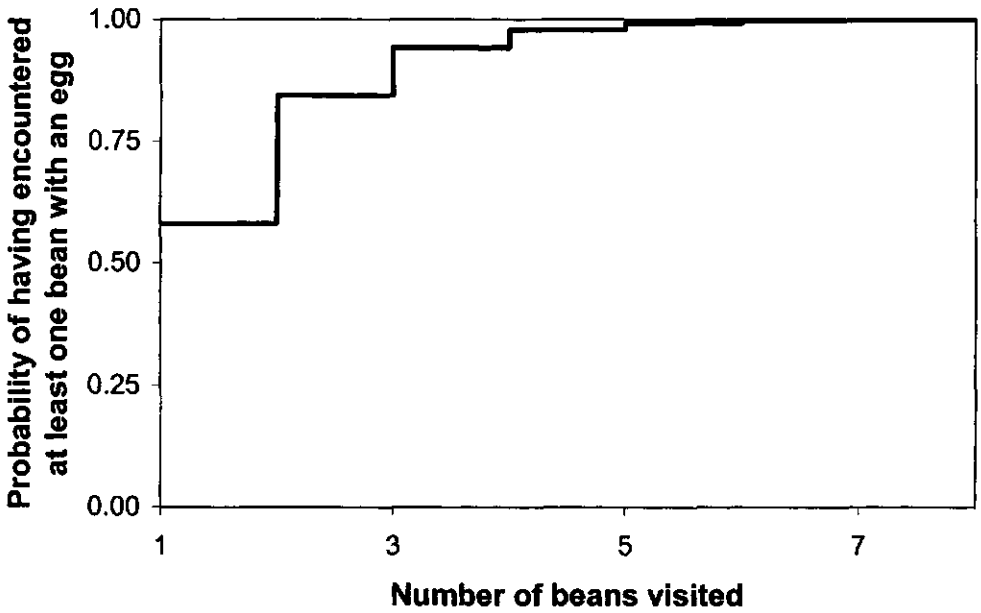

Figure 9. The estimated probability of having encountered at least one bean with an egg as a function of the number of visited beans, starting from a bean with an egg.

ber of neighbouring seeds. Figure 5 shows a peak in the abundance of nearest neighbour distances at 7 mm. This peak is probably not an artefact of the discretisation of the beakers into layers, since this peak also occurs if nearest neighbour distances are calculated for single layers of beans (data not shown). It coincides with the average distance (6.9 mm) of the center of a bean to the next. Based on Figure 5 and on the bean dimensions, we can assume that most eggs that occur within a radius of 3.5 mm around an egg are on the same bean. On the same basis, we can also assume that most eggs that occur within the surrounding shell (radius up to 10.5 mm) are on a neighbouring bean. We have re-analyzed the data under these assumptions to show that inside an egg cluster each bean with one or more eggs is surrounded by on average 5.5 beans which also carry eggs. Because a bean has on average 9.5 'neighbours', the probability of landing on a bean with one or more eggs is $\frac{5.5}{9.5} = 0.58$ if the parasitoid randomly moves to a new bean after a parasitization.

We also estimated this probability for a bean without eggs within a cluster. For this purpose, we randomly selected, within egg clusters, 49 locations that had no eggs in a 3.5 mm radius around it and we estimated the number of neighbouring beans with eggs for these 'empty beans'. We found that, within an egg cluster, a bean without eggs itself has 6.0 neighbouring beans with eggs. Thus, once a parasitoid is on an empty bean, the probability of landing on a bean with an egg is $\frac{6.0}{9.5} = 0.63$.

If a parasitoid, after having parasitized an egg on a bean, moves randomly from bean to bean, the probability to have encountered an egg after passing over n beans then equals

$$1 - \left[\begin{array}{c}\text{probability of not encountering}\\ \text{an egg on n subsequent beans}\end{array}\right]$$

$$= 1 - \left[\begin{array}{c}\text{probability of landing on empty}\\ \text{bean from a bean with an egg}\end{array}\right] \cdot \left[\begin{array}{c}\text{probability of landing on}\\ \text{another empty bean}\end{array}\right]^{(n-1)}$$

$$= 1 - (1 - 0.58)\cdot(1 - 0.63)^{(n-1)}$$
$$= 1 - 0.42\cdot(0.37)^{(n-1)}$$

This implies that, within an egg cluster, even after randomly visiting three beans, almost 95% of the parasitoids will have encountered already at least one bean with an egg (Figure 9). This result emphasizes the effectiveness of area-restricted search after a parasitization for this parasitoid.

Acknowledgements

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CHAPTER 3

C. Stolk, W. van der Werf and A. van Huis

Host searching behaviour of *Uscana lariophaga* in stored cowpea

Abstract

We describe the foraging behaviour of the trichogrammatid egg parasitoid *Uscana lariophaga*, a natural enemy of the stored-product pest *Callosobruchus maculatus*, in artificial arenas with a single layer of cowpea seeds. Search trajectories were recorded at a spatial resolution of single beans, while behavioural components were recorded at a temporal scale of seconds. The most important factor influencing the behaviour of *U. lariophaga* was an encounter with a host egg: this changed the walking trajectory from 'straight' to 'tortuous' and it increased the residence time per bean. *U. lariophaga* seemed attracted to host eggs from a distance of about 4-6 beans, and it showed a preference to move onto beans with an egg. Once it was on a bean with an egg, however, it often failed to find the egg during one visit.

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Introduction

Insect pests of stored food products are responsible for considerable damage. In addition to quantitative losses, there is often loss of quality of the stored product due to secondary infestations with fungi and contamination with insect debris (Boxall, 1991). Stored products represent a high economic value compared to crops in the field, because of the amount of energy that has already been invested into a crop once it has been harvested and stored. Therefore, losses in storage result in relatively high economic damage. Usually, stored-product pests are controlled chemically. Safety concerns in industrialized countries and limited availability of appropriate pesticides in developing countries have, however, caused an increased attention for alternative control methods, such as biological control (Hodges, 1999; Cox & Wilkin, 1998; Schöller *et al.*, 1997).

An example of a storage system in which biological control is an option is that of stored cowpea (*Vigna unguiculata* Walp.), which is often infested with *Callosobruchus maculatus* F. (Col.: Bruchidae) (Jackai & Daoust, 1986). *C. maculatus* oviposits on legume seeds and pods; hatched larvae penetrate into the seed and complete their development inside the seed (Singh *et al.*, 1990). *C. maculatus* is in particular an important pest of traditionally stored cowpea in West Africa (Van Alebeek, 1996b). One of the natural enemies of *C. maculatus*, *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae), parasitizes the eggs of *C. maculatus* and has been suggested as a biological control agent for this system (Van Huis *et al.*, 1991a). In experimental storage containers this parasitoid is capable of reducing *C. maculatus* numbers by 86% over a 3-month period (Van Huis *et al.*, 1998). Previous research has focused on the functional response of *U. lariophaga* in host clusters (Van Alebeek & Van Huis, 1997; Van Alebeek *et al.*, 1996a,b). *U. lariophaga* has been shown to be attracted to odours that are associated with host eggs (Van Huis *et al.*, 1994b; Ormel *et al.*, 1995).

The role of space in this three-dimensional pest - natural enemy system has as yet received little attention, whereas spatial processes may have a profound impact on biological control. In particular, it has been shown that *C. maculatus* oviposits in clusters (Chapter 2), and *U. lariophaga* obviously needs to find these clusters before it can parasitize the eggs. Foraging, in other words, takes place in a spatial environment. Since it is impossible to observe the searching behaviour of these 0.5 mm small wasps inside a three-dimensional, dark bean stock, we observed the foraging behaviour of *U. lariophaga* in a two-dimensional setting.

In this chapter we describe and quantify the foraging behaviour of *U. lariophaga* females in a single layer of cowpea seeds. The aim of this research is to obtain behavioural parameters which can be used to simulate the behaviour in three

dimensions. We explore various factors which might influence these behavioural parameters. The most important of these factors are (1) the presence and size of a host patch, (2) the presence of walking traces of *C. maculatus* and (3) an encounter with a host. We also investigate whether *U. lariophaga* shows directed search for a host patch or whether it searches randomly and is only arrested by contact with hosts. We define the behavioural parameters in terms of single beans (we study, for example, the residence time of wasps per bean). Reasons for doing so are: (i) beans are a natural unit for this system, (ii) previous observations have indicated that *Uscana* often walks around a bean several times before moving to a next bean, and (iii) the resulting behavioural parameters can easily be used in a spatial simulation model.

Materials and Methods

Observations

The behaviour and the walking path of individual *Uscana lariophaga* females was observed in arenas consisting of a petri dish (diameter 13.6 cm) filled with one tightly packed layer of cowpea seeds. The petri dish was placed on a sheet of glass that was fixed at 17 cm above a table. A magnifying mirror, placed underneath the glass sheet, enabled us to monitor the wasp continuously on both sides of the seeds. The wasp was released by opening a gel capsule at the center of the petri dish. The behaviour was recorded using a hand-held computer (Psion Workabout, Psion PLC, London, UK) equipped with the computer program The Observer for Windows 5.0 (Noldus Information Technology, Wageningen, The Netherlands). The behavioural elements and the types of locations that were distinguished are shown in Tables 1 and 2. The position of the wasp during the observation was recorded manually on a photographic map of the arena (scale 2:1). A pair of coordinates (measured in mm) was assigned to the center of gravity of each seed that the wasp had walked on to facilitate analysis of the walking track. The criteria that were used for ending an observation are shown in Table 3.

The arenas that were used each belonged to one of four treatments: (1) arenas with a small host patch (altogether 3-6 eggs on 2-4 beans); (2) arenas with a large host patch (18-22 eggs on 7-10 beans); (3) arenas with a small host patch plus 'walking traces' of female *Callosobruchus maculatus* beetles; (4) empty arenas, *i.e.* no host eggs or beetle traces present. *Callosobruchus maculatus* eggs were 0-20 h old. The center of each host patch was located at a distance of about 5 cm from the release point. The position of the host patch with respect to the release point varied among replicates. Arenas in treatment 3 had a trail of beans over which *C. maculatus*

Table 1. Behavioural elements that were distinguished in the observations of *U. lariophaga* females.

name	description
stand	not moving (sometimes interrupted with short moments of walking of up to about 2 seconds)
fly	flying way or jumping
walk	walking (sometimes with short stops of up to about 2 seconds)
enc	encounter with unparasitized host egg
par	parasitization
post	post-oviposition behaviour (grooming and walking on the host)
enc2	encounter with already parasitized host egg
lost	the wasp is out of sight of the observer

Table 2. Different types of locations that were distinguished in the observations of *U. lariophaga* females. Coordinates were measured for individual beans (*bean, egg,* and *trace*) and for the gel capsule (*gcap*).

name	description
gcap	gel capsule (release site at the center of the petridish)
bean	uninfested bean
egg	bean with one or more eggs
trace	bean on which a female beetle has walked
petri	petridish bottom or wall
lid	petridish lid

Table 3. The criteria that were used for ending an observation on an *U. lariophaga* female.

criterion	action
wasp stays inside gel capsule for 5 min	wasp is removed; a new wasp is released in the same arena
wasp does not move (behavioural element *stand*) for 30 min	wasp is removed; if the wasp has not walked on more than 8 beans a new wasp may be released (with a maximum of 3 releases per arena)
wasp walks ca. 4 cm (absolute distance) on petridish	wasp is removed; if the wasp has not walked on more than 8 beans a new wasp may be released (with a maximum of 3 releases per arena)
wasp flies away and does not return on seed layer with about 20 s	wasp is removed; if the wasp has not walked on more than 8 beans a new wasp may be released (with a maximum of 3 releases per arena)
after 1.5 hour of observation	wasp is removed; the arena is not used again

females had walked, leading from the release point to the host patch. These beans with 'walking traces' of *C. maculatus* were obtained just prior to the experiment by allowing individual *C. maculatus* females to walk over beans. The moment these females would start to oviposit they were transferred to another petridish with beans. For each treatment, 7-11 replicate trials were made (Table 4).

The experiment was carried out in a climate room at 30±1°C and 40±2% RH. High frequency fluorescent lighting provided light at an intensity of $3.0 \cdot 10^3$ lux at

the level of the arena. The lids of the petri dishes that were used for the arenas had notches, which allowed for some air circulation; the air in the climate cell flowed at a constant speed of 0.2 $m \cdot s^{-1}$. The wasps that were used were 2-23 h old females that been allowed to mate during at least one hour. They were reared at 30±1°C and at 12L:12D. *C. maculatus* was reared on cowpea seeds at a temperature of 35±1°C during photophase and 25±1°C during scotophase (12L:12D). *C. maculatus* females that were used were 0-2 d old. The cowpea seeds that were used in the rearing and in the experiment were of the variety 'Black Eye'. Before use, they were frozen at -18°C and subsequently dried at 45°C, both for at least three days. This was done to exclude any possible contamination by insects. The seeds that were used in the experiment had not been used before in any experiment.

Data processing

The total amount of time that wasps spent on beans (t_{beans}), and the time they were active (t_{active}), was calculated and expressed as a percentage of the total observation time per treatment. All behaviours except 'not moving' (*stand*) were considered active. For the wasps that found the host patch, we distinguished t_{beans} and t_{active} before and after the first encounter with a host. The amount of time that the wasps were out of sight of the observer (*lost*) was also calculated and expressed as a percentage of the total observation time.

We calculated the residence time for each visit of *U. lariophaga* to a bean. Residence times were calculated in terms of total residence time, the time spent walking (behavioural element *walk*) during that visit, the time spent standing (*stand*), the time spent walking and standing, and the time walking, standing and *lost* during that visit. These residence times were calculated for the different categories of beans (beans with eggs, beans with *C. maculatus* walking traces, and uninfested beans). For the beans with eggs, we also distinguished residence times according to whether or not the wasp had encountered an egg on that bean during that visit.

Based on the spatial coordinates of the beans that the wasps had walked upon, and using standard goniometric rules, we calculated the angular changes (in the range of -180° to +180°) in the walking trajectories of the wasps. A clockwise turning was defined negative and an anticlockwise turning was defined positive. U-turns were defined as +180°.

For each crossing from one bean to another we also calculated the cosine of the angle α between the actual crossing and a straight line from the previous bean to the host patch (Figure 1). This $\cos(\alpha)$ ranges from -1 to +1 and is a measure of the 'directedness' of the wasps towards the host patch for each crossing: it is +1 if the wasp moves straight towards the host patch and -1 if it moves in the opposite direc-

tion. The directedness parameter was plotted as a function of the distance from the center of the host patch, until the first encounter with a host.

The number of visits per bean was calculated separately for beans with and without eggs. We distinguished the number of visits per uninfested bean before and after a wasp first encountered a host. We calculated the straightness of the walking trajectories of the wasps that reached the host patch by dividing the actual walking path (in terms of number of beans and number of visits to beans) by the shortest possible route (in the same terms) to the host patch.

For each wasp that reached the host patch, we calculated the observed probability of moving to a bean with an egg ($p_{observed}$):

$$p_{observed} = \frac{\sum_{i=1}^{k}\left(\frac{m_{egg,i}}{m_{total,i}} \cdot m_{total,i}\right)}{\sum_{i=1}^{k}\left(m_{total,i}\right)} = \frac{\sum_{i=1}^{k}\left(m_{egg,i}\right)}{\sum_{i=1}^{k}\left(m_{total,i}\right)} \quad (1)$$

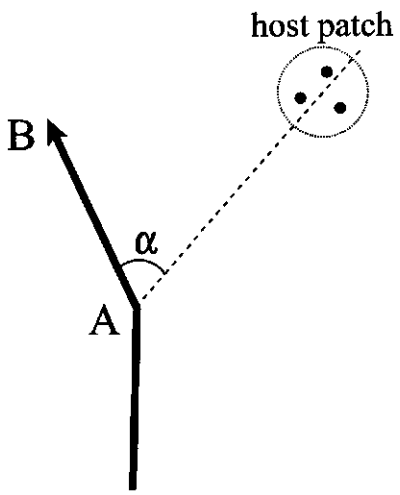

Figure 1. The 'directedness' of the wasp towards the host patch is defined as follows: If α denotes the angle between the walking path of the wasp from A to B and the line from A to the center of the host patch, then cos(α) is a measure of the directedness of the wasp towards the host patch. (The center of the host patch is calculated as the arithmetic mean of the respective x- and y-coordinates of the host eggs). Cos(α) equals 1 if the wasp moves straight towards the host patch and -1 if it moves in the opposite direction.

with

$m_{egg,i}$ = the number of times the wasp moved from bean i to a bean with an egg,
$m_{total,i}$ = the total number of visits to bean i, and
k = the total number of beans in and around the host cluster (beans that were in touch with a bean with an egg).

If, for example, a wasp had visited a certain bean nine times, and of those nine times it subsequently moved to a neighboring bean with an egg six times, then the observed probability of moving to a bean with an egg for that particular bean was $6/9 = 0.67$. Equation 1 weighs these probabilities for the number of times a bean is visited by a wasp.

We also calculated, for each wasp, the expected probability of accidentally moving to a bean with an egg if the wasps would randomly choose one of the neighboring beans to move to (p_{random}):

$$p_{random} = \frac{\sum_{i=1}^{k}\left(\frac{n_{egg,i}}{n_{total,i}} \cdot m_{total,i}\right)}{\sum_{i=1}^{k}(m_{total,i})} \qquad (2)$$

with

$n_{egg,i}$ = the number of neighbouring beans with an egg for bean i, and
$n_{total,i}$ = the total number of neighbours for bean i.

If, for example, a particular bean was surrounded by six other beans of which two carried an egg, then, if the wasp would be moving around randomly, the probability of accidentally landing on a bean with an egg would be $2/6 \approx 0.33$.

The probability that k eggs were parasitized in one visit to a bean with n unparasitized eggs, and the acceptance ratio (number of parasitizations divided by the number of host encounters) were calculated based on all observations combined.

Statistical analysis

The effect of the treatment on the probability of finding the host patch was tested using a Chi-square test. The effect of the treatment on overall indicators of behaviour, such as total observation time, percentage of time spent on beans, and percentage of time active, was tested using a Kruskal-Wallis test. For the wasps that found the host patch, we compared several behavioural parameters before and after the first encounter with a host. For each wasp we calculated the mean parameter values before and after the first host encounter (for residence times we used

the harmonic instead of arithmetic mean to reduce sensitivity to outliers). The means before and after host encounter were treated as paired observations and were analysed using the Wilcoxon signed ranks test with individual wasps as experimental units. Similarly, the Wilcoxon signed ranks test was used to compare the residence time on beans without eggs with the residence time on beans with eggs, to compare the number of left-turns with the number of right-turns, to compare $p_{observed}$ to p_{random}, to compare the number of visits per bean for uninfested and infested beans, and to compare the number and frequency of visits to the same bean before and after the first host encounter. Using the same approach, we compared the values of the directedness parameter for distances to the center of the host patch ≤ 4 cm, versus distances > 4 cm. We excluded wasps which had fewer than five values of the directedness parameter at ≤ 4 cm or > 4 cm from the patch, and we used only values of the directedness parameter before a host was encountered.

In order to test whether residence times were a function of time, of distance to the host patch, or of residence time on the previous two beans, we plotted residence times as a function of each of these factors. Similarly, we tested whether angular changes were a function of time, of the number of visits to beans, of residence time on the previous bean, of the distance to the center of the host patch, or of the previous two turning angles.

The actual route that wasps took towards the host patch and the shortest possible route, both in terms of number of beans visited and in terms of number of visits to beans, were compared using the Wilcoxon signed ranks test. The distribution of turning angles was compared before and after the first encounter with a bean with an egg using the Kolmogorov-Smirnov test. The Kolmogorov-Smirnov test was also used to compare the distribution of turning angles to a uniform distribution (for this purpose, we allocated half of the +180° angles to -180°).

Table 4. Summarized results of the observations on individual *Uscana lariophaga* females in an arena with cowpea seeds, specified for the four treatments. Small h.p. = arena with a small host patch; large h.p. = arena with a large host patch; *C.m.* traces = arena with a small host patch plus walking traces of female *Callosobruchus maculatus*; empty = empty arena. Differences were not significant.

Treatment	Number of releases	Number of wasps that reached the host patch	Duration of an observation (min) for wasps that walked on more than 8 beans (mean ± SE)	Time spent on beans for all wasps (% of total observation time)	Time active (not standing still) for all wasps (% of total observation time)
Small h.p.	8	2	74 ± 15	74.5	46.1
Large h.p.	11	2	73 ± 17	78.8	59.3
C.m. traces	10	3	76 ± 14	71.0	55.2
Empty	7	–	72 ± 6	73.3	48.2

Wasps which had visited fewer than nine beans were excluded from the analyses of residence times, turning angles and directedness parameters. All tests were evaluated at a significance level of 0.05.

Results

General

No effect of the different treatments on the overall indicators of searching behaviour of the wasps was found (*e.g.* total observation duration, percentage of time active, percentage of time on beans; $p>0.5$; Table 4). The presence of beans with beetle traces and the size of the host patch did not influence the host finding ($p=0.82$). We did find, however, significant differences in behaviour before and after an encounter with a host (see below).

On average, the wasps spent about 75% of the observed time on the beans and they were active during about 50% of the observed time (Table 4). Activity and the amount of time spent on beans both increased significantly after the first encounter with a host ($p=0.018$ and $p=0.043$, respectively). Before the first encounter, the wasps were active during 43% of the time and they spent 76% of the time on beans; after an encounter they were active during 97% of the time and on the beans during 99% of the time. The average time until the first encounter with a host was 38 minutes, after which the wasps were observed for another 53 minutes on average (data from all treatments pooled). The wasps were out of sight of the observer (*lost*) for less than 0.1% of the time.

Residence times per bean

More than 50% of the visits to a bean lasted less than 20 s (Figure 2a). Wasps stayed longer on beans with eggs than on beans without eggs, even if the time spent on host encounters, parasitizations and post-oviposition behaviour is subtracted from the total time on a bean (Figure 2b; $p=0.028$). The residence time on beans with host eggs is especially longer on beans on which the host is encountered (Figure 2c; $p=0.018$). The time wasps spent on these beans until the host encounter is shorter than the total residence time on beans on which the host was not encountered (7.9 versus 15.5 s harmonic mean residence time). Once a host had been encountered, residence times on uninfested beans also increased (Figure 2d; $p=0.028$). Figures 2a and -b are based on all datasets from all treatments whereas Figures 2c and -d are based on the datasets in which the wasp found the host patch. Residence times were not analysed for the beans with beetle traces because the wasps almost never visited these beans. There was no effect of time since start

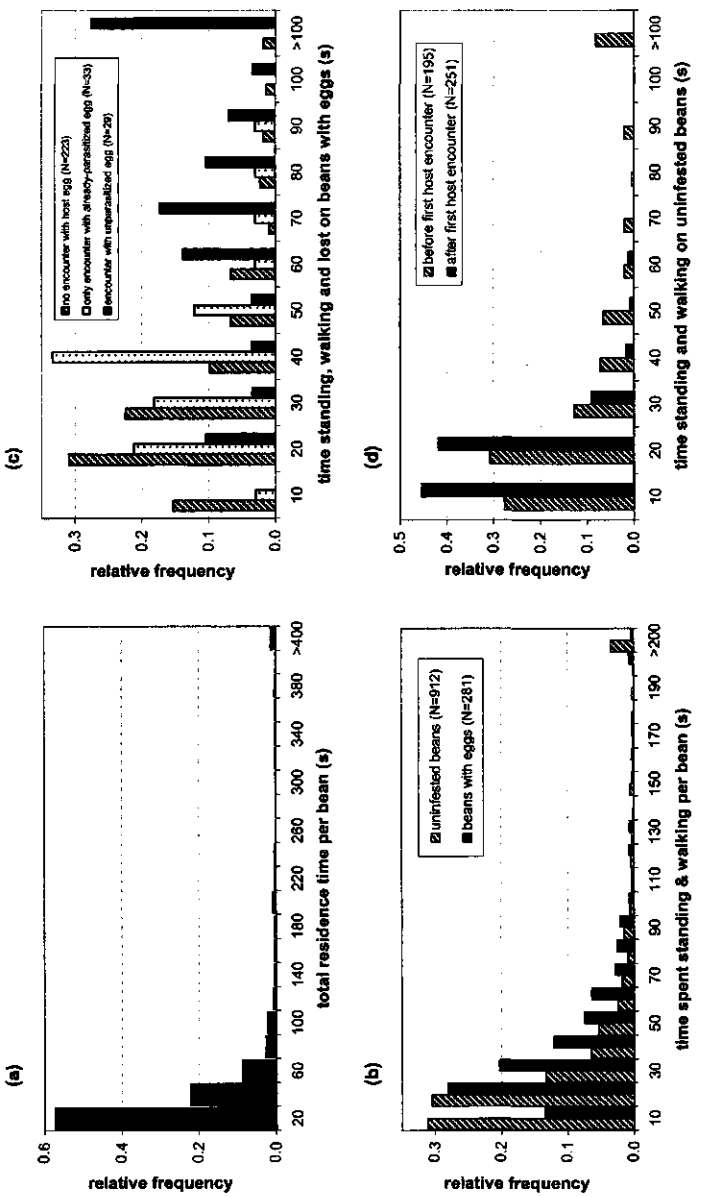

Figure 2. Relative frequency distributions of the residence times (s) per bean: **(a)** total residence time on all beans (N=1185); **(b)** time spent standing and walking on uninfested beans versus beans carrying host eggs; **(c)** total residence time (minus the time spent on host encounters, parasitizations and post-parasitization behaviour) on beans with eggs for three cases: (i) the host egg is not encountered on this bean during this visit, (ii) there is only an encounter with an already parasitized host egg, and (iii) there is an encounter with an unparasitized host egg during this visit; **(d)** time spent standing and walking on uninfested beans before and after the first encounter with a host egg.

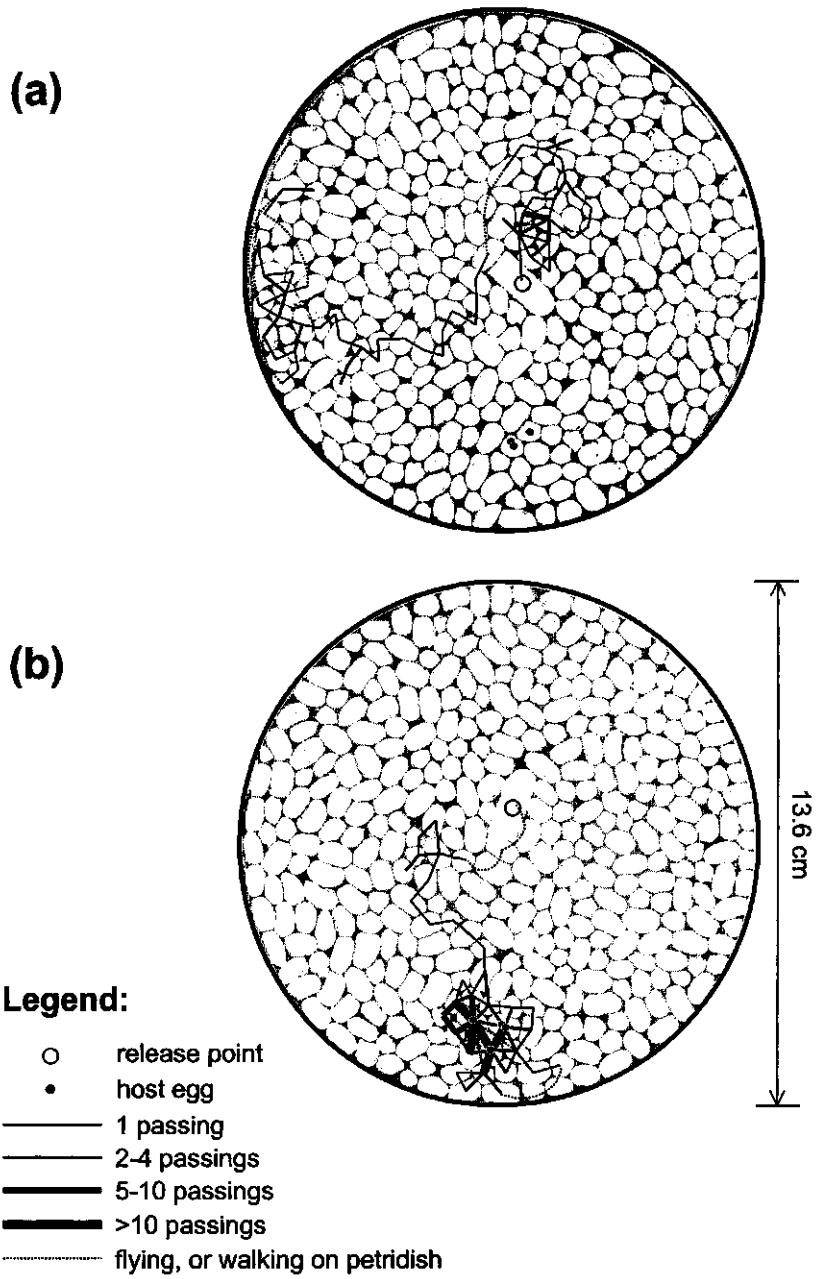

Figure 3. Walking tracks of two individual *Uscana lariophaga* females in an arena consisting of a petri dish filled with a single layer of cowpea seeds. Both examples belong to the treatment 'small host patch' and the observation time is in both cases 90 min. **(a)** host patch not found; **(b)** host patch found.

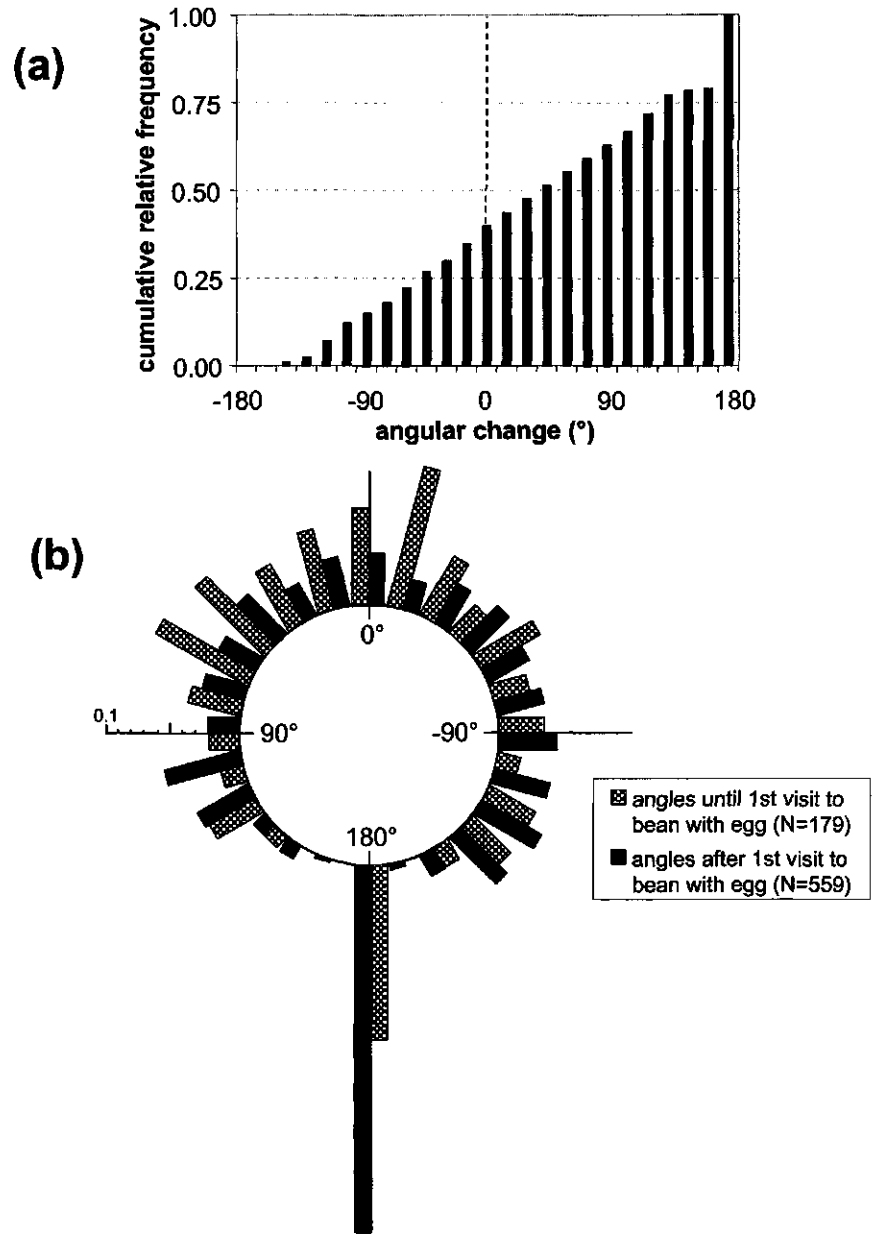

Figure 4. **(a)** Cumulative frequency distribution of angular changes (°) during transitions from one bean to another, based on 1090 transitions in all datasets in which the wasp walked on more than 8 beans. **(b)** Relative frequency distributions of angular changes (°) during transitions from one bean to another before and after the first visit to a bean with an egg. This Figure is based on the datasets in which the wasp found the host patch (see Table 4).

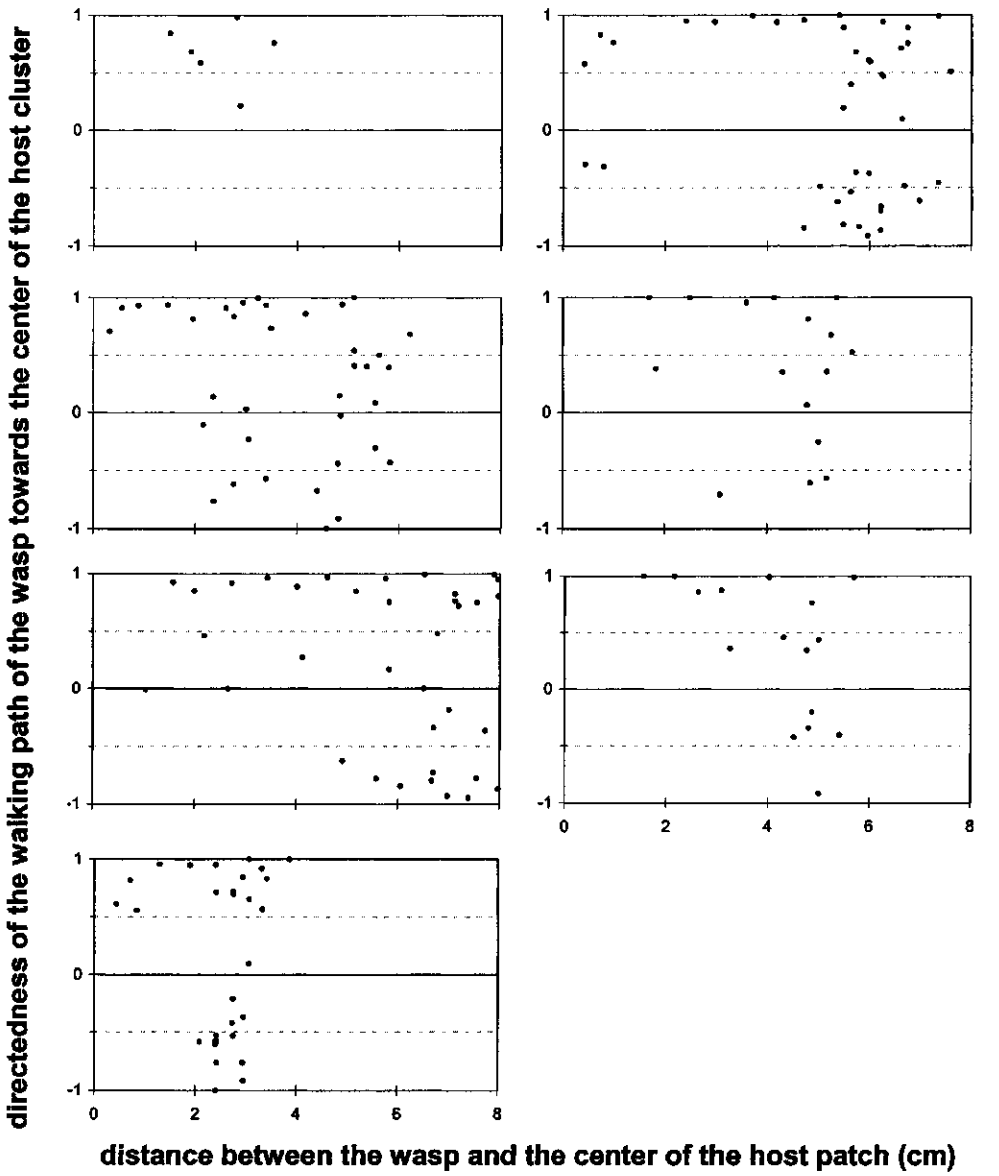

Figure 5. The 'directedness' of the wasp towards the center of the host patch, expressed on a scale of -1 (moving away from the host patch) to +1 (moving towards the host patch), plotted as a function of the distance from the host patch for the seven wasps which found the host patch. Data are shown up to the moment the first host is encountered. See Figure 1 for an explanation of how the measure of directedness is calculated.

of the observation, of distance to the host patch, and of the residence time on the preceding two beans on the residence time per bean.

Walking trajectories

Two examples of walking trajectories of *U. lariophaga* females are shown in Figure 3. Turning angles in the walking trajectories follow a near-uniform distribution (Figure 4a). The distribution is, however, significantly different from uniform, even when half of the +180° angles is assigned to -180° ($p<0.01$). Especially absolute angles of 180° are over-represented at the cost of absolute angles between 150 and 180°. This is probably due to the discretization of the available space into beans, which makes it virtually impossible to turn at angles of 150-179°, given the dimensions of the cowpea seeds. If we compensate for this by replacing angles of 180° with randomly generated angles in the range of 150-180° (and similarly so for -180°), the distribution of angles does not differ any more from a uniform distribution ($p=0.26$). There was no preference for clockwise or anticlockwise turnings ($p=0.73$). The angular distributions before and after the first encounter with a bean with an egg are significantly different from each other (Figure 4b; $p<0.01$). After the first encounter, angles of 180° occur more frequently than before the first encounter; this seems to be at the cost of angles around 0°. There was no effect of time since start of observation, of the number of visits to beans, of residence time

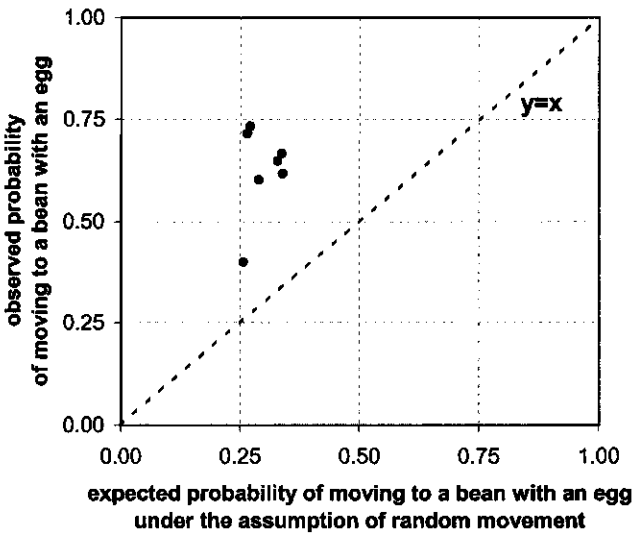

Figure 6. The observed probability of moving to a bean with an egg versus the expected probability of moving to a bean with an egg under the assumption of random movement of the wasp. Weighted averages are shown for the seven wasps that found the host patch. Each data point is based on 15-156 transitions from one bean to another.

Table 5. Probability of parasitization of k eggs during one visit to a bean with n eggs. Averages of observations on six wasps that reached the host patch are shown.

Number of eggs parasitized during this visit (k)	Number of unparasitized eggs on this bean (n)		
	1	2	3
0	0.67	0.58	0.71
1	0.33	0.25	0.00
2	–	0.17	0.14
3	–	–	0.14
N (# observations):	42	12	7

on the previous bean, of distance to the host patch, and of the previous two turning angles on the current turning angle.

The route that wasps took towards the host patch was 3.1 ± 1.5 (SD) times longer than the shortest possible route in terms of the number of beans (p=0.010). In terms of the number of visits to beans, the walking trajectory was 4.3 ± 2.6 (SD) times longer than the shortest possible route to the host patch (p=0.016).

There is a trend that wasps became more directed towards the host patch as they came closer to the host patch (Fig. 5): high values of the directedness parameter are relatively more common at smaller distances between the wasp and the center of the host patch. The directedness of these wasps towards the host patch especially seemed to be increased within a distance of 2-4 cm from the center of the host patch; this corresponds to about 4-6 beans between the wasp and the edge of the host patch. Values of the directedness parameter, averaged per wasp for all distances $\leq$ 4 cm, were indeed higher than the averages for distances $>$ 4 cm (p=0.046).

We often observed *U. lariophaga* walking in short hops in circles on top of a bean, stopping frequently, and thereby moving the antennae. This behaviour could last several minutes. Eventually the wasp would choose a neighboring bean and pursue its course, but it also happened sometimes that it later came back to that same bean and displayed the same type of behaviour again, and then chose another bean to move to. We have called this set of behaviours 'orientation behaviour'. This behaviour was only observed before a host was encountered in an experiment.

Behaviour in and around the host patch

Wasps moved on average 2.1 times more often onto a bean with an egg than they would have done if they had randomly chosen new beans to move to (Figure 6; p=0.018). In conjunction with this, it was often observed that a wasp would run across the surface of a bean, stopping briefly at all points where a neighbouring bean

touched the bean it was standing on, and touching these neighbouring seeds with its antennae. If one of these seeds carried eggs, the wasp would often move immediately to this bean. As a result, beans with eggs were visited much more often than uninfested beans (12 vs 2 visits per bean, p=0.018). After the first host encounter, the walking trajectory became limited to a relatively small number of beans. This appears from the fact that uninfested beans received more visits per bean after the first host encounter than before the host had been encountered (1.4 vs 2.7 visits per bean, p=0.018). The number of visits per bean per hour, however, did not differ before and after the first encounter with a host (p=0.61), probably due to the increased amount of time spent per visit.

Once a wasp landed on a bean with an unparasitized egg, it often did not find the host egg (Table 5). It was often observed that a female missed the egg by about one mm. But once an unparasitized, healthy egg was encountered, it was always accepted for parasitization (N=41). Only one unparasitized egg was consistently rejected, but this egg later appeared to have been damaged during handling.

Discussion

Host patch searching behaviour

The analysis of walking trajectories and our direct observations of orientation behaviour both suggest that *U. lariophaga* shows directed search towards hosts. This confirms the findings of Ormel *et al.* (1995) and Van Huis *et al.* (1994b) who have shown that *U. lariophaga* females are attracted to odours related to *C. maculatus* eggs from a distance of 5-7 cm. They measured attraction in a petri dish and in a glass tube, both without a bean layer. In the current setup, which mimicks a storage situation more closely, the effect of directed search could be measured from a distance of about five beans from the host patch. The orientation behaviour was, however, often observed at longer distances from the host patch, when walking trajectories still seemed to be random, and it was also observed in arenas without a host patch. We therefore think that in the current setup, *U. lariophaga* searching behaviour may to some degree have been triggered by host-related odours; but odours became a reliable 'road sign' only from a short distance from the host patch. In other words, *U. lariophaga* seemed to walk towards the host patch in a directed way once predominantly random searching behaviour had brought it within a relatively short range from the host patch. The odour source that attracts *U. lariophaga* might be a marker pheromone left by the ovipositing *C. maculatus* female to deter conspecific females (Ormel *et al.*, 1995; Credland & Wright, 1990).

The number of eggs in the host patch did not influence the probability of find-

Table 6. Observed differences in the behaviour of the *Uscana lariophaga* females before and after the first encounter with a host or a host patch.

Behaviour before first encounter with host (or bean with host egg)	**Behaviour after first encounter with host (or bean with host egg)**
(long) periods of standing still	wasp is almost continuously moving
only 76% of the time spent on beans	almost 100% of the time spent on beans
'orientation behaviour'	no 'orientation behaviour' observed
short residence time on beans	residence time on beans is increased (especially so on beans with an egg, and even more if the egg is encountered)
walking path is often relatively straight;	walking path becomes limited to a few, mainly infested
each beans are not often revisited	beans which are visited very often
turning angles around 0° are slightly more common	turning angles of 180° become more abundant

ing the host patch. Although Van Alebeek & Van Huis (1997) found that host finding probability was influenced by the number of eggs in a host patch, in another experiment Van Alebeek *et al.* (1996b) did not find this relationship. It seems likely therefore that the influence of host patch size on host finding probability depends on the details of the experimental setup, especially those which influence the odour diffusion process.

Beans on which *C. maculatus* females had walked did not help *U. lariophaga* in finding the host patch. In previous experiments, *U. lariophaga* reacted to odours left by *C. maculatus* females over a period of 24 h in a diffusion olfactometer (Van Huis *et al.*, 1994b). In the current experiment, however, *C. maculatus* females had walked only for several minutes on the beans which were used to construct a trail of beans with beetle-odors. Natural residence times of *C. maculatus* on beans are likely to be of the order of magnitude of minutes. Such short residence times per bean are apparently not long enough to leave a recognizable trace for *U. lariophaga*, or perhaps the odours emanating from the host patch were dominant.

Behaviour in and around the host patch

Large differences were observed in behaviour before and after the first encounter with a host (Table 6). After the first encounter, the wasps became more active, the residence time per bean increased and the walking trajectory became more tortuous. This is in line with Van Alebeek & Groot (1997) who reported that walking trajectories of *U. lariophaga* became more tortuous after an encounter with a host and that walking speed increased. They, however, recorded the actual walking behaviour on bean surfaces at a very fine resolution, while we recorded walking trajectories only at a resolution of individual beans. At both spatial scales the walk-

ing trajectory becomes more tortuous after an encounter with a host. This type of behaviour is known as area-restricted search and has been described for many parasitoids, including at least six *Trichogramma* spp. (Suverkropp, 1997). It is adaptive if the host has an aggregated distribution, because in that case, encountering a host means that more hosts can probably be found in the vicinity. Indeed, *C. maculatus*, which is probably the main host of *U. lariophaga*, oviposits in clusters (Chapter 2).

Residence times on beans on which an unparasitized egg was encountered were longer than on beans on which the egg was not encountered, even if the time spent on encounters, parasitization and post-parasitization behaviour is excluded (Figure 2c). In principle, this increased residence time could be the result of two processes: (1) a longer stay on a bean increases the probability that an egg is encountered on that bean, and (2) an encounter with a host extends the residence time on that bean. The average time until encounter was, however, shorter than the total residence time on beans on which the egg was not encountered. Moreover, residence times on beans on which a parasitized egg was encountered were shorter than on beans on which an unparasitized egg was encountered (Figure 2c). Both arguments contradict the position that most eggs were encountered on beans on which the wasps stayed longer. Therefore our data strongly suggest that an encounter with a host extends the residence time on that bean. This, again, is adaptive if beans with more than one egg are relatively common. For parasitoids of solitary hosts it is adaptive to leave immediately after a parasitization (Vos *et al.*, 1998).

It would be interesting to know how long this change in behaviour, or arrestment response, lasts. A closely related question is: how long does it take before the wasp leaves the host patch? This time span is called the giving up time (GUT) and is measured from the last oviposition. We cannot calculate the GUT from the data presented here because of the limited number of observations and because wasps were often still in the host patch at the maximum observation time of 90 min. We have, however, carried out additional observations for some wasps that had found the host patch, after the experiment had ended at 90 min. These observations were carried out at regular time intervals, up to two hours after the end of the experiment. They show that, 15-60 minutes after the last parasitization, *U. lariophaga* was not found in the host patch any more and that the walking trajectory had become more straight again (data not shown). This is in line with Van Alebeek & Groot (1997) who found that the walking trajectory of *U. lariophaga* was more straight at 12-30 min than at 0-2 min after oviposition. GUT, however, is usually not constant, but is influenced by previous and current experience of the parasitoid (Godfray, 1994). In addition, one may wonder how GUT should be defined in this system. In stored cowpea, patch leaving is not a discrete, one-time event; rather it is a gradual process in which the wasp increasingly chooses non-infested beans to

move to. As a consequence, GUT can be accurately measured for single infested beans but not for a patch of infested beans. For single beans we have shown that an encounter with a parasitized host induces a slight increase in residence time (or GUT) and an encounter with an unparasitized host induces a substantial increase in residence time (or GUT) (see Figure 2c). We assume that the same effects will be present at patch level.

When it comes to stepping from one bean to the next, *U. lariophaga* chooses beans with eggs significantly more often than might be expected if it would move

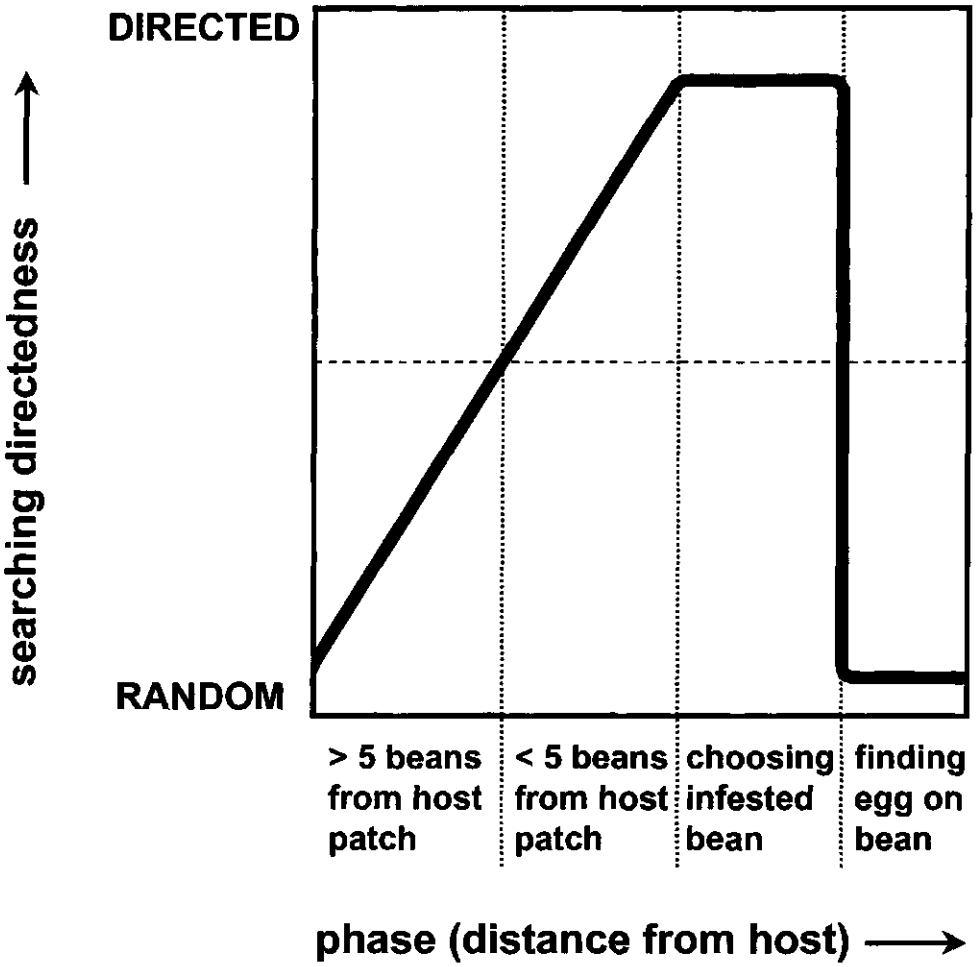

Figure 7. Diagram of the directedness of *U. lariophaga* females towards hosts during different parts of the searching behaviour. Note that, although the walking trajectory on beans seems to be random, females spend more time on beans with eggs than on beans without eggs. This may also be a form of 'directed search'.

about randomly. We assume that *U. lariophaga* was able to select these beans based on chemical traces left by the *C. maculatus* female. This could be the same marker pheromone which is used by *C. maculatus* females to restrain oviposition of other females on the same bean (Ormel *et al.*, 1995). A short range or contact response to host kairomones has already been described for other trichogrammatids (Boo & Yang, 2000; Garnier-Geoffrey *et al.*, 1996; Renou *et al.*, 1992; Thomson & Stinner, 1990).

Once *U. lariophaga* landed on a bean with an egg, it often missed the egg by a very short distance. For several *Trichogramma* spp. it has been reported that they use visual cues to locate host eggs from 2-4 mm distance (Suverkropp 1997:55). Our results, however, suggest that in *U. lariophaga* visual cues do not play an important role in host-finding at short range.

The different phases of host searching behaviour of *Uscana lariophaga* in stored cowpea are summarized in Figure 7. We think that directedness towards hosts increases as *U. lariophaga* approaches hosts, except on an infested bean itself. This increasing directedness as *U. lariophaga* gets closer to hosts serves as a positive feedback: the closer *U. lariophaga* is to a host, the easier it becomes to get even more close (except during the very final stage of host finding).

Methodology

It is striking that almost all of the time that was spent on active searching by the wasps was spent on beans. Walking on the petri dish instead of on beans would have been a much faster way of displacement for the wasps, but this was rarely observed. Apparently the current setup provided enough stimuli for the wasps to search on the beans. It is indeed known that *U. lariophaga* is attracted not only by infested but also by clean cowpea seeds (Ormel *et al.*, 1995; Van Huis *et al.*, 1994b).

A main characteristic of our methodology is that individual beans were used as the spatial unit for observation. The methodology also allowed us to observe the wasps almost 100% of the time, while at the same time the wasps could walk across the whole bean surface (*i.e.*, get underneath the beans), and step from one bean to the next. In other experiments in which *U. lariophaga* behaviour was observed, beans were placed with their bottom half in fine sand to prevent *U. lariophaga* from getting underneath the seeds (Van Alebeek & Groot, 1997; Chapter 5). In the current setup we often observed *U. lariophaga* walking in circles from the upper side to the lower side, and back again. This behaviour would of course not have been possible if the beans would have been buried in sand. Therefore, if we would have used beans buried in sand, we might have underestimated residence times. It is possible, on the other hand, that residence times were overestimated in the current setup compared to a three-dimensional storage system, because in the current set-

up the wasps had fewer opportunities to move to a neighbouring bean than in a three-dimensional setting. In the current setup, each bean had almost six neighbouring beans (data not shown), while in a three-dimensional setting each bean is surrounded by almost ten beans (Chapter 2). Another deviation from a three-dimensional bean stock is the presence of light in our setup; but without light observations would of course not have been possible.

We have used individual beans as the spatial unit for the analysis of *U. lariophaga* searching behaviour. This has allowed us to express elements of the searching behaviour in terms which are not only relevant from the perspective of the parasitoid, but which can also be used in a spatially explicit simulation model of *U. lariophaga* foraging behaviour.

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We are grateful to Frans van Aggelen, Leo Koopman and André Gidding for rearing the insects. We thank H.P. Spijkerboer for his help in the goniometric analysis of walking trajectories. We gratefully remember the late Peter Mols, who gave advice on data processing. Antoon Loomans is thanked for helpful advice during the experiments. Joop van Lenteren and members of the PE&RC PhD discussion groups 1 and 5 gave valuable comments on an earlier version of the manuscript.

CHAPTER 4

C. Stolk, M.N. Ghimire, S. Souquié, W. van der Werf & A. van Huis

Host finding by *Uscana lariophaga* in stored cowpea: the effect of distance, time interval, host patch size and spatial orientation

Abstract

We measured host finding and parasitization by *Uscana lariophaga* (Hym.: Trichogrammatidae), a potential biocontrol agent of the storage pest *Callosobruchus maculatus* (Col.: Bruchidae), in stored cowpea. Host finding is shown to be a function of distance, of time, of host patch size and of the spatial position of *U. lariophaga* relative to the host patch. *U. lariophaga* females were able to find hosts up to 75 cm horizontal distance from the host patch, which was the largest distance tested. The probability that a host patch was found when an individual *U. lariophaga* female was released at 2.5 cm horizontal distance from the host patch ranged from 0.6 at 2 h foraging time to 0.9 at 8 h foraging time. At 10 cm from the host patch, host finding probability ranged from 0.2 to 0.45 at these respective foraging times. Finding probabilities doubled compared to horizontal distances when *U. lariophaga* was released below the host patch, and halved when she was released above the host patch. We show that this negative geotaxic response is not an artefact of the release method. The median net displacement rate in the direction of the host patch was two beans per hour (1.4 $cm \cdot h^{-1}$). Our results suggest that *U. lariophaga* started searching for hosts regardless of the quality of the olfactory information she received. Additional observations indicate that *U. lariophaga* is adapted to a host with a patchy distribution, which implies that host finding over larger distances is indeed relevant for *U. lariophaga*.

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Introduction

Cowpea (*Vigna unguiculata* Walp.) is an important crop in Africa (Nwokolo & Ilechukwu, 1996). It is attractive to subsistence farmers because it is relatively resistant to dry periods (Turk *et al.*, 1980), all green parts can be used for human or animal consumption or as manure (Duke, 1990), and because nitrogen-binding symbiotic bacteria in the roots improve soil fertility (Summerfield *et al.*, 1974). After harvest, cowpea seeds are stored during the dry season for home consumption, for trade, and to provide seeds for the next growing season. This dry season may last up to eight months in the Sahel region (Van Huis *et al.*, 1990). During storage, cowpea is often infested with *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae) (Jackai & Daoust, 1986). This may result in a loss of nutritional value (Modgil & Mehta, 1994, 1996), secondary infestations with toxin-producing fungi (Sinha & Wallace, 1966; Siwela, 1996), and in a decreased market value (Jackai & Daoust, 1986). Although quantitative losses are difficult to determine (Boxall, 1991), it has been estimated that, on a yearly basis, 20-40% of the stored cowpea seeds in northern Nigeria become infested with *Callosobruchus maculatus* (Caswell, 1981).

C. maculatus oviposits on the surface of seeds or pods (Singh *et al.*, 1990). After a few days the eggs hatch and the emerging larvae penetrate into the seed, where they complete their development. The adult emerges through an emergence hole in the bean. The total development time is about 25 d at 30°C (Giga & Smith, 1983). Adult females live for about a week, during which they lay up to about 100 eggs (Singh *et al.*, 1990).

In West Africa, eggs of *C. maculatus* are parasitized by *Uscana lariophaga* Steffan (Hymenoptera: Trichogrammatidae). In the field and in storage *U. lariophaga* is responsible for substantial mortality of *C. maculatus* (Lammers & Van Huis, 1989; Sagnia, 1994; Van Alebeek, 1996b; Van Huis *et al.* 1998). Therefore *U. lariophaga* has been suggested as a candidate for biological control of *C. maculatus* in stored cowpea (Van Huis *et al.*, 1991a). Total development time of *U. lariophaga* is 8-11 days at 30°C; the adult lives for about two days and can lay 40-80 eggs (Van Huis *et al.*, 1994a; C. Stolk, unpublished data). *C. maculatus* eggs turn black within a few days after parasitization.

Previous research on *U. lariophaga* has focused on the biology, the role of odours in host location, the functional response, and population dynamics in small experimental storage containers (Van Huis *et al.*, 1994b; Van Alebeek & Van Huis, 1997; Van Alebeek *et al.*, 1996a,b; Van Huis *et al.*, 1998, in press). *U. lariophaga* has been shown to be attracted by odours emanating from host eggs (Van Huis *et al.*, 1994b; Ormel *et al.*, 1995). In functional response experiments *U. lariophaga* was able to parasitize up to about 25 eggs in 4 h or 40 eggs in 24 h (Van Alebeek *et al.* 1996b).

In these experiments *U. lariophaga* showed strong negative geotaxis when navigating in a small cowpea stock. This was indicated by the fact that the wasps parasitized substantially more eggs in clusters above the release point than in clusters below the release point (Van Alebeek & Van Huis, 1997).

No attention has as yet been given to the capacity of *U. lariophaga* to find hosts in stored cowpea over distances other than 5 or 6 cm, and little attention has been paid to net displacement rates of *U. lariophaga* in stored cowpea. An effective host-finding capacity is, however, one of the main determinants of successful biological control. This is especially true if the host has a patchy distribution, which may be the case if *C. maculatus* occurs in low densities early in the storage season (Chapter 2). In addition, it is not clear to what extent the behaviour that has been studied for *U. lariophaga* might have been influenced by the release method that was used in these studies (*e.g.* Van Alebeek & Van Huis, 1997). Negative geotaxis in particular might have been induced by the release method, since the method may have disturbed normal behaviour and disturbance can induce negative geotaxis in insects (Surtees, 1963). In this chapter we therefore investigate the host finding capacity of *U. lariophaga* as a function of the distance to the host patch, the size of the host patch, the time that *U. lariophaga* is allowed to forage, and of spatial orientation. We do so using two different methods of introducing the wasps into a cowpea stock: one which resembled the usual release method and another one which was designed to minimize disturbance of *U. lariophaga*.

Materials and methods

General procedure

In six experiments, described in detail below, we used tightly closed opaque containers, filled with cowpea and containing either one or two host patches. The host patches consisted of small nylon gauze bags which contained beans carrying fresh *C. maculatus* eggs. *U. lariophaga* wasps were introduced into these containers at varying horizontal or vertical distances from the host patch or patches. Each experiment was ended by opening the containers and removing the host patches for determination of parasitism. Both the release site and the host patches were then inspected for the presence of *U. lariophaga*. Any wasp present in the host patches was removed and the host patches were incubated in sealed petridishes at 30°C. After 3 days of incubation we counted the numbers of parasitized and unparasitized eggs in each host patch. The host patch was considered found by *U. lariophaga* if it had parasitized eggs or if a wasp was found in the host patch. All experiments were replicated over time.

Cowpea seeds (*Vigna unguiculata*) of the cultivar 'Black Eye' were used in all experiments and in the rearings. Before use, these beans were frozen at -18°C and subsequently dried at 45°C, both for three days, to exclude contamination with insects. Beans that were used in Experiments 3-6 were used only once in an experiment; they were not used again because they might have been tainted with beetle egg odours. For the same reason, we did not re-use beans within a distance of 7.5 cm from the host patch in Experiments 1 and 2. The remaining beans in Experiment 1 and 2 were kept at 45°C for at least two days before they were used again in an experiment. This was done to kill any remaining parasitoids.

Callosobruchus maculatus was reared at 35±1°C during photophase and at 25±1°C during scotophase (12L:12D). *C. maculatus* eggs were obtained from 0-2 d old females. If the eggs would not be used within 24 h after oviposition, they were stored at 5±1°C for up to one day. *Uscana lariophaga* was reared at 30±1°C and at 12L:12D. Female adult wasps that were used in the experiments were 2-17 h old and had been allowed to mate for about 2 h.

Two methods of introducing U. lariophaga *into the cowpea stocks*

In four of the experiments described in this chapter (Experiments 2, 3, 5 and 6, see Table 1), individual female parasitoids were released inside the cowpea stock using small glass vials which were open at both ends (length about 2.5 cm, inner diameter 0.5 cm). Each vial contained a single wasp and the vial was closed with two plugs of cotton wool before release. The plugs of cotton wool had strings attached which ran through the cowpea stock and through a tiny hole in the wall of the container. By pulling these strings the vial would open, thereby releasing the parasitoid inside the cowpea stock. After release of the wasp, the holes in the walls of the container were closed with clay. This method is similar to the method used in previous experiments (*e.g.* Van Alebeek & Van Huis, 1997).

In Experiments 1 and 4, rather than releasing individual adult females, we inserted beans carrying parasitized eggs, about to emerge, at one location into the cowpea stock. This method is more natural and minimizes disturbance for *U. lariophaga*.

Experiments 1 and 2: Host finding over long horizontal distances

In Experiment 1 we investigated host finding of *U. lariophaga* over a series of distances of up to 75 cm. The containers that were used measured 95×20×20 cm (l×w×h). The host patch contained 20 beans carrying 565±146 (SD) fresh *C. maculatus* eggs and was placed at 10 cm from one end of the container, at the centre of the bean mass. We chose this high number of eggs in the host patch with the

Table 1. Overview of the experiments.

Expt	Effect tested	Container type	*Uscana* introduction method	Host patch size (approx. # eggs)	Nb. of patches	Treatments
1	distance	box	batch of parasitized eggs	560	1	5, 15, 25, 40, 75 cm
2	distance and host patch size	box	single female, from vial	560 or 30	1	15, 75 cm; large or small host patch
3	time and distance	cylinder	single female, from vial	25	1	2.5, 5, 10 cm; 2, 4, 8 h
4	geotaxis	cylinder	batch of parasitized eggs	400	2	choice: top vs. bottom patch
5	geotaxis	cylinder	single female, from vial	25	1	no-choice: 'vertical' (top, bottom) and 'horizontal' host finding (analysed together with Expt 3)
6	geotaxis	cylinder	single female, from vial	25	2	choice: top vs. bottom patch

intention that each wasp that reached the host patch could lay all her eggs. At either 5, 15, 25, 40 or 75 cm from this host patch about 10 beans holding altogether 15 parasitized *C. maculatus* eggs were placed at the centre of the bean mass inside a small nylon gauze bag (the 'release bag'). After 66 h the containers were opened. The black eggs in the release bag were inspected for the number of parasitoid emergence holes. The host patches were incubated and the number of parasitized eggs was counted as indicated above. Each distance was replicated five times, except the treatment of 5 cm, which had four replicates.

Parallel to this experiment we incubated 50-100 parasitized eggs, from the same batch as those in the release bag, in darkened petri dishes to check emergence and sex ratio. Wasps that emerged from these eggs were counted and sexed every day. These emergence and sex ratio data were used to express parasitism in the host finding experiment in terms of 'per female per day'. The number of parasitized eggs per female per day was calculated as the total number of parasitized eggs in the host patch, divided by the estimated number of 'female·days'. The number of female·days was calculated as the integral over time of the estimated number of females present in each container. Each female was assumed to live for 2 d after emergence (Van Huis *et al.*, 1991a).

In Experiment 2 we compared host finding at two distances, using host patches

of two different sizes. We used containers measuring 94 × 18 cm at the top and 91 × 14 cm at the bottom. The height of the containers was 16.5 cm. The host patch was placed at 7.5 cm from one end of the container and contained either 40 beans carrying 562 ± 108 (SD) fresh *C. maculatus* eggs or 10 beans carrying 29 ± 4 (SD) eggs. The large host patch resembled the host patches of Experiment 1; the number of eggs in the small host patch resembled the number of fresh eggs in a 'natural' host patch (Chapter 2; Boeke, submitted). At either 15 or 75 cm from the host patch one female *U. lariophaga* was released from a glass vial. After 24 h the experiment was ended. Each treatment combination was replicated 11 times.

Experiment 3: Host finding over short horizontal distances

In Experiment 3 we investigated host finding for three distances and three time intervals. We used horizontally placed cylindrical containers (length 14.5 cm, diameter 11 cm) which contained one host patch at 2.3 cm from one end of the container. The host patch contained 26 ± 4 (SD) eggs on 10 beans. One *U. lariophaga* female was released from a glass vial at either 2.5, 5 or 10 cm from the host patch. After 2, 4, or 8 h the experiment was ended. The nine resulting treatment combinations were each replicated 20 times.

Experiments 4, 5 and 6: Vertical host finding

In Experiment 4 we investigated up- and downward host finding in an upright cylinder with two host patches. One host patch was placed at 5 cm from the bottom end of the cylindrical container (height 20 cm, diameter 12 cm), the other host patch was placed at 5 cm from the top end. The host patches contained 387 ± 182 (SD) eggs on 10 beans. A few beans with in total seven parasitized eggs which were about to emerge were placed at the centre of the cylinder in a gauze bag. To increase the probability of mating (which might influence host finding behaviour), the parasitized eggs were accompanied by an open gel capsule containing three *U. lariophaga* males. After 72 h the experiment was ended. The experiment was replicated 11 times.

In Experiment 5 we investigated up- and downward host finding in upright cylinders with one host patch. The host patch contained 26 ± 4 (SD) eggs on 10 beans and was placed at 2.3 cm from either the top or the bottom of the cylindrical container (height 14.5 cm, diameter 11 cm). An *U. lariophaga* female was released from a glass vial at the center of the container, at 5 cm distance from the host patch. After 4 h the cylinder was opened. Both treatments were replicated 20 times. Experiments 3 and 5 were carried out simultaneously. This allowed us to analyze this experiment together with the '5 cm, 4h' treatment from Experiment 3 to investigate the effect of the horizontal versus vertical spatial orientation on host finding.

Experiment 6 was similar to Experiment 5, except for the fact that we used both top and bottom host patches in one container. The experiment was replicated 20 times.

Statistical analysis

The probability that the host patch was found was analysed as a function of distance for Experiment 1 and 2, and as a function of distance and time for Experiment 3, using binary (logistic) regression. We also tested whether the spatial position of the host patch in Experiments 3 and 5 (above, below, or level with the release site) had an effect on host finding probability using Cochran's test. For Experiment 3 we also tested the effect of distance and time on the probability that the wasp was found still at or near the release point, using binary regression.

For Experiments 1, 2, 3 and 5 we investigated the effect that the factors involved had on the number of parasitized eggs. For Experiments 1 and 5 we used the Kruskal-Wallis test and for Experiment 2 and 3 we used Friedman's test with multiple observations per cell, corrected for ties. For Experiments 3 and 5 we also tested for an effect on the number of parasitized eggs for those host patches which were found, using the test of Bernard and Van Elteren for Experiment 3 (Bernard & Van Elteren, 1953) and the Kruskal-Wallis test for Experiment 5. The test of Bernard and Van Elteren is a generalized form of Friedman's test which allows for unequeal numbers of observations per cell (missing values). We used non-parametric tests in all these cases because in these experiments the variance appeared to be not homogeneous among all treatments (according to Levene's test) and/or the error term was not normally distributed (according to the Kolmogorov-Smirnov test with Lilliefors correction). For Experiment 3 we also analyzed the relationship between the number of parasitized eggs and the number of beans with parasitized eggs using linear regression.

For those cases in Experiments 4 and 6 where only one host patch was found by *U. lariophaga*, we compared the probability for the top and the bottom cluster to be found using a Chi-square test. The number of parasitized eggs was compared for the top and the bottom clusters using Wilcoxon Signed Ranks test. For Experiment 4 we also compared the number of beans with parasitized eggs for the top and bottom clusters using the same test.

For Experiment 3 we estimated for each individual wasp the time it had taken to reach the host patch, assuming the following relationship:

$$t_{arrival} = t_{total} - t_{par} \quad (1)$$

with $t_{arrival}$ = time at which the wasp arrived at the host patch (s), t_{total} = total time available (s) and t_{par} = time spent on parasitizations (s).
The total time available was either 2, 4 or 8 h, depending on the treatment. The time spent on parasitizations consisted of the time spent on ovipositions and of time in between ovipositions (*i.e.*, searching for new hosts). We assumed the time needed for an oviposition to be constant (156 s), whereas the time in between subsequent ovipositions increases as more eggs in a cluster are parasitized (based on the experiments of Chapter 3). Thus,

$$t_{par} = t_{ovip} \cdot n + \sum_{i=1}^{n-1} t_{search,i} \tag{2}$$

with t_{ovip} = the time spent on one parasitization (s), $t_{search,\,i}$ = the time in between parasitizations (s) and n = the number of parasitizations.
$t_{search,\,i}$ increases as fewer eggs are left unparasitized:

$$t_{search,i} = -273 \cdot \ln(f_{unp,i}) + 83 \tag{3}$$

with $f_{unp,\,i}$ = the fraction of unparasitized eggs in the host cluster after the i-th parasitization + 0.001 to avoid zeros (based on the experiments of Chapter 3).

Based on the arrival times and the distance between the release site and the host patch (2.5, 5 or 10 cm), we calculated net displacement rates in the direction of the host patch for those wasps which arrived at the host patch in Experiment 3:

$$\text{net displacement rate} = \frac{\text{distance between release site and host patch}}{\text{arrival time}} \tag{4}$$

The estimated arrival times were analyzed using Cox regression analysis. Cox regression analysis is a type of survival analysis which can be used to analyze time spans until a certain event, or 'failure', occurred (Kalbfleisch & Prentice, 1980). In this case the event of interest is 'arrival at the host patch'. The analysis focuses on the 'hazard rate', or the probability per unit of time that a failure occurs for particular values of covariates. The hazard rate is the derivative of the log-survival curve; in our case the fraction 'surviving' is defined as the fraction of wasps that has not yet arrived at the host patch. According to Cox regression analysis, the probability of arriving at the host patch per unit of time can be expressed for the current situation as

$$h(t,z_1,z_2) = h_0(t) \cdot e^{\beta_1 z_1 + \beta_2 z_2} \tag{5}$$

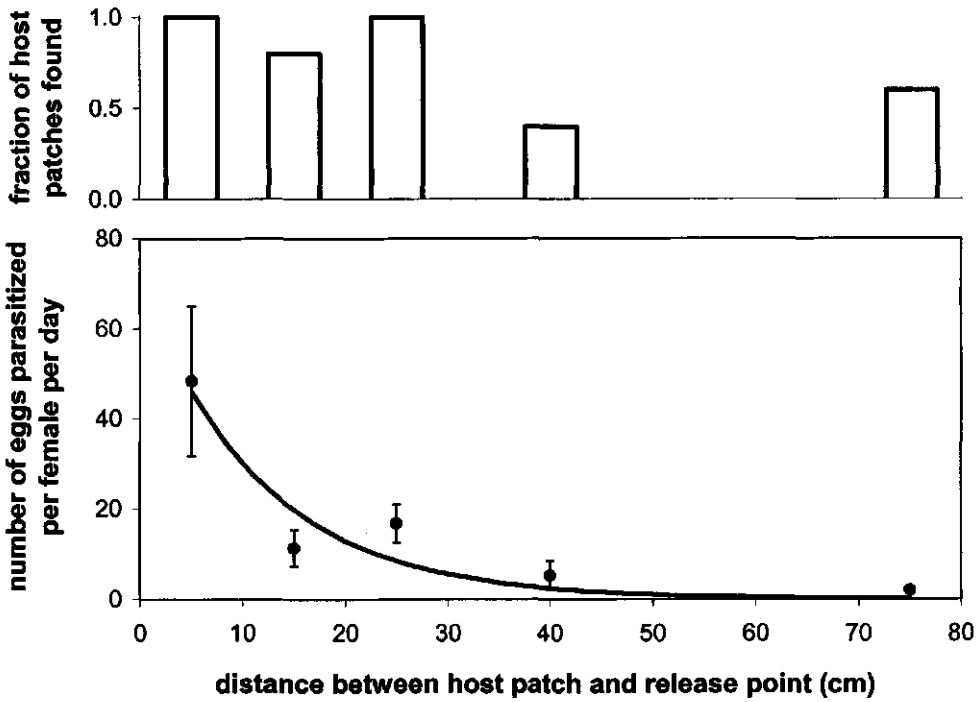

Figure 1. Results of Experiment 1. Top: the fraction of host patches that were found by *U. lariophaga* as a function of distance between the host patch and the *Uscana* release bag. Bottom: the number of parasitized *C. maculatus* eggs per female per day as a function of distance between the host patch and the *Uscana* release bag. Error bars indicate the standard error (N=5 for each distance except at 5 cm, where N=4). The curve shows the function $y = 70 \cdot e^{-0.085 \cdot x}$ (R^2=0.49), with y = the number of parasitized eggs per female per day and x = distance (cm).

where h, the hazard rate, is a function of time t and of the covariates z_1 and z_2. The latter covariates are indicator variables which can only take values of 0 or 1 for a given distance; 2.5, 5 and 10 cm are coded as (z_1, z_2) = (0,0), (1,0) and (0,1), respectively. The baseline hazard, $h_0(t)$, is the probability per unit of time of arriving at the host patch at distance 2.5 cm. The effects on the hazard rate of release at 5 and 10 cm distance, compared to the hazard rate at 2.5 cm, are given by $\exp(\beta_1)$ and $\exp(\beta_2)$, respectively. For example, the probability per unit of time to arrive at the host patch from 5 cm distance after a given amount of time is $h_0(t) \cdot e^{\beta 1}$. The baseline hazard $h_0(t)$ is not specified but is estimated from the data.

Experiment 1 was carried out over a period of seven weeks with replicates over time. The constancy of the sex ratio of the emerging wasps over this period was tested by calculating the probability of the observed sex ratio for each week, using

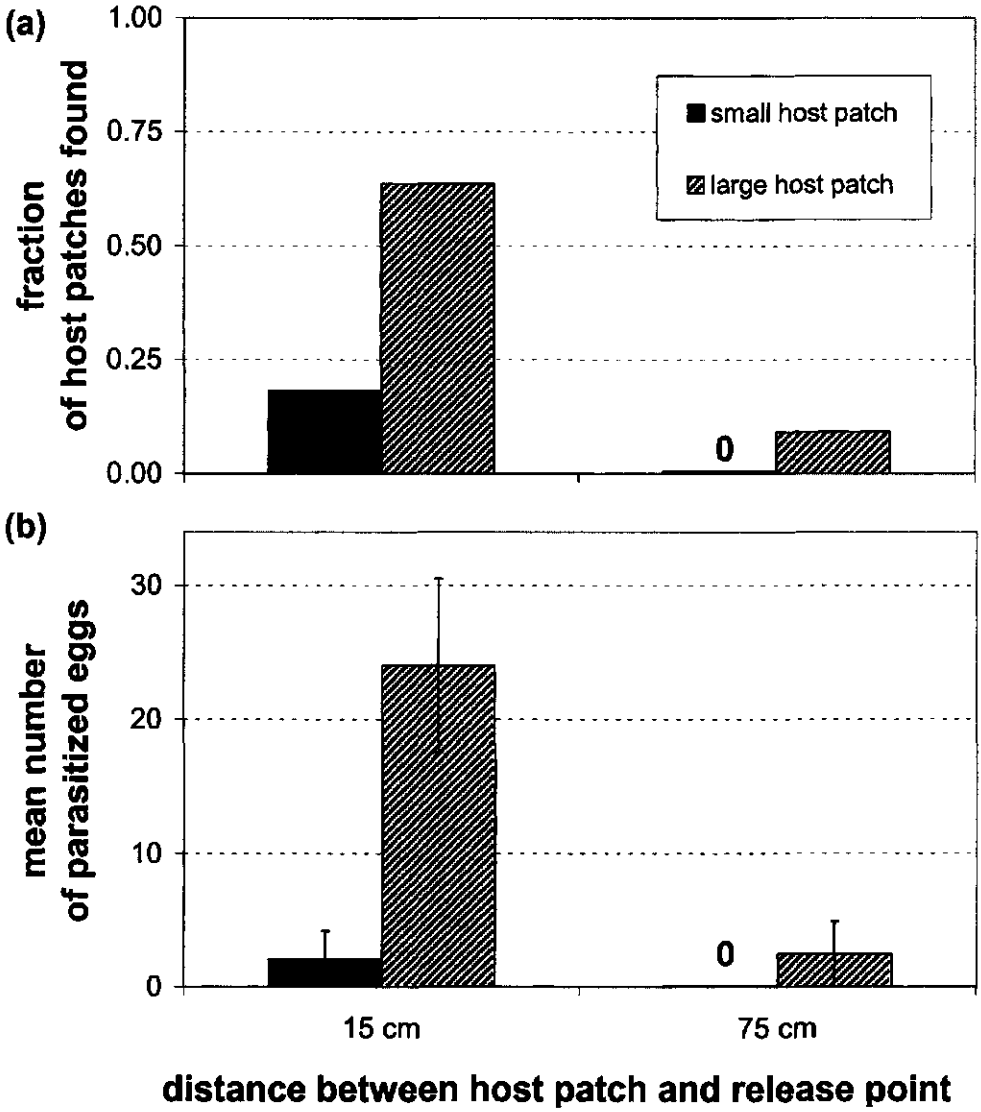

Figure 2. **(a)** The fraction of host patches found and **(b)** the mean number of eggs parasitized by individual females during one day at two distances from the release site (Experiment 2). Error bars indicate the standard error (N=11 for each treatment).

the binomial distribution with the average sex ratio over seven weeks as the expected value.

All tests were evaluated at a significance level of 0.05.

Results

Experiments 1 and 2: Host finding over long horizontal distances

In Experiment 1 there was no effect of distance to the host patch on the probability that the host patch was found ($p=0.49$; Figure 1), but distance did have a significant, negative effect on the number of parasitized eggs ($p<0.01$; data not shown) and on the number of eggs that were parasitized per female per day ($p<0.01$) (Figure 1). The non-linear regression function $y = 70 \cdot e^{-0.085 \cdot x}$, with y = the number of parasitized eggs per female per day and x = distance, described the data reasonably well (Figure 1).

On average 11.2 ± 2.8 (SD) of the 15 black eggs in each release bag emerged during the experiment. The parallel experiment showed that the sex ratio was constant over the whole duration of the experiment (fraction females = 0.57; $p>0.05$). All 450 black eggs that were used in the parallel experiment emerged during the experiment; 59% of the females emerged within 24 h. The average time of emergence was 1.1 d after the start of the experiment for females, and 0.9 d for males. In 12 out of the 24 replicates, one or more males were found still in or on the release bag at the moment the containers were opened (66 h after the start of the experiment).

In Experiment 2, both host patch size and distance had a significant effect on the probability of finding the host patch ($p=0.025$ and $p=0.010$, respectively). The effect of host patch size was positive and the effect of distance negative (Figure 2a). Host patch size and distance also had a significant effect on the number of parasitized eggs ($p<0.001$ and $p<0.01$, respectively) (Figure 2b).

Experiment 3: Host finding over short horizontal distances

Both distance and available time had a significant effect on the probability of finding the host patch at short range ($p<<0.001$ and $p<0.001$, respectively). Distance had a negative effect on host patch finding while time had a positive effect (Figure 3a). There was no interaction between time and distance in their effect on host finding probability ($p=0.47$).

Distance and available time also had a significant effect on the number of parasitized eggs ($p<<0.001$) (Figure 3b). If we omit, however, the replicates in which the host patch was not found by the wasp, distance does not have an effect any

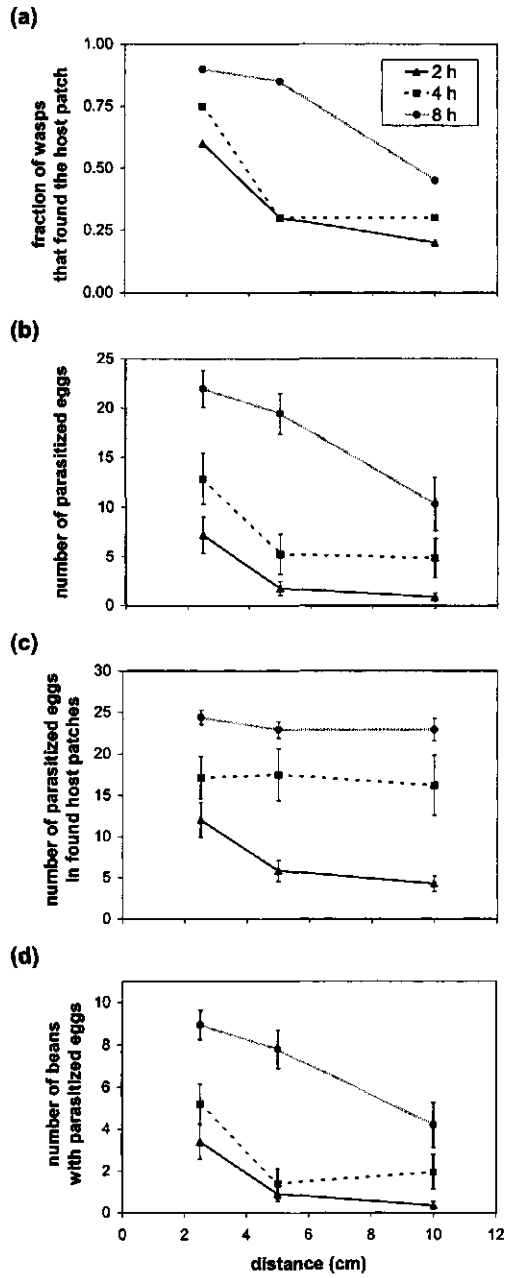

Figure 3. Results of Experiment 3, in which the effect of distance and time on host finding probability and parasitization was investigated: **(a)** the fraction of host patches found, **(b)** the mean number of parasitized eggs per host patch, **(c)** the mean number of parasitized eggs for host patches that were found, and **(d)** the number of beans with parasitized eggs. Error bars indicate the standard error (N=20 for each data point).

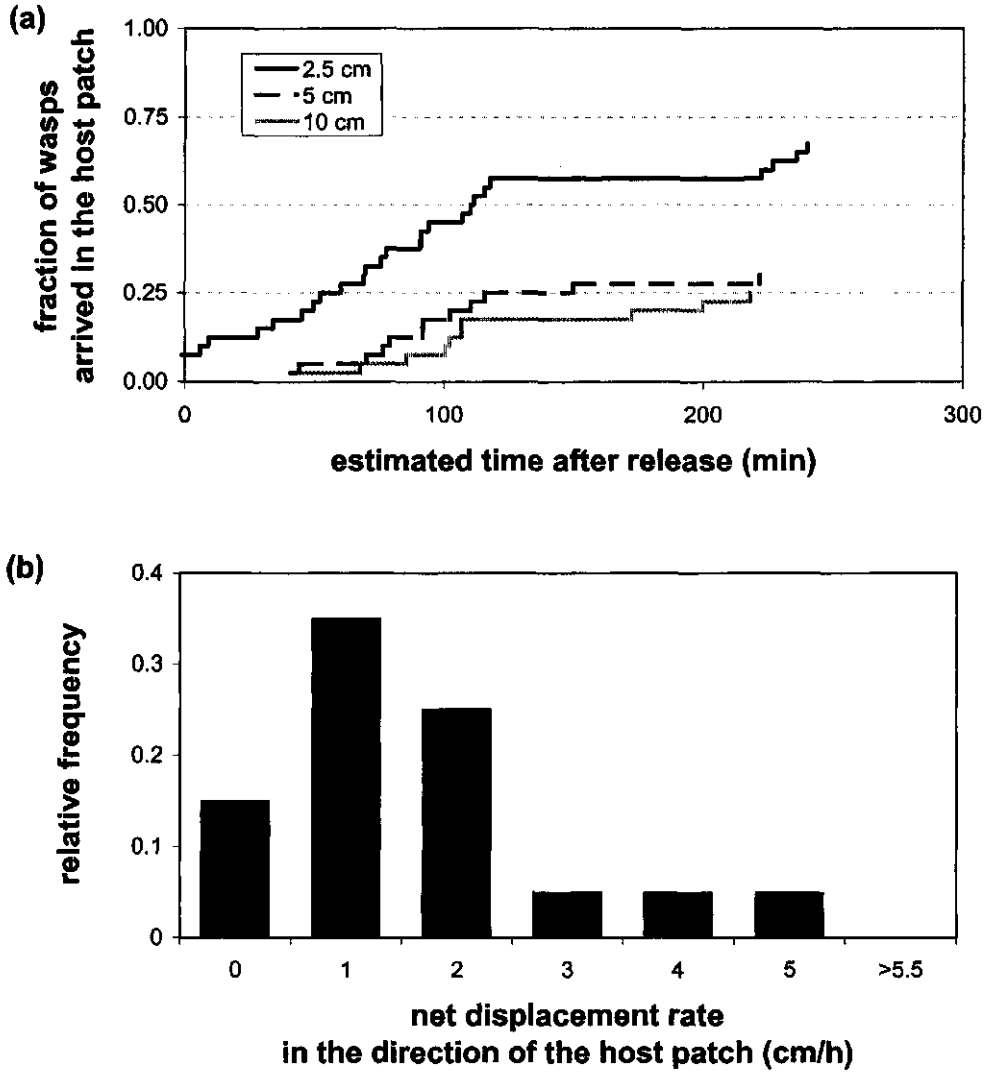

Figure 4. Arrival times and net displacement rates as estimated for the 2 and 4 h treatments of Experiment 3. (a) The fraction of wasps arriving at the host patch as a function of time. Arrival times are based on the time that was available for foraging, the number of parasitized eggs, and the estimated time needed for these parasitizations. (b) Frequency distribution of net displacement rates of wasps in the '2.5 cm, 4 h' treatment. Net displacement rates are calculated from the estimated arrival times and the distance between host patch and release point. The frequency of wasps with a zero net displacement rate is based on the number of wasps that was found at or near the release site. Two wasps, which were not found back at the release site and had also not reached the host patch, are not included in this distribution. Their net displacement rate in the direction of the host patch was lower than $2.5/4 = 0.63$ cm·h^{-1}; possibly they moved away from the host patch.

more on the number of parasitized eggs ($p=0.25$) while available time still does have a positive effect ($p<<0.001$) (Figure 3c). The number of beans with parasitized eggs behaved much the same as the number of parasitized eggs (Figure 3d). Indeed, the number of beans with parasitized eggs (x) could be used to predict the number of parasitized eggs (y) according to the linear regression function $y = 2.4 \cdot x$ ($R^2 = 0.84$ if the host patches which were not found are omitted, $R^2 = 0.95$ if they are included; data not shown).

Altogether 39 females were found still at or near the release point when the cylinders were opened at the end of the experiment. Distance had no effect on the probability that the wasp was found at the release point ($p=0.56$; data not shown), but time did have an effect: After both 2 and 4 h 27% of the released wasps was found back at the release site; after 8 h this figure had shrunk to 12% ($p=0.032$).

The estimated time of arrival in the host patch (Equation 1) for wasps released at 2.5, 5 and 10 cm distance are shown in Figure 4a. Data from the 8 h treatments are omitted from this Figure since those wasps had parasitized almost all eggs, regardless the distance between the host patch and the release point. In this case, because almost all eggs were parasitized, the fraction of parasitized eggs could not be used to give an accurate estimate of the time at which wasps had arrived in the host patch. Arrival times that were calculated for wasps in the 2 and 4 h treatments, on the other hand, were very similar for each respective distance (data not shown). This indicates that these estimates were probably fairly accurate, and arrival times from the 2 and 4 h treatments were pooled in Figure 4a.

Net displacement rates in the direction of the host patch were calculated from the data shown in Figure 4a. Most wasps progressed towards the host patch at net rates of about 1-2 $cm \cdot h^{-1}$, as is shown by the representative frequency distribution of net displacement rates for the '2.5 cm, 4 h' treatment (Figure 4b). The median net displacement rate for the 2.5 cm data was 1.4 $cm \cdot h^{-1}$. The median net displacement rate could not be calculated for the other distances, because the percentage of wasps that reached the host patch was never as high as 50% at those distances. The time at which 25% of the wasps reached the host patch was 52, 116 and 216 min for 2.5, 5 and 10 cm, respectively (Figure 4a). The corresponding 'quartile' net displacement rates are almost equal at 2.9, 2.6 and 2.8 $cm \cdot h^{-1}$, respectively.

Effects from Cox regression analysis were calculated as $\exp(\beta_1) = 0.32$ and $\exp(\beta_2) = 0.25$. This means that the rate at which the host patch is found at 5 cm, for instance, is 0.32 times the rate at which the host patch is found at 2.5 cm. $\exp(\beta_1)$ and $\exp(\beta_2)$ were both significantly different from 1 ($p<0.01$, Wald test, df=2) but did not differ significantly from each other ($p=0.88$, Wald test, df=2).

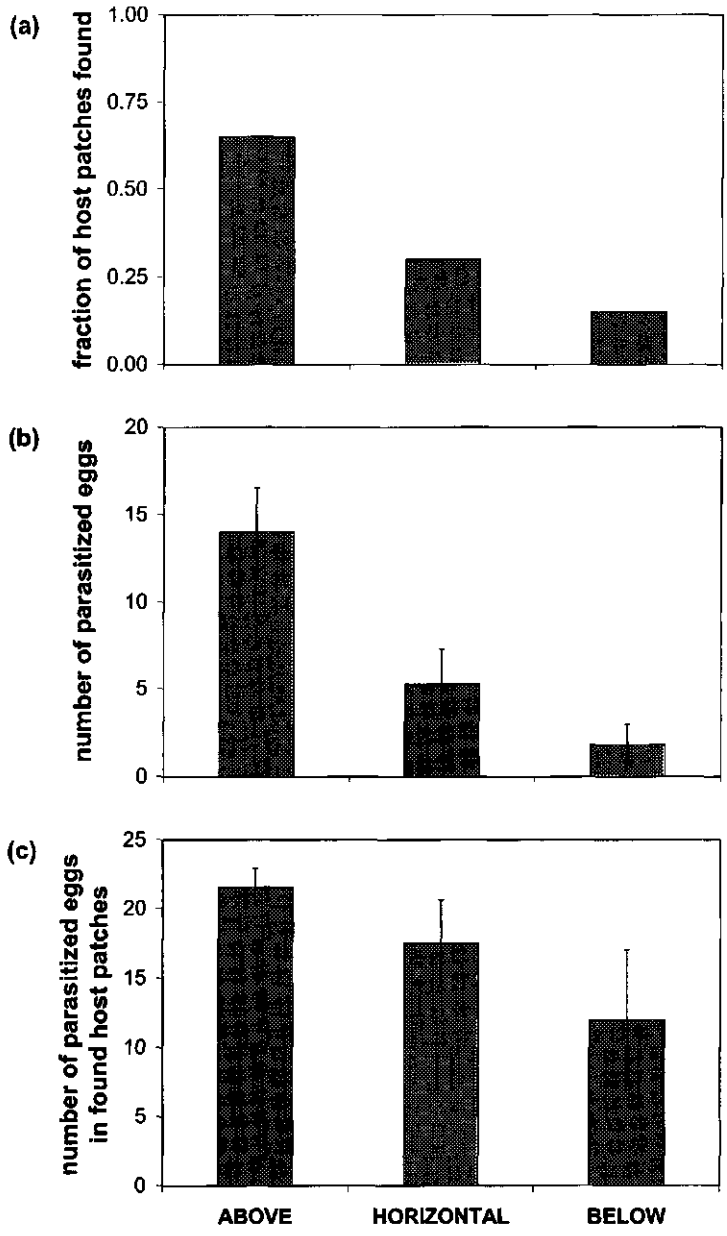

Figure 5. (a) The fraction of host patches found, (b) the mean number of parasitized eggs per host patch, and (c) the mean number of parasitized eggs for host patches that were found, as a function of the position of the host patch with respect to the release site. The 'horizontal' data are taken from Experiment 3 and the other data are from Experiment 5 (which had been carried out as a sub-experiment within Experiment 3). Error bars indicate the standard error (N=20 for each treatment).

Experiments 4, 5 and 6: Vertical host finding

In seven out of the twelve replicates of Experiment 4 both host patches were found while in the remaining five cases only the top patch was found. Thus, the top patch had a significantly higher probability of being found than the bottom patch ($p=0.025$). The mean number of parasitized eggs was higher for the top patch than for the bottom patch, but this difference was not significant (60 versus 31, $p=0.062$). The mean number of beans with parasitized eggs was, however, significantly higher for the top than for the bottom patch (8.8 versus 4.4, $p=0.012$).

In Experiment 5, which was a no-choice experiment with only one host patch per cylinder, the position of the host patch with respect to the release point had a significant effect on host finding probability ($p<0.01$). The top patch was found most often and the bottom patch was found least often (Figure 5a). The position of the host cluster also had a significant effect on the number of parasitized eggs ($p<0.01$) (Figure 5b). For host patches that were found, there was no effect of orientation on the number of parasitized eggs ($p=0.15$) (Figure 5c).

In Experiment 6, in 14 out of the 20 replicates only one of the two host patches was found; in the remaining replicates no host patch was found. The top patch was found significantly more often than the bottom patch: 13 times for the top patch versus only once for the bottom patch ($p<0.01$). Also, more eggs were parasitized in the top cluster than in the bottom cluster (13 vs 0; $p<0.01$).

Discussion

Host finding

Experiments 1 and 2 show that *U. lariophaga* can find host patches over distances of 75 cm. So far, host patch finding by *U. lariophaga* in stored cowpea had only been measured for distances of 5 and 6 cm, although in a dispersal study it had been shown to be able to travel at least 20 cm through stored cowpea (Van Alebeek, 1996a:129; Van Alebeek & Van Huis, 1997; Van Alebeek *et al.*, 1996a,b). Our results also suggest, however, that it takes *U. lariophaga* a long time to cover 75 cm. This appears from the fact that very few eggs were parasitized at this distance, even though a substantial fraction of the host patches was found at 75 cm in Experiment 1. It seems therefore that, when the wasps finally arrived in the host patch, they had little time or energy left before they died or before the experiment ended, and/or most eggs were not parasitizible any more. At 30°C, *U. lariophaga* lives for about two days (Van Huis *et al.*, 1991a; Van Huis *et al.*, 1994a) and eggs can be parasitized until they are about two days old (Van Huis *et al.*, 1991b). Indeed, according to the net

displacement rate of 1.4 cm·h^{-1} that we found in Experiment 3, it would have taken *U. lariophaga* about two days to reach the host patch from 75 cm distance.

For Experiment 3, Cox regression analysis showed that the host finding rate was significantly lower at 5 and at 10 cm compared to 2.5 cm, and that there was no significant difference between host finding rates at 5 and 10 cm distance. This result is based on pooled arrival times from the 2 and 4 h treatments. Indeed, Figure 3a shows that host finding probabilities were similar at 5 and 10 cm for the 2 and 4 h datasets. Figure 3a, however, also suggests that at 8 h foraging time finding rates did differ between 5 and 10 cm distance from the host patch. Thus, there seems to be a critical distance within which the host patch is rapidly found, and over time, more wasps from longer distances appear to accidentally cross this critical distance through random search -- simply because they have had more time available. This confirms our findings of Chapter 3 which suggest that from a short distance from a host patch *U. lariophaga* shows directed search. This directed search is probably mediated by odours related to *C. maculatus* eggs (Ormel *et al.*, 1995; Van Huis *et al.*, 1994b).

The probability of finding the host patch doubles if the host patch is above the release site instead of level with the release site, and this probability halves if the host patch is underneath the release site (Experiment 5; Figure 5a). This result, and Experiment 6, confirm the findings of Van Alebeek & Van Huis (1997) and Van Alebeek & Conteh (unpublished results) who showed that *U. lariophaga* has a strong negative geotaxic response. Experiment 4 shows that this negative geotaxis is also present when the parasitoid is released in a more natural way, instead of from a vial or a gel capsule. A negative geotaxic response has also been reported for other parasitoids of stored product pests, such as *Trichogramma* spp. (Quednau, 1958), *Eupelmis vuilleti* (Cortesero *et al.*, 1997) and *Anisopteromalus calandrae* (Press, 1988). An exception is *Lariophagus distinguendis*, which does not show a clear geotaxic response and which is able to find hosts in stored grain up to a depth of 4 m (Steidle & Schöller, 2002).

Results from the different experiments are generally in good agreement with each other and with results from previous experiments. For example, when females were released at 5 cm from the host patch in Experiment 1, they parasitized an estimated 16 eggs in 8 h (derived from Figure 1). This corresponds well to the 19 eggs that were parasitized in 8 h at the same distance in Experiment 3 (Figure 3). The host patch finding probability of 65% that was found for the top cluster in Experiment 5 (Figure 5a) corresponds well to Van Alebeek & Van Huis (1997), who reported host patch finding probabilities of 58-75% in an almost identical experimental setup.

Net displacement rates

We estimated the median net replacement rate of *U. lariophaga* in Experiment 3 at 1.4 cm·h^{-1}. Van Alebeek (1996a:130) estimated the median net displacement rate in stored cowpea seeds at $0.62 \cdot 10^{-5}$ m·s^{-1}, which corresponds to 2.2 cm·h^{-1}. He, however, measured displacement in the upward direction; given the negative geotaxis displayed by *U. lariophaga* it is not surprising that the upward net displacement rate is somewhat higher than the horizontal net displacement rate. In the same experiment, the fastest individuals covered 20 cm in 1 h, while in another experiment reported by Van Alebeek & Van Huis (1997) the fastest wasps traveled at least 50 cm in all directions in 24 h (~2.1 cm·h^{-1}).

The average diameter of the cowpea seeds we used is 0.7 cm (Chapter 2). If we take this into account, the median net displacement rate of 1.4 cm·h^{-1} can also be expressed as 2 beans·h^{-1}. The latter expression may be more useful because crossing from one bean to another is a distinct event in the foraging behaviour of *U. lariophaga* (Chapter 3).

Females and males staying at the release site

It is interesting to note that the distance between the host patch and the release site in Experiment 3 did not have an effect on the probability that the wasp was found back at the release site at the end of the experiment. Distance did, however, have an effect on the probability that the wasp found the host patch. We might assume that the quality of olfactory information that the wasps perceived is related to the distance from the host patch, because host odours must have reached the wasp almost exclusively by diffusion (the containers were hermetically sealed off, which excludes ventilation, and convection within the small containers can equally be ruled out). If this assumption is true, our results imply that females started searching for hosts regardless of the quality of the olfactory information, whereas the probability of 'getting lost' while searching for the host patch increased with distance.

In Experiment 1, males were often still found on or in the release bag three days after the start of the experiment, while females were only found back in the host patch, at distances of up to 75 cm from the release bag. In addition, it is known that *U. lariophaga* males emerge slighly earlier than females (Van Huis & Appiah, 1995; see also Experiment 1). These observations indicate that *U. lariophaga* males usually mate with wasps which emerge at the same location. This type of behaviour is usually associated with patchily distributed organisms. This behaviour of *U. lariophaga* therefore suggests that it is adapted to a patchily distributed host. It is not clear, however, whether it experiences this patchy host distribution primarily in the field or in storage (Chapter 2).

Methodology

We used two different methods of introducing *U. lariophaga* into the experimental storage containers, because we were concerned that releasing adult wasps from a small vial might disturb the wasps and induce unnatural behaviour. The experiments in which wasps were introduced in a more natural way, however, showed very similar end results. This suggests that *U. lariophaga* is not disturbed by the 'vial' release method and it strengthens confidence in previous work in which this method was used (*e.g.* Van Alebeek & Van Huis, 1997).

For Experiment 3 we showed that the number of beans with parasitized eggs is a good predictor of the actual number of parasitized eggs. Analyzing the number of beans with parasitized eggs always yielded similar results as analyzing the number of parasitized eggs itself (compare *e.g.* Figure 3b and 3d). Since counting only the number of beans with parasitized eggs is much less labour-intensive than counting the number of parasitized eggs, this would be a better response variable to use in this type of experiments, provided that the number of eggs per bean is not too high and not too variable.

Other parasitoids in stored products

In this chapter we have studied host finding abilities, or displacement, of *U. lariophaga*. The result of a displacement or dispersal process is the spatial distribution of the organism involved. Very little is known of the spatial distribution of *Uscana* spp. or other parasitoids in stored products – either naturally occurring or artificially introduced. Delobel (1989) mentions that, in an experimental study, parasitization of *Caryedon serratus* eggs in stored groundnut by *Uscana caryedoni* decreased rapidly with depth: no parasitization was found below the 4th seed layer from the top. It seems likely that this is the result of negative geotaxis in this species. In a study by Sedlacek *et al.* (1998), significantly more *Anisopteromalus calandrae* and *Pteromalus* sp. were found at the center of commercial corn bins than near the walls. Flinn *et al.* (1992) mention unpublished field data for a 351 m^3 grain bin. Unidentified hymenopteran parasitoids were found at the central regions of this bin and apparently not in the periphery. Howe (1943) investigated a heating bulk of stored grain, and showed that most of the *Lariophagus distinguendus* individuals were found in the top 25 cm of that grain bulk. Based on this limited amount of information, it might seem that parasitoids of stored product insects tend to occur at the center and near the surface of the stored product. It is likely, however, that their distributions simply follow those of their hosts. The five pest species that were most frequently encountered in a study by Hagstrum *et al.* (1985), for instance, were also most abundant at the central regions of stored wheat.

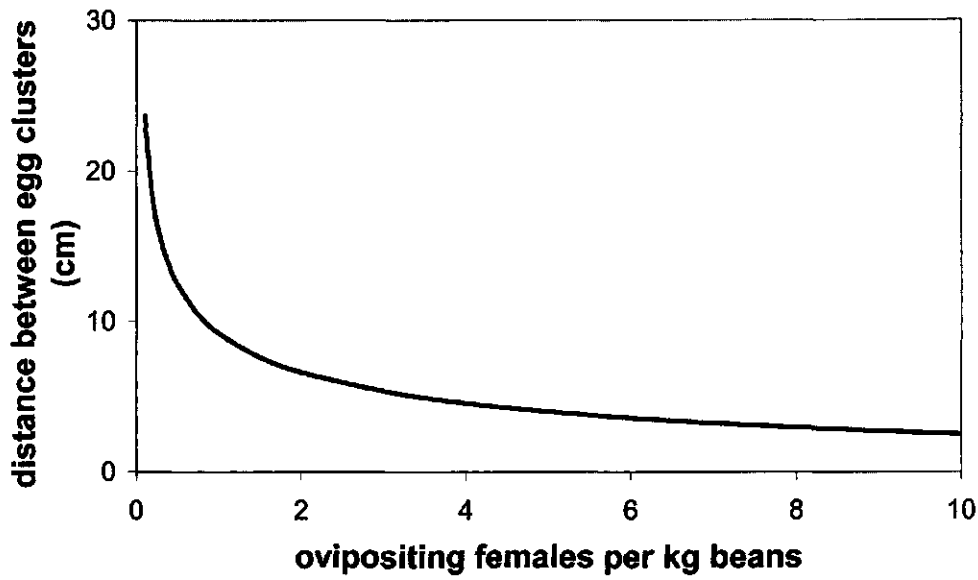

Figure 6. Distance between egg clusters if they are homogenously distributed through a cowpea mass, as a function of *C. maculatus* female density. The specific weight of cowpea is set at 700 kg·m^{-3}.

Host finding capacity in stored product has been studied for several parasitoid species (Schöller *et al.*, 1994, 1996; Schöller, 2000; Brower, 1990; Verma, 1990; Cortesero *et al.*, 1997; Press, 1988, 1992). In most cases, however, parasitoids are released on top of the stored product and only vertical penetration into the stored product is measured. It has, for instance, been shown that *Eupelmis vuilleti* can find bruchid larvae down to at least 77 cm depth when she is released on top of stored cowpea (Cortesero *et al.*, 1997), and that *Trichogramma evanescens* is able to find host eggs up to at least 55 cm depth (Schöller *et al.*, 1994). Exceptions are Steidle & Schöller (2002) and Schöller (2000) who investigated lateral dispersal of *Trichogramma evanescens* and *Lariophagus distinguendus*, respectively, when they were released on top of the stored product. Steidle & Schöller (2002) showed that *L. distinguendus* can find hosts in stored grain at a depth of 50 cm and at a horizontal distance of 4 m (but not at 6 m) from the release point. *T. evanescens* was able to find host patches in stored grain at a depth of 4 cm and at a lateral distance of 80 cm from the release point (Schöller, 2000). The number of parasitized eggs decreased as a function of distance. It is, however, difficult to compare host finding capacity of *U. lariophaga* with these parasitoids because of large differences in methodology.

Relevance of this study for biological control

Lateral dispersal, as studied in this chapter, is relevant if parasitoids should travel from one host patch to another. This is especially important if one does not intend to practice inundative biological control but a conservation strategy of biological control instead (Van Huis *et al.*, 1991a). In an inundative strategy, new parasitoids are regularly released in or on the stored product, and they should move around or down into the seed mass in order to find hosts. If enough parasitoids are released, it is sufficient if each parasitoid finds only one host patch. In a conservation strategy, however, it is important that parasitoids that emerge from hosts inside the stored product also find hosts themselves, which may imply host finding over some distance in the stored product. A conservation strategy has been proposed for *U. lariophaga* (Van Huis *et al.*, 1991a).

It would be interesting to know what distances *U. lariophaga* needs to cover in stored cowpea to find new hosts. We can use the model proposed by McCoy & Powelson (1974) to estimate the distances between ovipositing *C. maculatus* females if we assume that they are uniformly distributed in a cowpea mass:

$$D = 2^{\frac{1}{6}} \left(\frac{V}{\mathcal{N}} \right)^{\frac{1}{3}} \tag{6}$$

where V = volume (cm^3), $\mathcal{N}$ = the number of beetles in volume V, and D = distance between beetles (cm). If we also assume that each female oviposits in a single, spherical cluster with a volume of 19.1 cm^3 (Chapter 2) and with a resulting radius of 3.3 cm, the distance between fresh egg clusters is

$$D = 2^{\frac{1}{6}} \left(\frac{V}{\mathcal{N}} \right)^{\frac{1}{3}} - 3.3 \tag{7}$$

Figure 6 shows the distance between egg clusters as a function of female density according to equation 7. As this Figure shows, the distance between egg clusters declines rapidly as the beetle density increases. In other words, it becomes disproportionally difficult for *U. lariophaga* to find new hosts as host densities become lower. It will therefore be difficult for *U. lariophaga* to exterminate *C. maculatus* in stored cowpea, especially if a conservation strategy of biological control is used.

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CHAPTER 5

C. Stolk, J.J. van der Hout, W. van der Werf & A. van Huis

Host discrimination, superparasitism, and sex allocation by *Uscana lariophaga*

Abstract

In two experiments, we investigated superparasitism in *Uscana lariophaga* (Hym.: Trichogrammatidae), an egg parasitoid of the stored-product pest *Callosobruchus maculatus* (Col.: Bruchidae). In the first experiment, experienced females were individually released into an arena with 15 host eggs. Two series were created: a 'self' series, in which females were confronted with eggs that had been parasitized by themselves only, and a 'conspecific' series, in which females were confronted not only with eggs that had been parasitized by themselves but also with eggs that had been parasitized by others. Logistic regression analysis showed that an encounter with an unparasitized egg in the same arena significantly reduced the probability that a parasitized host egg would be superparasitized. As a result, self superparasitism occurred only twice, whereas conspecific superparasitism was observed 40 times. In the second experiment, experienced females were confronted with a single host egg that had been parasitized by either herself or by another female. In this experiment, self- and conspecific superparasitism were both equally rare: superparasitism occurred in 6% of these no-choice tests. Finally, we show that sex of offspring could be predicted, with 95% reliability, based on observation of the oviposition behaviour. A detailed, quantitative description of this behaviour is given. A short period (4 s) without movement, while the ovipositor was deeply inserted into the host egg, signaled fertilization (females develop from fertilized eggs whereas unfertilized eggs result in males). Using this technique, we show that females had a higher survival probability than males in host eggs that contained both a male and a female parasitoid egg. In addition, females allocated a higher fraction of daughters when superparasitizing compared to when they were ovipositing in non-parasitized hosts. This shows that superparasitizing females can discriminate between parasitized and non-parasitized hosts, implying that superparasitism is not a result of failure in host discrimination but possibly adaptive behaviour.

A slightly modified version of this chapter will be submitted to an international scientific journal as: Stolk, C., Van der Hout, J.J., Van der Werf, W. & Van Huis, A. Host discrimination, superparasitism and sex allocation by *Uscana lariophaga*, parasitoid of *Callosobruchus maculatus* eggs.

Introduction

Callosobruchus maculatus F. (Col.: Bruchidae) is an important pest of stored cowpea (*Vigna unguiculata* [L.] Walpers) in West Africa. Each year, 20-40% of the cowpea seeds that are stored for human consumption in Northern Nigeria become infested with this bruchid (Caswell, 1981). Traditional control methods are usually not sufficient and safe chemical control is often not available for subsistence farmers (Van Huis, 1991). The egg parasitoid *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae) has therefore been proposed as a biological control agent of this pest (Van Huis *et al.*, 1991a).

In experimental cowpea granaries, *U. lariophaga* suppresses *C. maculatus* populations by up to 86% as compared to a control treatment without this parasitoid. Unfortunately, *U. lariophaga* offers a lower level of control at low initial densities of the host (Van Huis *et al.*, 1998). This might be due to difficulties in host finding Chapter 4). Another aspect of low host densities is that superparasitism is more likely to occur, especially if the number of available host eggs is low compared to the number of parasitoids.

Superparasitism is defined as one host being parasitized more than once by either the same individual (self superparasitism) or by another individual of the same species (conspecific superparasitism). Because *U. lariophaga* is a solitary endoparasitoid, superparasitism may influence population dynamics: only one parasitoid can emerge from each *C. maculatus* egg, and the fraction of hosts from which a parasitoid emerges may be lower for superparasitized eggs than for singly parasitized eggs. The prevalence of superparasitism in *U. lariophaga* is debated. Van Huis *et al.* (1991b) found that *U. lariophaga* almost never superparasitized, but superparasitism by *U. lariophaga* was frequently observed in a setup in which females were released sequentially in an arena with host eggs (M.W. van Es & F.A.N. van Alebeek, unpublished data).

Superparasitism by solitary parasitoids has often been regarded as non-adaptive because of reduced survival of the offspring of the superparasitizing female and because many parasitoid species can discrimate between parasitized and non-parasitized hosts (Van Lenteren, 1981). Conspecific superparasitism is, however, adaptive if hosts are scarce and if other females superparasitize as well. A female that does not superparasitize in this case, while the other females do, suffers from a reduced fitness because her offspring is victim of superparasitism of others while she herself, by not superparasitizing, does not increase the relative contribution of her own genes to the next generation (Van Alphen & Visser, 1990). (Note that superparasitized hosts often suffer from increased mortality, killing both the first and the second parasitoid, and that the second larva may sometimes win the com-

petition with the first). Conspecific-superparasitism is also adaptive if a time-limited parasitoid arrives in a host patch that has already been depleted by others and if searching for other patches would probably not increase her number of offspring. Even self-superparasitism, in which a parasitoid parasitizes a host which had already been parasitized by herself before, can sometimes be adaptive, for instance if hosts are thrice parasitized (which can occur when host densities are very low compared to the number of parasitoids). If such triple parasitizations are likely to occur, parasitizing an unparasitized host twice makes it more likely that one of these two eggs will develop into maturity instead of a third egg that is later added by a conspecific female (Van Alphen & Visser, 1990). In general, however, conditions under which conspecific superparasitism is adaptive are wider than those for self-superparasitism.

In this chapter we address the following questions concerning superparasitism of *C. maculatus* eggs by *U.lariophaga*:

(1) Which factors influence the probability that a parasitized host is superparasitized? Superparasitism might be favoured if more hosts in the patch are parasitized. We expect conspecific superparasitism to occur more readily than self superparasitism.
(2) What is the fraction of superparasitized hosts from which neither the first nor the second parasitoid emerges?
(3) Does one of the sexes have a survival advantage in superparasitized hosts? Males could have a higher survival probability than females because they develop slightly faster than females (Van Huis & Appiah 1995; Chapter 4). This has for example been found in *Trichogramma chilonis* (Suzuki *et al.*, 1984).
(4) In conjuction with question (3): Does *U. lariophaga* show a preference to lay a male or female egg when superparasitizing? *U. lariophaga* is an arrhenotokous parasitoid: females are diploid and males are haploid. Consequently fertilized eggs result in females, and unfertilized eggs result in males. Eggs are fertilized individually during oviposition using sperm from the spermatheca.

And, in order to be able to answer questions (3) and (4):

(5) Is fertilization visible during an oviposition? In *Trichogramma* spp., fertilization is visible as a period without movement during the oviposition process (Suzuki *et al.*, 1984; Liu & He, 1991; Luck *et al.*, 2001).

In addition, we give a detailed, quantitative description of the oviposition behaviour of *U. lariophaga.*

Material & methods

General

Cowpea seeds of the variety 'Black Eyes' were used in the experiments and in the rearings. Before use, the beans were frozen and subsequently dried at 45°C, both for at least three days, to exclude any contamination by insects. Splitted cowpea seeds were used in the experimental arenas (see experiments). For convenience we call these seed halves 'cotyledons'.

Callosobruchus maculatus was reared in large petri dishes on cowpea seeds. Each petri dish contained one age cohort, produced by about 35 ovipositing females. The temperature was kept at 35±1°C during photophase and at 25±1°C during scotophase (L12:D12). Beetle eggs were obtained by allowing 0-2d old females to oviposit on cowpea seeds (rearings) or on cowpea cotyledons (experiments). Eggs that were used in the experiments were less than 24 h old.

Uscana lariophaga was reared in glass vials (length 10 cm, ∅ 2 cm) containing cowpea seeds with fresh *C. maculatus* eggs. The rearing was kept at 30±1°C and L12:D12. Unless mentioned otherwise, wasps that were used in the experiments were 1½ - 24 h old and unfed. Females were given opportunity to mate during at least one hour before use in the experiments.

All experiments were carried out at 30±1°C, 30-45% RH, and at a light intensity of $2 \cdot 10^3$ lux, unless mentioned otherwise. All observations were carried out using a stereomicroscope.

Experiment 1: Is fertilization visible?

An unfed and presumably mated 1½ - 8 h old female was released into a glass petridish (∅ 5.4 cm) containing a single cowpea cotyledon carrying 1-3 *C. maculatus* eggs. The behaviour of the wasp during each oviposition was observed; a short period with no movement in the abdomen was assumed to signal fertilization, as in *Trichogramma* spp. (Suzuki *et al.*, 1984). After a training period of 20 observations, sex of the offspring was predicted for 68 ovipositions of 34 females. Each parasitized egg was individually reared, and sex of emerging wasps was determined without prior knowledge of the predicted sex. A 2×2 test of independence, using the G-stastistic, was applied to test for association between predicted and emerged sex (Sokal & Rohlf, 1995).

Experiment 2: Sequential release of females into arenas with 15 hosts

Glass petridishes (∅ 5.4 cm), containing three uninfested cotyledons and five cotyledons with each three *C. maculatus* eggs, were used as arenas. The cotyledons were placed on a layer of fine white sand to prevent wasps from getting underneath

the cotyledons. Each cotyledon was in touch with 2-3 neighbouring cotyledons. A female was released onto one of the uninfested beans. Prior to the experiment the female had been offered, together with other females, a large surplus of unparasitized hosts during one hour, and just before release the female was kept in isolation for about half an hour. The wasp was removed after either 5 ovipositions, 35 minutes of observation (or at most 45 minutes if the last oviposition started just before the 35th minute), if the wasp flew away and remained on the glass wall or lid of the petridish for more than 5 minutes, or if the wasp was standing still (including grooming) for more than 10 minutes. Two to four minutes after the female had been removed, it was either released again, or a new female was released into the same arena. This procedure was executed three times in a row in the same arena. Two treatments, or series, were set up: A 'self' series, in which one female was released three times into the same arena; and a 'conspecific' series, in which three different females were released sequentially into one arena (Figure 1). The host encounters, rejections and parasitizations were recorded for each wasp and each egg. Each egg was individually reared to determine survival and sex of the offspring. We also recorded the behaviour and location of the wasps using a Tandy 102 computer and the computer program The Observer 2.0 (Noldus Information Technology, Wageningen, The Netherlands). The following behavioural elements were distinguished: walking, standing, grooming, flying, and (as part of the the oviposition behaviour) insertion of the ovipositor, rapid movement of the abdominal tip, deepening of the ovipositor (drilling), fertilization, and end of fertilization. The following locations were distinguished: the uninfested cotyledons (taken as one location), the infested cotyledons (numbered 1 through 5), the host eggs (numbered 1 through 3 for each cotyledon), the sand in the petridish, and the walls and lid of the petridish. The 'self' series consisted of 19 replicate arenas and the conspecific series had 22 replicate arenas (66 wasps).

We analyzed a number of covariates for a possible superparasitism-promoting effect, using binary logistic regression. The logistic regression model is defined as (Neter et al., 1996):

$$\text{Probability(event)} = \frac{e^{Z}}{1 + e^{Z}}$$

with $Z = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_p X_p$.

Here X_0 ... X_p are the independent variables or covariates and β_0 ... β_p are the regression coefficients. The covariates may be indicator variables. The regression coefficients are estimated by the maximum likelihood method. The interpretation

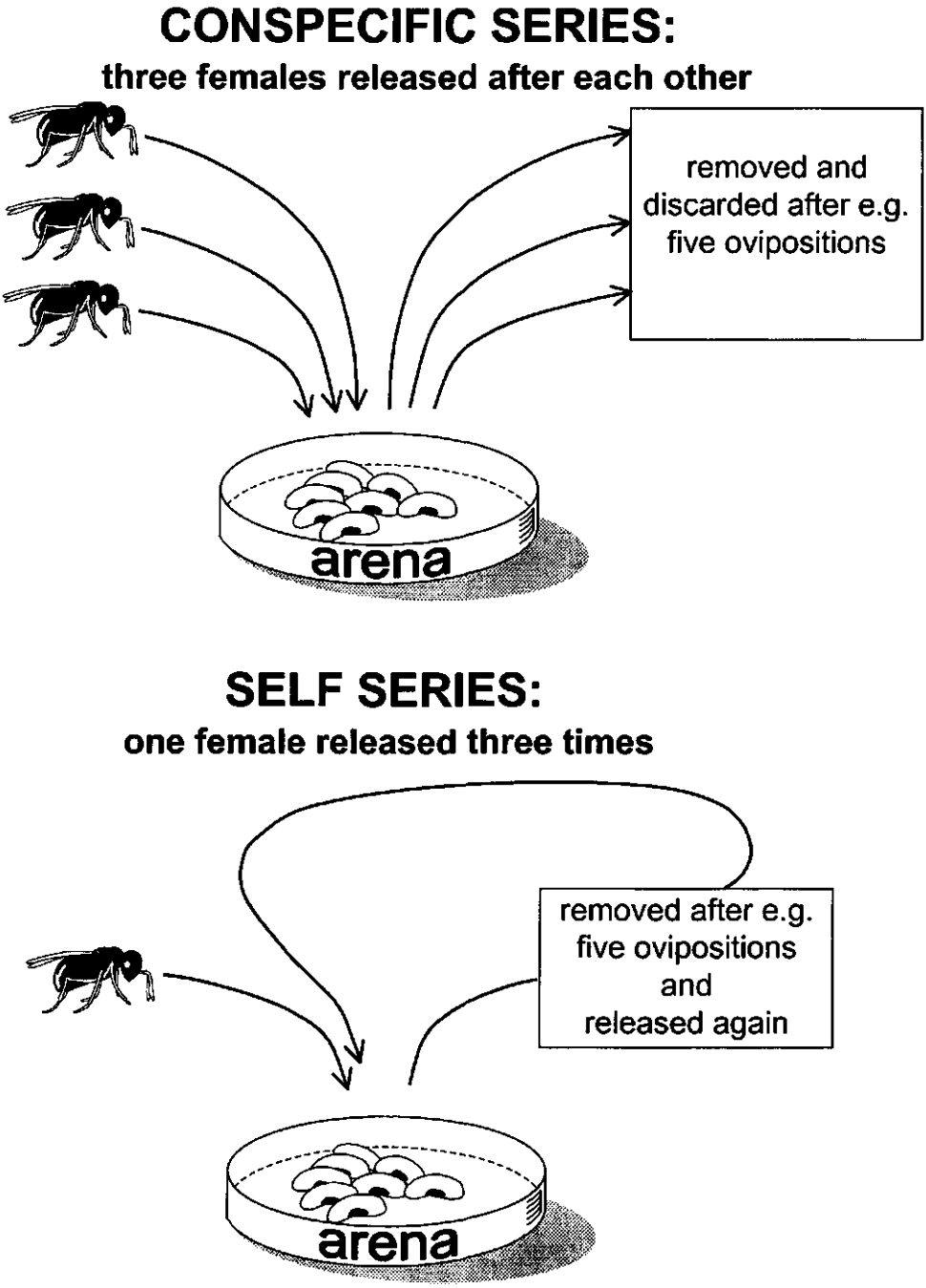

Figure 1. Schematic overview of releases of *U. lariophaga* females in Experiment 2.

of the regression coefficients β_i in this model can be clarified by rewriting the model as

$$\frac{\text{Probability(event)}}{\text{Probability(no event)}} = e^{\beta_0} \cdot e^{\beta_1 X_1} \cdot e^{\beta_2 X_2} \cdot \ldots \cdot e^{\beta_p X_p}$$

The left-hand term in this expression, the probability of the event occurring divided by the probability of the event not occurring, is called the 'odds'. Now e raised to the power β_i has a clear interpretation: it is the factor by which the odds change if the covariate increases by one unit.

The outcomes of encounters with parasitized hosts were the events used as the response variable in the logistic regression model. Each encounter resulted in either rejection (0) or superparasitizion (1). If, during a parasitoid release, a given parasitized host was encountered more than once, only the decision at the first encounter was used as dependent variable, to avoid pseudoreplication. From now on we use the term "first encounter" to indicate, for each parasitized host, the first time this particular host was encountered by a parasitoid during one release. The covariates that were used in the model are: was the host parasitized by the current female or by a conspecific female (0 or 1); had the current female already encountered an unparasitized host in this arena (0 or 1); the number of encounters with unparasitized hosts, with parasitized hosts, and with all hosts; the number of rejections; the number of parasitized hosts encountered; the number of parasitized hosts present in the arena; and the release number (1, 2 or 3). Covariates to be included in the regression model were selected using an automatic forward stepwise procedure, with the likelihood ratio as selection criterion (SPSS 10.0 for Windows). Non-parasitized eggs that were consistently rejected by the parasitoids were omitted from the analysis because these eggs appeared to have been damaged during handling.

The superparasitizations that occurred in this experiment were also used to study whether the fraction of hosts from which a parasitoid emerges is lower for superparasitized than for singly-parasitized eggs, whether one of the sexes has higher survival probabilities in superparasitized eggs, and whether females preferentially allocate one of the sexes during superparasitism. These questions were addressed using G-tests (Sokal & Rohlf, 1995).

Experiment 3: Exposure of females to a single host

A glass petridish (∅ 5.4 cm), lined with a layer of fine sand and containing one uninfested cotyledon and one cotyledon with one *C. maculatus* egg, served as arena. A female which had had access, together with other females, to a large susplus of fresh hosts during about one hour, was released onto the uninfested cotyledon,

and it was observed until it found and parasitized the egg on the other cotyledon. After parasitization, it was removed, and after at least 30 minutes it was released again into either the same arena (containing the egg parasitized by herself), or into an arena which contained an egg parasitized by another female, or into an arena which contained an unparasitized egg. This second release was the actual experiment. The wasp was observed until she found the egg and either rejected it or accepted it for parasitization. The 'self' treatment had 17 replicates, and the 'conspecific' and 'unparasitized' treatments each had 18 replicates. Due to hot and humid weather conditions, the temperature during this experiment was 34±1°C and the relative humidity varied between 45 and 50%.

Results

Experiment 1: Is fertilization visible?

Fertilization was visible as a period of about 4 seconds without movement while the ovipositor was deeply inserted into the host egg. Table 1 shows that sex of the offspring could be predicted with 61/64 = 95% reliability ($p<0.001$, G-test). See Experiment 2 for a detailed description of the oviposition behaviour.

Experiment 2: Sequential release of females in arena with 15 hosts

Factors favouring superparasitism. In the experiment in which females were sequentially released into arenas, about four or five parasitizations occurred during each release (Table 2). Superparasitism was more common in the conspecific series than in the self series. In the self series, only one out of 343 first encounters with parasitized hosts resulted in (self-)superparasitism. It was therefore not possible to analyse which factors favoured superparasitism in the self series.

In the conspecific series, 41 superparasitizations by 24 females were observed. Of these 41 superparasitizations, 37 occurred on first encounter (out of 324 first encounters) and were included in the binary logistic regression analysis. The remaining four superparasitizations occurred on second encounter. Of all 324 first encounters, 166 were encounters with conspecific-parasitized eggs. Except for one, the 41 superparasitizations in this series were conspecific-superparasitizations. Of those 40 conspecific-superparasitizations, 35 were carried out by females which had not yet encountered an unparasitized egg in the arena. Two eggs were thrice parasitized.

Based on the likelihood ratio, two covariates were included in the binary logistic regression model: (1) whether the egg had been parasitized by the current female (self, coded as 0) or by a previous female (conspecific, coded as 1) and (2)

Table 1. Number of wasps emerging from parasitized hosts in Experiment 1: predicted and 'emerged' sex.

Predicted sex	Emerged sex		
	female	male	not emerged
female	39	2	3
male	1	22	1
Total	40	24	4

Table 2. Average number of eggs parasitized and superparasitized per release in Experiment 2.

release or female	# eggs singly parasitized	# eggs superparasitized
	SELF SERIES (N = 19)	
release 1	4.8	0.1
release 2	4.4	0.1
release 3	4.2	0.0
	CONSPECIFIC SERIES (N = 22)	
female 1	5.0	0.0
female 2	3.6	1.0
female 3	3.4	0.9

whether or not the current female had already encountered an unparasitized host in this arena (coded as 1 and 0, respectively). The odds of superparasitism increased, but not significantly, if the egg was parasitized by a conspecific female ($\exp[\beta]=4183$, $p=0.7$, Wald-test), and the odds of superparasitism decreased significantly if the female had encountered an unparasitized egg in the arena ($\exp[\beta]=0.054$, $p<<0.001$, Wald-test). Thus, the odds of superparasitization occurring upon an encounter with a parasitized host decreased by 95% if the parasitoid had already encountered an unparasitized egg in the arena. The intercept $\exp(\beta_0)$ was estimated as 0.0 ($p=0.7$, Wald-test). The Nagelkerke R^2 is 0.52, which means that 52% of the 'variation' is explained by this model.

Survival in superparasitized hosts. No parasitoid emerged from 22% of the superparasitized hosts ($\frac{8}{39\text{-}2}$, Table 3) whereas this figure is 9% for singly parasitized hosts ($\frac{45}{518\text{-}27}$, Table 4). This difference is significant ($p=0.03$, G-test). There was no difference in mortality between males and females in singly parasitized hosts (Table 4, $p=0.20$, G-test). In superparasitized hosts that contained both a male and female egg, the female survived in 13 out of 16 cases (Table 3). Although in these cases the female egg was also often the second egg (see next paragraph), a binary logistic regression analysis showed that oviposition order (first vs. second oviposition) did not determine which sex survived ($p=0.25$, Wald-test). In superparasitized eggs,

Table 3. Emergence data for superparasitized hosts in Experiment 2.

Predicted:	1st oviposition	Female		Male		Total
	2nd oviposition	Female	Male	Female	Male	
Emerged	Female	11	4	9	0	24
	Male	1	1	2	1	5
	Not emerged	4	0	4	0	8
	Lost	1	0	1	0	2
	Total	17	5	16	1	39

Table 4. Emergence data for singly parasitized hosts in Experiment 2.

Emerged	Predicted		Total
	Female	Male	
Female	291	12	303
Male	15	128	143
Not emerged	27	18	45
Lost	20	7	27
Total	353	165	518

therefore, females had a significantly higher survival probability than males (13 vs. 3, p=0.02, G-test). Unfortunately, some uncertainty was involved in sex prediction. Table 4 shows that 9% of the predicted males in Experiment 2 turned out to be females. This implies that some predicted males in the mixed-parasitized eggs might actually have been females, causing an overestimation of the survival probability of females. Stochastic simulations in which this inaccuracy in sex prediction is taken into account, show, however, that the probability of detecting a survival advantage of one of the sexes while both sexes actually have equal survival probabilities, is only 2.4% (see Appendix). We may therefore safely conclude that females had a survival advantage in hosts that contained both a male and a female parasitoid egg.

Sex allocation during superparasitism. In 85% of the cases, the superparasitizing wasp deposited a female egg in an already parasitized host ($\frac{17+16}{39}$, Table 3), whereas the average sex ratio for single parasitizations is 68% females ($\frac{353}{518}$, Table 4). The sex ratio, however, also changes with oviposition number (Figure 2). The first egg that is laid by a female in an arena is usually a male. Since most superparasitizations were also first ovipositions, we compared the sex ratio of ovipositions in unparasitized hosts with the sex ratio of ovipositions in parasitized hosts, for the first oviposition in releases 2 and 3 of the conspecific-series. If the first oviposition occurred in an unparasitized host, the sex ratio was 36% females; if it occurred in

a parasitized host, the sex ratio was 95% females (Figure 2b). Thus, the fraction of females that was allocated during oviposition was significantly higher for parasitized hosts than for unparasitized hosts ($p<0.001$, G-test). For the combined second and third ovipositions, on the other hand, the percentage females did not differ between superparasitizations and ovipositions in unparasitized host (59% versus 77%, N=17 and 43, respectively; $p=0.29$, G-test)

Some hosts were damaged during handling and a few parasitoids escaped before they could be sexed; this accounts for te 'lost' parasitoids in Tables 3 and 4.

Oviposition behaviour. Several stages in oviposition behaviour could be distinguished. The following description is based on the observation of 556 ovipositions that took place in Experiment 2. When a female encountered a host egg, the wasp walked onto the egg and started walking in circles while drumming with the antennae on the surface of the egg. This behaviour lasted 10 ± 5 s (mean ± SD) before an oviposition and 1 ± 5 s before a rejection. If the egg was accepted for oviposition, the behaviour continued with *insertion of the ovipositor* into the egg (54 ± 75 s). The ovipositor was extended and placed on the surface of the egg. The wasp moved back and forth while the tip of the ovipositor remained on the same spot. If one or more legs slided, the wasp often shifted these legs, but occasionally oviposition was restarted at another location on the egg. Once the ovipositor had been inserted into the host egg, the tip of the abdomen showed *rapid movement*, both up and down and from side to side (20 ± 15 s). On the whole, body movement was slower than during insertion of the ovipositor. This behaviour was followed by a *slow jerky movement*, during which the abdominal tip was lowered and jerked back up again, several times in a row (for duration of this stage and following stages, see below). Body movement slowed down and although the ovipositor was moving, it was not inserted any deeper until the next stage. The next stage was therefore called *deepening of the ovipositor.* The ovipositor was inserted more deeply, often in a sliding fashion, and the abdomen was stretched until it almost touched the surface of the egg. In fact, hairs on the tip of the abdomen frequently touched the egg. If a female egg was laid, deepening of the ovipositor was followed by *fertilization.* During fertilization, body movement ceased completely for 4 ± 2 s. The abdomen remained stretched and was slightly contracted. The hind legs were pressed to the sides of the abdomen. If a male egg was laid, no fertilization occurred and deepening of the ovipositor was followed immediately by the next stage, *withdrawal of the ovipositor.* The moment when the egg was laid was not visible, but after the deepest point had been reached, frequency of body movements increased, after which the ovipositor was withdrawn. If no fertilization occurred, it was not clear when withdrawal of the ovipositor started; for this reason duration of withdrawal was measured sepa-

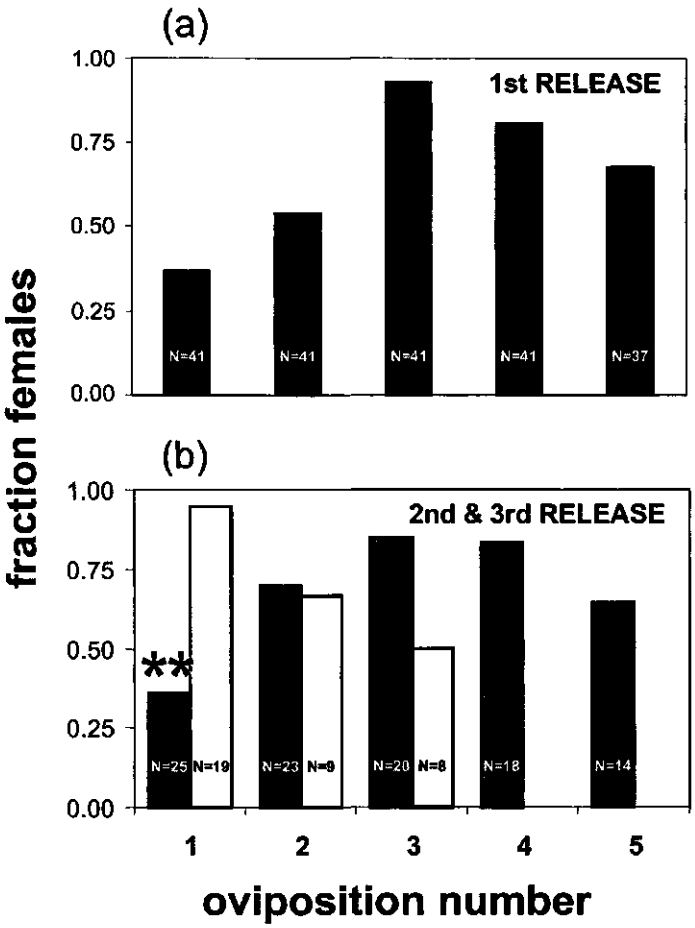

Figure 2. Sex ratio as a function of oviposition number for **(a)** the first release in the conspecific and self-series, and **(b)** the combined second and third releases in the conspecific-series for ovipositions in unparasitized hosts (grey columns) and ovipositions in parasitized hosts (white columns). Females that started superparasitizing were omitted from the 'unparasitized host' data from that moment onwards. Note: if the second or third oviposition was a superparasitization, then this does not imply that the preceding ovipositions were also superparasitizations. ** = $p<0.01$.

rately only for female eggs (17 ± 8 s) and not for male eggs. Moreover, slow jerky movement and deepening of the ovipositor were not separated during observations. For female eggs, these two behavioural elements lasted 24 ± 11 s in total. For male eggs, slow jerky movement, deepening and withdrawal of the ovipositor were taken together and lasted 36 ± 15 s. When the ovipositor had been withdrawn from the egg, the wasp walked back a few steps and immediately started intensive grooming of especially the abdominal sides, using the hind legs. Antennae were

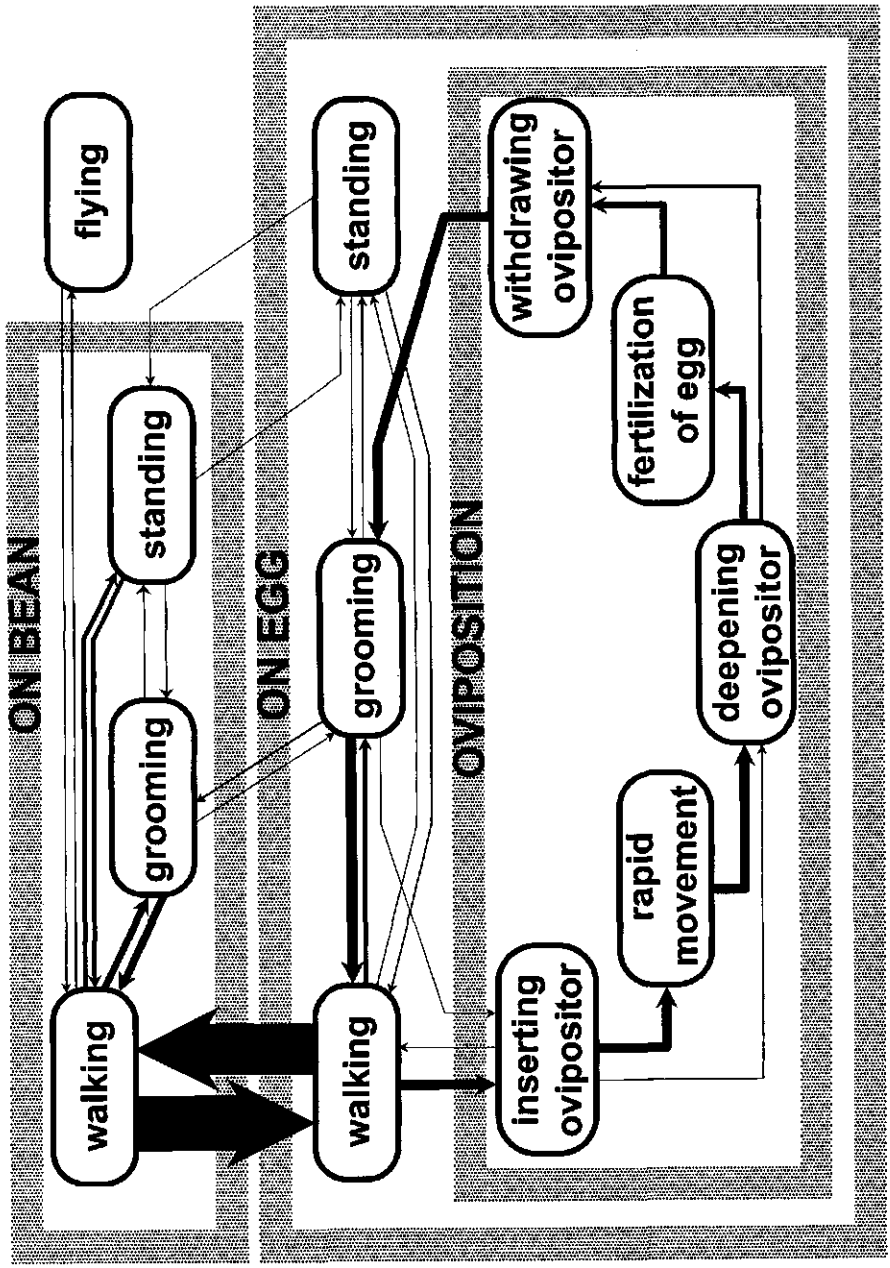

Figure 3. Flow diagram of the behaviour of *U. lariophaga* as observed in Experiment 2. Arrow width is proportional to the frequency with which the involved transitions between behavioural elements occurred. The diagram is based on 11,257 transitions and involves 556 ovipositions by 85 females. Transitions that occurred fewer than 10 times are not shown.

groomed only occasionally, using the front legs. This *post-oviposition behaviour* lasted 38 ± 75 s.

Figure 3 shows that the most important behavioural transitions were between walking on bean and walking on egg, between walking on egg and oviposition, between oviposition and grooming on egg, between grooming and walking on egg, between walking and grooming on bean, and between walking and standing on bean.

Experiment 3: Exposure of females to a single host

Females almost always rejected hosts that had already been parasitized by either herself (16 rejections out of 17 replicates) or by a conspecific female (17 rejections out of 18 replicates). Unparasitized hosts, on the other hand, were always accepted for parasitization (N=18). Conspecific-superparasitism occurred less frequently than in Experiment 2, where 22% of the encounters with conspecific-parasitized hosts had led to superparasitism; but the difference was not significant ($p=0.11$, G-test). The frequency of conspecific superparasitism in Experiment 3 is, however, lower than the frequency with which it occurred if we look at only the first oviposition in the 2nd and 3rd release of the conspecific series of Experiment 2 ($\frac{19}{25+19}$ = 43%, see Figure 2) ($p<0.001$, G-test).

Discussion

Sex of offspring of *U. lariophaga* could be predicted based on the presence or absence of visible fertilization behaviour during oviposition. Although similar behaviour has been described for among others *Trichogramma* spp. and for pimpline ichneumonids (Suzuki et al., 1984; Ueno, 1995), it had not yet been described for *Uscana* spp.

Conspecific-superparasitism occurred more often than self-superparasitism. Yet the only factor which significantly influenced superparasitism was whether an unparasitized host had been encountered in the arena or not. If no unparasitized host had yet been encountered, an encounter with a parasitized host often resulted in superparasitism. Self-superparasitism, therefore, hardly occurred in the 'conspecific' series: once an unparasitized host had been encountered and parasitized it was almost never parasitized again by the same female. A decreased tendency to superparasitize after recent encounters with unparasitized hosts has also been found in many other parasitoids, including trichogrammatids (Van Lenteren, 1981; Klomp *et al.*, 1980).

Superparasitism occurred much more often in the conspecific series than in the

self series. These two series can, however, not be compared directly, since the parasitoids in the self series gained more parasitization experience during the experiment than those in the conspecific series. Experiment 3 did not have this disadvantage. In this experiment self and conspecific-superparasitism were both equally rare. The fact that one female (out of 17) in this experiment self-superparasitized is somewhat unexpected, since she had already encountered an unparasitized host in that arena. This seeming discrepancy between the two experiments might be explained from the fact that parasitoids in the self series of Experiment 2 often had several encounters with unparasitized hosts before they re-encountered a parasitized host, and before they were re-released in the arena they had had up to five ovipositions, whereas in Experiment 3 they had had only one oviposition in the arena before they were re-released. Experiment 3 does confirm, in any case, that whether a host is parasitized by 'self' or 'conspecific' does not significantly influence the probability of superparasitism.

The fact that *Uscana lariophaga* adapts the sex ratio of the offspring when it is superparasitizing indicates that superparasitizing females can distinguish between parasitized and unparasitized hosts. Superparasitism by experienced females is therefore probably not due to failure in host discrimination; it might even be adaptive behaviour. The pattern of superparasitism that is displayed by *U. lariophaga* could be an adaptive strategy if *U. lariophaga* is time-limited and unable to distinguish self from conspecific-parasitized hosts. If the first host that is encountered in a patch is already parasitized, it is quite likely that more hosts in the patch are also already parasitized. A parasitoid that has many eggs to spend but that has little time available to search for another patch or for unparasitized hosts in the same patch should then superparasitize that first host with relatively high probability. For this first host it is also certain that she has not yet parasitized it herself. As soon as she has encountered an unparasitized host, however, chances are that more unparasitized hosts are available. Because more unparasitized hosts may be availabe, and to avoid the risk of self-superparasitism, she should then become more reluctant to superparasitize. This simple rule seems adequate to describe many of the oviposition decisions of *U. lariophaga*. Indeed, this short-lived (48 h) parasitoid with high fecundity (40-80 eggs) will mostly be time-limited; and based on our results, it is not clear whether *U. lariophaga* can distinguish between self- and conspecific-parasitized eggs. It is quite possible that self-superparasitism is avoided as a result of this rule. There is a number of trichogrammatids which seem to lack the ability to distinguish self- from conspecific-parasitized hosts (Van Dijken & Waage, 1987; Luck *et al.*, 2001). A prerequisite for this rule to be successful is that either the probability of visiting one patch more than once should be very low, or females have to be able to recognize host patches that they have visited earlier. If, on the

other hand, a second female enters the patch while the first is still there, and if the second female starts superparasitizing, this rule does not suffice. It would then be advantageous for the first female to be able to distinguish self- from conspecific parasitized eggs.

We do not know how females can distinguish parasitized from unparasitized hosts, but it is likely that the host is somehow marked by ovipositing females. Such marking behaviour has been described for many parasitoids, and often involves the use of pheromones (Nufio & Papaj, 2001; Hoffmeister, 2001; Rosi *et al.*, 2001; Höller *et al.*, 1993). In *U. lariophaga*, marking might take place during the post-oviposition behaviour, which consists of a combination of grooming and walking on the host. M.W. van Es and F.A.N. van Alebeek (unpublished data) found that *U. lariophaga* spends significantly less time on post-oviposition behaviour after a superparasitization than after an oviposition in an unparasitized host. Host marking might also take place during one of the other stages of the oviposition behaviour (Figure 3). In addition to a chemical marker, the presence of an oviposition hole and the physical presence of an egg inside the host might somehow be perceived by the parasitoid (Van Lenteren, 1981).

The sex allocation in unparasitized and parasitized hosts displayed by *U. lariophaga* is unusual. Many parasitoid species typically produce a female at the beginning of an oviposition sequence in a patch (Godfray, 1994), whereas in our experiments the first egg was usually a male. A similar exception has also been found in the scelionid egg parasitoid *Gryon pennsylvanicum* (=*atriscapus*) (Waage, 1982). According to Godfray (1994), the production of males early – but not as first – in an oviposition sequence may be a good strategy "for small egg parasitoids if they are unable to assess the size of the egg mass prior to oviposition because it ensures that they never run out of host eggs before they have laid at least one son to inseminate their daughters". As for sex allocation in parasitized hosts, Local Mate Competition (LMC) theory predicts that superparasitizing parasitoids should produce a more male-biased sex ratio than those parasitizing unparasitized hosts (Godfray, 1994). This has indeed been found for many parasitoids. *U. lariophaga* has all the other characteristics that are usually associated with LMC: it develops on clustered hosts, males develop slightly faster than females, and males tend to stay at the emergence site for perhaps their entire lifetime (Chapter 4). The current study shows, however, that superparasitizing *U. lariophaga* females produce a more female-biased sex ratio, at least during the first oviposition. Perhaps an argument, similar to Godfray's (1994), can be set up for superparasitizing females: If the first host they encounter in a patch is already parasitized, there is a high probability of a male egg already being present, because the first egg laid in unparasitized hosts is usually a male. The first egg for superparasitizing females that are

unable to assess the size of the host patch prior to oviposition should then be a female (which, by the way, also has the highest survival probability). Subsequent ovipositions in parasitized hosts, however, should produce more males which can compete with the other males, as LMC theory predicts.

Belinsky (cited in Southgate, 1979) mentions an unidentified *Uscana* sp. in which up to four wasps can emerge from one host egg, the host being *Caryedon serratus paleastinicus*. *Uscana lariophaga*, on the other hand, is a strictly solitary parasitoid on *C. maculatus* eggs. Never did more than one parasitoid emerge from a superparasitized egg. Contrary to our expectation, females had a higher survival probability than males in such superparasitized hosts. The mechanism by which females are able to outcompete males is not clear.

Based on our results, the importance of superparasitism for population dynamics and biological control will in most cases be limited. Superparasitism was a relatively rare event in our experiments, even in cases where the host patch was largely depleted. Avoidance of superparasitism can be regarded as a form of mutual interference, which generally has a stabilizing effect on population dynamics (Visser & Van Driessen, 1991; Godfray & Hassell, 1994). On the other hand, *U. lariophaga* tends to parasitize almost all hosts in a host cluster (Van Alebeek *et al.*, 1996b). If a female that has never encountered an unparasitized host then finds such a depleted host patch, she is likely to superparasitize many hosts in that patch. In those cases where hosts are double-parasitized, substantial mortality occurs: out of two eggs, only 0.8 wasp develop (Table 3). This implies a mortality of 60%. In cases where host densities are low and many naive parasitoids occur, superparasitism may therefore have a negative impact on *U. lariophaga* population persistence.

Appendix

The simulation model that was used to assess the implications of the uncertainty in sex prediction follows an approach advocated by Hilborn & Mangel (1997). The program mimics part of Experiment 2 by simulating 37 cases of superparasitism, including sex allocation, the (not always accurate) observation of sex allocation by an observer, and survival of one of the sexes in mixed-parasitized eggs. The program assumes equal survival probabilities for males and females and it calculates in how many cases a G-test wrongly detects inequal survival probabilities for males and females. A pseudocode for these stochastic simulations, in conformity with Hilborn & Mangel (1997), is:

1. Parasitize a host with either a female egg ($\frac{17+5-1}{39-2} = 57\%$ probability, Table 3) or a male egg (43% probability).

2. Parasitize the same host again (superparasitism) using either a female (84% probability) or a male (16% probability) egg.
3. Simulate the determination of the sex of both eggs by an investigator. If the actual sex of an egg that was allocated during an oviposition is female, then this is accurately predicted in $\frac{291}{303} = 96.0\%$ of the cases; if it is a male, the sex is accurately predicted in $\frac{128}{143} = 89.5\%$ of the cases (Table 4).
4. If a host is predicted to contain both sexes, then:
   a. Both parasitoids die in $\frac{8}{39-2} = 22\%$ of the cases (Table 3);
   b. For the remaining 78%, randomly choose one of the parasitoids to survive. Both sexes have assumed equal survival probabilities. If the egg actually contained two females, a female survives, and if it contained two males, a male survives.
5. Repeat steps 1 to 4 until a series of 37 superparasitized hosts has been created ($39 - 2 = 37$, Table 4).
6. Calculate in how many cases a female survived from mixed-parasitized eggs and in how many cases a male survived, and perform a G-test using these data.
7. Repeat steps 5 and 6 until 100,000 series of superparasitized hosts are obtained, and calculate in how many cases the G-test ascribed a survival advantage to one of the sexes (while both sexes actually had equal survival probabilities in the simulations).

Note that steps 1 - 4b are stochastic processes: each time the program comes across these steps, the involved events have the same probability of occurring.

In only 2.4% of the 100,000 iterations did the G-test wrongly detect a survival advantage for one of the sexes. If we look at only the 5,449 cases in which exactly 16 mixed-parasitized hosts survived (as in Experiment 2), this percentage is 2.3%. Thus, the probability that males and females had equal survival probabilities in mixed-parasitized eggs in Experiment 2 is very small, considering the outcome of the experiment. The null hypothesis of equal survival probabilities for males and females is therefore rejected.

Acknowledgements

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CHAPTER 6

C. Stolk, S.A. Khamis, W. van der Werf & A. van Huis

A slightly modified version of this chapter will be submitted to an international scientific journal as: Stolk, C., Khamis, S.A., Van der Werf, W. & Van Huis, A. Survival and walking activity of *Uscana lariophaga* at different host densities.

Survival and walking activity of *Uscana lariophaga* at different host densities

Abstract

We studied whether a trade-off between reproduction and survival exists in *Uscana lariophaga* (Hymenoptera: Trichogrammatidae), an egg parasitoid of *Callosobruchus maculatus* (Coleoptera: Bruchidae) in stored cowpea. We found, however, the opposite: females that had access to 98 hosts lived significantly longer than females that had access to 0 or 10 eggs (45 versus 33 h on average). Total lifetime fecundity was 61 parasitized eggs for females kept at high host density and 17 parasitized eggs for females kept at low host density. This reduced longevity at zero or low host density may be a consequence of intense searching, since females at low density displayed significantly more walking activity than females at high host density. The results are discussed in the light of biological control and of quality control of parasitoids.

Introduction

Callosobruchus maculatus Fab. (Coleoptera: Bruchidae) is an important pest of traditionally stored cowpea (*Vigna unguiculata* (L.) Walp.) in West Africa (Jackai and Daoust, 1986). In northern Nigeria, for instance, an estimated 20-40% of the stored cowpea seeds become infested with *Callosobruchus maculatus* every year (Caswell, 1981). Traditional methods to protect stored cowpea – such as the use of insecticidal plants – are often not effective, and safe chemical control is not within reach for many West African subsistence farmers (Van Huis, 1991). An indigenous parasitoid of *C. maculatus* eggs, *Uscana lariophaga* Steffan (Hymenoptera: Trichogrammatidae), has therefore been proposed as a biocontrol agent (Van Huis *et al.*, 1991a). In experimental granaries, a one-time inoculation of *U. lariophaga* suppressed *C. maculatus* populations by up to 86% after three months, as compared to the control (Van Huis *et al.*, 1998). It seems, however, that the density of the host is a critical factor in the level of control that is achieved by the parasitoid. *U. lariophaga* is less effective at low host densities, probably because it has more difficulty in finding hosts at these low densities (Van Huis *et al.*, 1998; Chapter 4).

It would be advantageous for the parasitoid if it were able to live longer at low host densities, since this would increase its chances of host finding. An increased longevity at low host densities occurs if there is a trade-off between reproduction and survival. Such a trade-off has been found in other insects (Bell and Bohm, 1975), and there is also evidence for its existence in *U. lariophaga*. Van Huis *et al.* (1991a) and H.M. Maes (unpublished results) mention that *U. lariophaga* lived slightly longer in a treatment without hosts and without cowpea seeds than in a treatment with cowpea cotyledons carrying host eggs. Because their 'no host' treatment also lacked cowpea cotyledons, however, we do not know for certain which factor was responsible for this increased longevity. In this chapter we therefore investigate in more detail whether a trade-off between reproduction and survival exists in *U. lariophaga* females, by measuring longevity at different host densities. We expect that females live longer when no or few hosts are present than when many hosts are present. Because results from our experiments caused us to suspect that survival might be linked to walking activity, we also investigated walking activity at different host densities.

Materials & Methods

General

Cowpea seeds of the variety 'Black Eyes' were used in the experiments and in the rearings. Before use, the beans were frozen and subsequently dried at 45°C, both for at least three days. This was done to exclude any contamination by insects.

Both the *Callosobruchus maculatus* and *Uscana lariophaga* strains had been collected around Niamey, Niger, and had been reared for about 100 generations. *Callosobruchus maculatus* was reared in petridishes on cowpea seeds at L12:D12. The temperature was kept at 35±1°C during photophase and at 25±1°C during scotophase. Beetle eggs for use in the experiments were obtained by allowing 0-2d old females to oviposit on cowpea seeds fitted into paper strips (survival experiment) or on cowpea cotyledons (walking experiment).

Uscana lariophaga was reared in glass vials containing cowpea seeds with fresh *C. maculatus* eggs. Wasps in the rearing were provided with honey, but wasps that were used in experiments did not have access to honey. The rearing was kept at L12:D12 and at 30±1°C throughout. *Uscana lariophaga* females for use in the experiments were obtained by isolating beans with parasitized eggs in a glass vial. Age of the wasps was standardized by removing these beans after a predetermined number of hours. The females which had emerged during this time interval were allowed at least one hour for mating before they were used in the experiments. In the survival experiments we wanted the age of the wasps at the start of the experiment to be as uniform as possible. For that reason we used used narrow time frames for emergence in these experiments: 4:00 - 8:00 am in the first experiment and 23:00 pm - 7:00 am in the second experiment (see below). In the walking experiments, wasps were allowed to emerge between 18:00 pm - 9:00 am.

All experiments were carried out at 30±1°C, 30-40% RH and at L12:D12. Any observations during scotophase were carried out using red light.

Survival

Freshly emerged *U. lariophaga* females were kept individually in glass vials (length 5.5 cm) which were closed with a plug of cotton wool. Each vial was supplied with a cowpea seed that held either no host eggs, or 10 ± 2 (SD) or 98 ± 19 (SD) host eggs. These treatments were called 'no host', 'low density' and 'high density', respectively. The cowpea seed was tightly wedged into a hole in a paper strip; the paper strip, in turn, fitted tightly into the glass vial. This prevented the seeds from rolling over, which might have killed wasps prematurely. The paper strip with the cowpea seed was replaced every 24 h, until the wasp was dead. Survival of the wasps was recorded every 12 h. Eggs that had been parasitized by the wasps were

counted after about four days, when the parasitized eggs had turned black. The experiment was carried out twice. The first experiment consisted of 7, 7 and 8 replicates for the no host, low density and high density treatments, respectively. In the second experiment these respective treatments had 28, 26 and 27 replicates.

Walking activity

Single *U. lariophaga* females were individually released in petridishes (diameter 5.3 cm) containing a piece of filter paper with four cotyledons glued onto it, using Pelikan gum. Cotyledons were used instead of complete seeds in order to facilitate video recordings of the parasitoid's walking behaviour. Each petridish contained either 8 ± 2 (SD) or 94 ± 24 (SD) host eggs, distributed over the four cotyledons. These treatments were called 'low density' and 'high density', respectively. Each petridish was recorded on videotape six times, at 1 h intervals, each time for 90 s. The amount of time that the wasp was walking out of those 90 s was measured using a stop watch. Later on, the videotape was played and the walking trajectory of each parasitoid during each 'take' was traced with a marker on a polyethylene sheet that was stuck onto the monitor screen. (We did not use automated video analysis software because previous experiments had shown that such software could not properly keep track of these 0.5 mm small wasps on cowpea seeds with black spots; see Van Alebeek and Groot, 1997). The length of the walking trajectory was measured and converted into mm using the appropriate conversion factor. The experiment was carried out twice, on two different days. On day 1 the low and high density treatments had 6 and 5 replicate wasps, respectively; on day 2 these respective treatments had 7 and 8 replicate wasps.

Statistical analysis

The response variable that was used in the survival experiment was longevity, measured in hours. Longevity was expressed for each individual as

$$\tfrac{1}{2}(t_{alive} + t_{dead}) - \tfrac{1}{2}(t_{emergence,start} + t_{emergence,\,end})$$

with t_{alive} = last time when the wasp was still observed to be alive, t_{dead} = time when the wasp was found dead, $t_{emergence,start}$ = the time when beans with emerging wasps were isolated, and $t_{emergence,end}$ = the time when emergence of new females was ended by removing the beans with parasitized eggs. If, for example, emergence of wasps started at time $t = 0$ h, and it ended at $t = 8$ h, and if a wasp which emerged in this time interval was last seen alive at $t = 58$ h and it was dead at $t = 70$ h, then the total longevity of this wasp was (58+70)/2 - (0+8)/2 = 60 h.

We tested for an effect of the treatment on the longevity of the wasps using a

parameter-free Kruskal-Wallis test. We also compared treatments pairwise using a Mann-Whitney U test. Longevity was further analyzed using Cox regression analysis (see Chapter 4). According to Cox regression analysis, the probability of dying per unit of time can be expressed for the current situation as

$$h(t,z_1,z_2) = h_0(t)\cdot e^{\beta_1 z_1+\beta_2 z_2}$$

where h, the hazard rate (time^{-1}), is a function of time t and of the covariates z_1 and z_2. The latter covariates are indicator variables which can only take values of 0 or 1 for a given treatment; no host, low density and high density are coded as $(z_1, z_2) =$ (0,0), (1,0) and (0,1), respectively. The baseline hazard, $h_0(t)$, is the probability of dying, per unit of time, when no hosts are present. The effects on the hazard rate at low and high density, compared to the baseline hazard, are given by $\exp(\beta_1)$ and $\exp(\beta_2)$, respectively.

For the walking experiment, we analysed the effect of host density on the number of times a wasp had walked (out of the six takes) using ANOVA with 'host density' as fixed factor, 'day' as random factor, and no interaction. Total time walked and the total distance walked during the six takes were first square root-transformed and then analyzed using the same ANOVA model. Square root transformation was necessary because the variance was not homogeneous among treatments according to Levene's test ($p<0.01$). After transformation variance was homogeneous ($p>0.05$, Levene's test) and the error term was normally distributed ($p>0.05$, Kolmogorov-Smirnov with Lilliefors correction). We furthermore compared the time and distance walked for each 'take' between low and high host density, using the Mann-Whitney U test. A t-test was not applicable because the response variables were not normally distributed ($p<0.05$, Kolmogorov-Smirnov with Lilliefors correction).

Unless mentioned otherwise, statistical tests were evaluated at a significance level of 0.05.

Results

Survival

In both survival experiments longevity was affected by host density ($p<0.01$ and $p<0.001$, respectively; Kruskal-Wallis) (Figure 1a,b). Averaged over both experiments, wasps lived 45 h at high host density, and only 33 h when no or few hosts were present. The difference in longevity between high host density on the one hand, and no hosts or low host density on the other hand, was significant in both

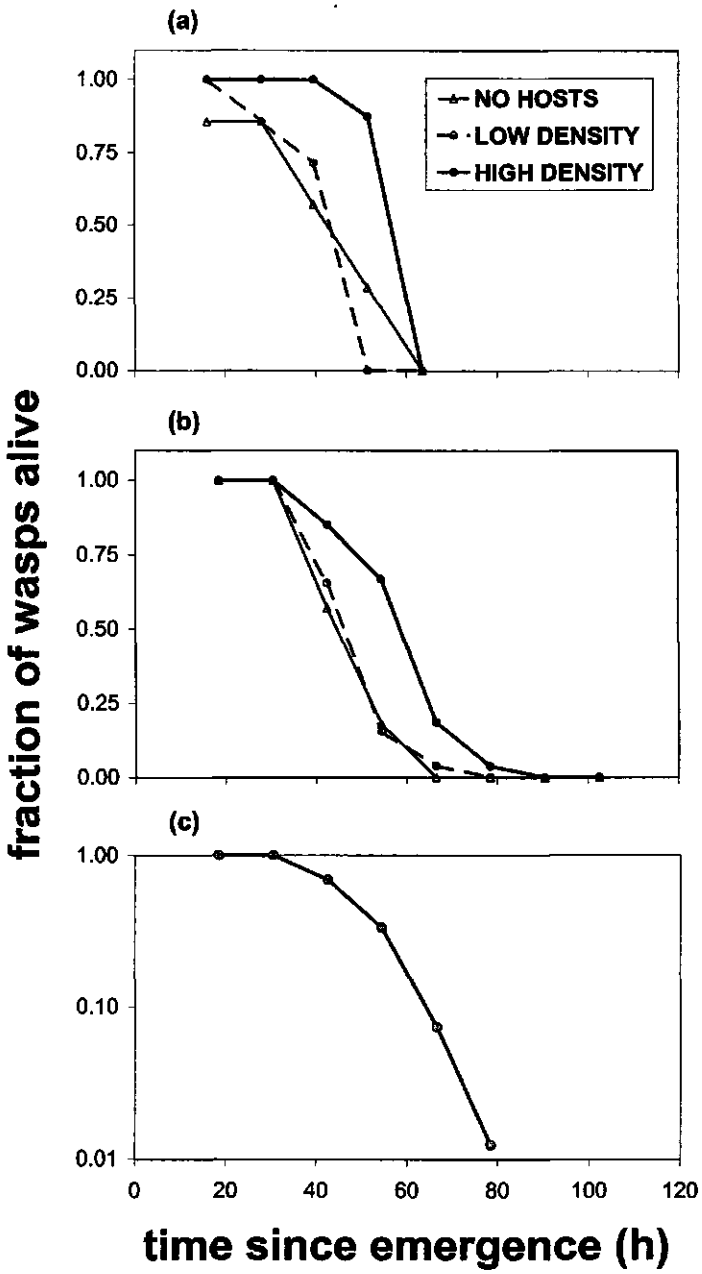

Figure 1. Fraction surviving *U. lariophaga* females as a function of time and host density. (a) First experiment, with 7-8 replicates per treatment, (b) second experiment, with 26-28 replicates per treatment, (c) all treatments from the second experiment combined.

experiments ($p=0.04$ and $p<0.01$, respectively, for the first experiment and $p<0.001$ for both comparisons in the second experiment; Mann-Whitney U). There was no difference between no hosts and low density in both experiments ($p=0.8$ and $p=0.7$, respectively; Mann-Whitney U).

Effects from Cox regression analysis in the first experiment were not significant ($p=0.6$ and $p=0.3$ for $\exp(\beta_1)$ and $\exp(\beta_2)$, respectively; Wald test, df=2). In the second experiment, effects from Cox regression analysis were calculated as $\exp(\beta_1) = 0.92$ and $\exp(\beta_2) = 0.46$; the latter was significantly different from 1 but the first was not ($p=0.03$ and $p=1.0$, respectively; Wald test, df=2). In other words, the rate at which wasps died at high host density was 0.46 times the rate at which wasps died when no hosts were present; and at low host density wasps died slightly, but not significantly, slower than at zero host density. $\text{Exp}(\beta_2)$ did not differ significantly from $\exp(\beta_1)$, which means that the dying rate did not differ between the high and low density treatments ($p=0.06$, Wald test, df=2). Mortality was, by the way, not constant, but increased over time, as is shown by the log-survival plot (Figure 1c).

In the low host density treatment, females parasitized on average (±SD) 17±8 (Experiment 1) or 17±3 (Experiment 2) eggs over their entire lifetime. In the high density treatment these figures were 68±16 and 58±9 for Experiment 1 and 2, respectively.

Walking activity

The number of times the wasps were observed walking (out of the six takes) was significantly affected by the host density but not by the day of observation ($p<<0.001$, and $p=0.13$, respectively; ANOVA). On average, wasps were observed walking in 4.5 out of the six takes in the low density treatment; in the high density treatment they walked in only 1.6 out of the six takes. At low host density, the wasps spent more time walking, covering larger distances, than at high host density ($p<<0.001$, ANOVA) (Figure 2). Averaged over both days, wasps walked 1.9 $mm \cdot s^{-1}$ at low host density and 1.5 $mm \cdot s^{-1}$ at high density.

Discussion

Contrary to our expectation, *U. lariophaga* lived shorter at low or zero host density than at high host density. Host feeding has never been observed in *U. lariophaga*; our results can therefore not be explained from wasps obtaining nutrients from hosts at high host density. We did notice, however, that wasps in the treatments without hosts or hosts at low densities were almost continuously walking, whereas

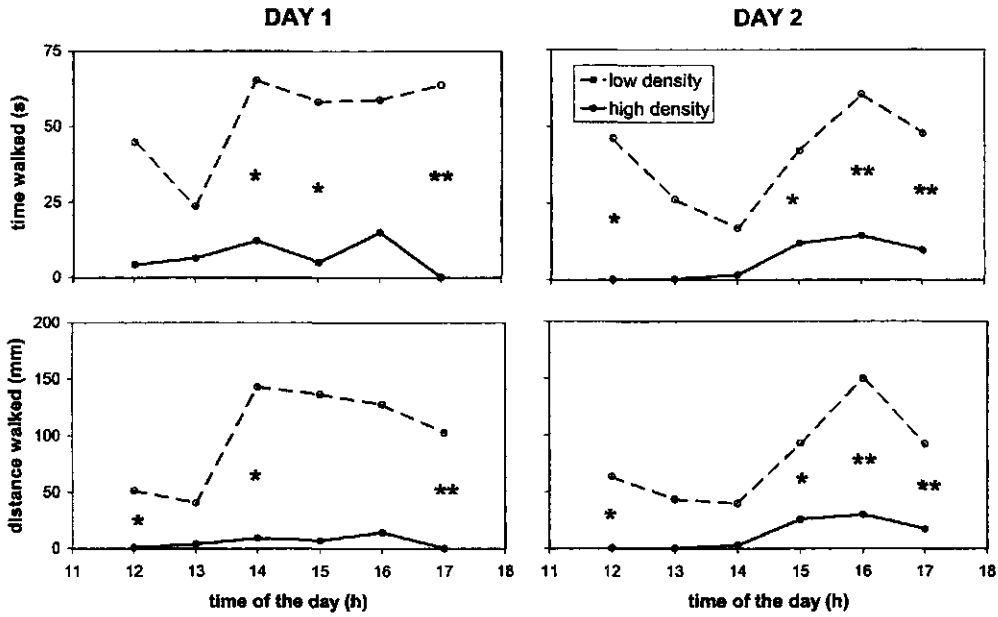

Figure 2. Average time and distance walked by *U. lariophaga* during six observation periods of each 90 s, at two host densities, on two days. Each treatment had 5-8 replicates per day. Asterisks indicate significant differences between the treatments according to the Mann-Whitney U test. * = $p<0.05$; ** = $p<0.01$.

the wasps in the high density treatment were mostly standing still or parasitizing. This was confirmed by the follow-up experiment in which we observed walking behaviour at low and high host densities. Our results suggest, therefore, that wasps lived shorter at zero or low host density because they spent more energy on locomotion. An additional explanation could be that parasitoids at low host density suffered physiological damage due to egg binding, although this has rarely been documented for insects (but see Taktak, 1984). Egg binding is the phenomenon that organisms suffer negatives consequences of not being able to deposit their eggs.

This result seems to contradict Van Huis *et al.* (1991a) who showed that *U. lariophaga* females lived slightly longer in the absence of hosts compared to the presence of hosts. Their 'no host' treatment, however, consisted of an empty paper card without a cowpea seed (H.M. Maes, unpublished results). Cowpea seeds constitute an olfactory stimulus for *U. lariophaga* (Ormel *et al.*, 1995; Van Huis *et al.*, 1994b). It may be speculated that wasps were standing still in the 'no host' treatment in the experiment reported by Van Huis *et al.* (1991a), resulting in a low energy usage and a longer lifespan, whereas the presence of a cowpea seed in our 'no

host' treatment induced walking behaviour in the wasps, increasing energy usage and reducing longevity.

A reversed trade-off between reproduction and survival has also been reported in a few other insects. Hegazi and Khafagi (2001), for example, mention that females of the non-host feeding species *Trichogramma cacoeciae* and *T. dendrolimi* that had had access to hosts unexpectedly lived longer than females that had been deprived of hosts. These are, however, exceptions. As a rule, a negative trade-off between reproduction and survival exists, as has indeed been found in many organisms, including hymenopteran parasitoids (Bell and Bohm, 1975; Reznick, 1985; Papaj, 2000). In parasitoid species that do not host feed such a trade-off is usually assumed to be mediated by egg resorption, although in some species it appears to be mediated by differential allocation of fat reserves (Ellers and Van Alphen, 1997). Egg resorption has been shown to occur in many insects, including trichogrammatids (Boggs and Ross, 1993; Fleury and Boulétreau, 1993; Ohgushi, 1996; Reznik *et al.*, 2001). For parasitoids, a trade-off between reproduction and survival is adaptive in a variable environment where hosts are sometimes scarce. We cannot yet decide whether the reverse effect, as we found for *U. lariophaga*, could be a functional adaptation to a natural environment, because we do not know enough about this natural environment. Increased walking activity at low host densities is probably adaptive, especially for a parasitoid of sessile host eggs. Whether increased walking activity is also adaptive if it implies reduced longevity depends on the distribution of host eggs that *U. lariophaga* normally encounters in the field. Lifetime fecundity is quite possibly higher for actively searching females than for females that are less active, even if searching reduces longevity.

On the other hand, we cannot exclude the possibility that reduced survival at low host densities is an adaptation to rearing conditions, where hosts are never scarce. Since rearings typically provide maximal conditions for reproduction, they tend to select for individuals that invest much in fast reproduction and little in survival. This might also make reared insects less flexible in postponing reproduction if current conditions do not allow reproduction. Indeed, Ellers and Van Alphen (1997) found that a strain of the hymenopteran parasitoid *Asobara tabida* that was collected from a relatively stable environment invested more in early reproduction, and was less flexible in allocating resources to survival, than a strain that was collected from a more variable environment. If this explanation for our results would appear to be correct, it could be useful to include survival at low host densities in quality control protocols of commercial parasitoid rearings.

Acknowledgements

We are indebted to Gerard Pesch for his assistence with the insect rearings and the literature references. We thank Piet Huisman for his help with the video equipment in the walking experiment. The critical comments of Felix Wäckers and Joop van Lenteren on an earlier version of the manuscript are gratefully acknowledged. We thank Joop van Loon, Hans Smid and Lia Hemerik for discussions and advice.

CHAPTER 7

General Discussion

From parasitoid behaviour to biological control

In this thesis, I have studied the behaviour of *Uscana lariophaga* against the background of biological control of *Callosobruchus maculatus* in stored cowpea, with biological control being, in a sense, an application of population dynamics. Behaviour of individual parasitoids determines population dynamics of host and parasitoid to a large extent (Vet, 2001). Which aspects of behaviour are essential, or most important, in determining population dynamics is still a matter of debate (Ives, 1995; Mondor & Roitberg, 2000). Ideally, one starts with a model that describes the system's population dynamics already fairly well. From such a model one can derive which elements in the parasitoid's behaviour are likely to have most influence on population dynamics, using sensitivity analyses (Mondor & Roitberg, 2000; Van Roermund *et al.*, 1997). Further attention should then be focused on these key behaviours. Some amount of knowledge of the system is, however, needed before one can build such a preliminary model. In addition, which behaviours need to be studied, and the amount of detail that is needed, also depend on the aim of the investigator. Many models are concerned with long term stability of host and parasitoid populations, whereas from the perspective of biological control it may be more interesting to know whether a given natural enemy can eradicate or suppress a pest over a relatively short time interval, such as a growing season (Ives, 1995; Mondor & Roitberg, 2000; Van Lenteren, 1986). The latter is particularly true for biological control in stored product: one would rather see complete and rather quick eradication of stored product pests, than a stable but substantial pest population which continues to inflict damage to the finite amount of stored product.

It is likely that at high densities of *C. maculatus*, existing host-parasitoid models, or adaptations thereof[1], will suffice to predict population dynamics of *C. maculatus* and *U. lariophaga* in stored cowpea (see models cited in Stolk *et al.*, 1999). Figure 1 shows typical dynamics of *C. maculatus* and *U. lariophaga* populations, at fairly high densities, in stored cowpea. However, by the time *C. maculatus* densities are high,

[1] At high densitites of insects in stored product, metabolic heat generated by the insects themselves becomes an important factor in population growth (Howe, 1943; Sinha *et al.*, 1966; Cofie-Agblor *et al.*, 1996). The metabolic heat that is retained by the stored product ill at first accelerate population growth, but eventually lethal temperatures can be reached (Van Huis *et al.*, 1998). This phenomenon would have to be included in a model that predicts population dynamics at high insect densitites in stored product. In addition, competitition between bruchid larvae in a single seed might have to be included (Bellows & Hassell, 1984).

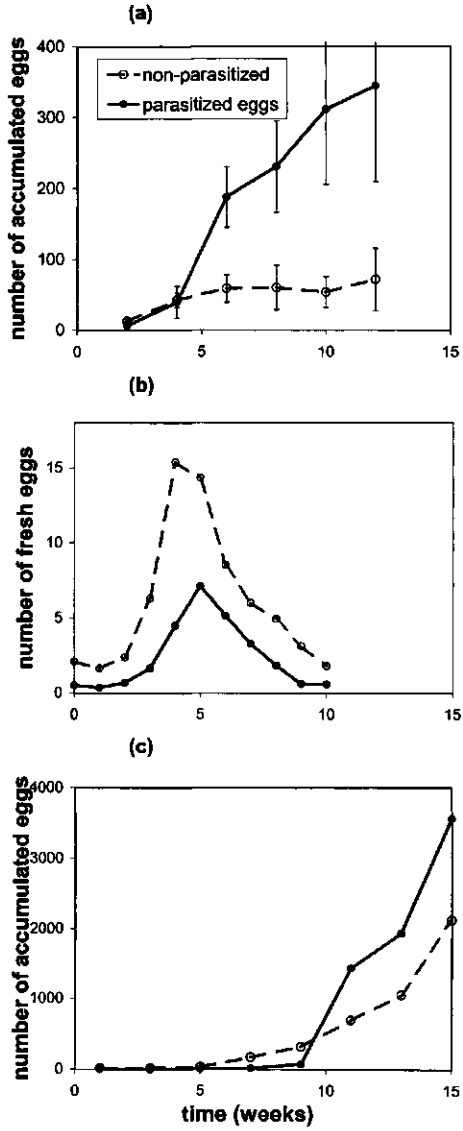

Figure 1. Examples of population dynamics of *Callosobruchus maculatus* and *Uscana lariophaga* in experimental cowpea stocks: **(a)** eggs and egg shells (either parasitized or unparasitized) in samples of 80 g, taken from 9 kg of cowpea seeds (each data-point is the average of five replicates, with two sub-samples per replicate; error bars show standard deviation; data from Van Huis *et al.*, 1998); **(b)** freshly deposited and freshly parasitized eggs per 1 m pod length in samples taken from 35-85 kg of cowpea pods (each data-point is the average of nine granaries, with six sub-samples per granary; data from Lammers & Van Huis, 1989); **(c)** eggs and egg shells (either parasitized or unparasitized) in samples of 40 g, taken from 9 kg of cowpea seeds (each data-point is the average of five replicates, with four sub-samples per replicate; data from Van Huis *et al.*, in press).

protection of the stored product has already failed and much irreversible damage has been inflicted on the stored cowpea. It is therefore much more interesting, and more relevant, to focus on the dynamics of low density populations of *C. maculatus* in the presence of *U. lariophaga*. It is also at low densities that biological control is most difficult (Van Huis *et al.*, 1998). At these low densities, spatial aspects of foraging behaviour are likely to play an important role. For instance, host finding by *U. lariophaga* may be hampered by the large distances between host patches, and the negative geotaxis of *U. lariophaga* may determine which host patches will be found. To my knowledge, a suitable model which takes such aspects into account does not yet exist. In the absence of such a model, studying aspects of the searching behaviour of *U. lariophaga* can be a good starting point.

In this thesis I have studied several aspects of the behaviour of *U. lariophaga* which are especially relevant at low host densities. In the next two sections I will highlight some aspects of the foraging behaviour of *U. lariophaga* females and I will discuss possible consequences for the prospects of biological control of *C. maculatus*. I will also indicate how findings of this thesis can be incorporated in a simulation model and I will show the utility of such a model.

Behaviour of *U. lariophaga* females

Mating

The fact that males develop slightly quicker than females (Chapter 4), and the fact that males remain at the site of their emergence for perhaps their entire lifetime (Chapter 4) both suggest that females probably mate soon after emergence. Indeed, only two out of the 85 females that were used in the experiments of Chapter 5 seemed not to have mated. This appeared from the fact that two females produced only male offspring, even though fertilization behaviour was observed (Chapter 5, data not shown).

Host patch searching

Uscana lariophaga starts searching regardless of the presence or concentration of host-related odours (Chapters 3 and 4). Searching seems to be random, until the parasitoid reaches a certain distance from a host patch. From that moment onwards, *U. lariophaga* seems to search in a directed way for the host patch, guided by odours emanating from beans with host eggs. In olfactometer tests, Ormel *et al.* (1995) had already shown that *U. lariophaga* is attracted by odours related to *C. maculatus* eggs, and Van Huis *et al.* (1994) showed attraction of *U. lariophaga* to a synthetic component of the sex pheromone of *C. analis*. This evidence is now

supplemented with results from setups that resemble the storage environment more closely. "Orientation behaviour", characterized by wavering of the antennae in the air, was described for *U. lariophaga* in Chapter 3. Moreover, in that same chapter, the searching trajectory of *U. lariophaga* became more directed towards the host patch when it had got as close as 2-4 cm from the host patch. There is, however, a danger of a circular argument in the interpretation of this result. For wasps that reached the host patch (through either directed or random search) it will always seem that the last part of the searching trajectory was aimed at the host patch. Further investigations could elucidate whether it is through chance, or because of directed search, that the last 4-6 visits to beans before finding the host patch were roughly on a straight line. In addition to the evidence from Chapter 3, there is some indirect evidence for directed search in Chapter 4. An analysis of Figure 3 in that Chapter suggested that the host patch was rapidly found within a certain critical distance. This critical distance could well be the result of an odour sphere around a host patch.

It would be interesting to know whether *U. lariophaga* is also attracted by kairomones associated with host egg shells and eggs that are too old to be parasitized, *i.e.*, bruchid eggs that are more than about three days old (Van Huis *et al.*, 1991b). In that case, a cowpea stock infested with *C. maculatus* could easily become saturated with kairomones: the number of fresh host eggs at any given time may be limited, but egg shells accumulate over time. The oviposition deterrent that is produced by *C. maculatus* is known to be highly persistent: the deterrent effect on *C. maculatus* oviposition can be measured at least up to 30 days after cowpea seeds have been marked with the product (Credland & Wright, 1990). If up to 30 days old eggs and egg shells would still be attractive to *U. lariophaga*, its searching behaviour could be confused rather than guided by these kairomones. It is also possible, on the other hand, that other, short-lived kairomones, play a role in the attraction of *U. lariophaga*.

Behaviour within a patch

Upon encountering a host, *U. lariophaga* shows a strong arrestment response: residence times per bean are prolonged, and the walking trajectory becomes tortuous (Chapter 3). It is not known exactly how long this arrestment response lasts, and which factors influence the giving up time or the tendency of females to leave the patch. It seems likely, however, that previous experience in other patches, or learning, does not play an important role in such a short-lived egg parasitoid (Van Alebeek, 1996a; Vet *et al.*, 1995). In addition, it is known that *U. lariophaga* exploits patches until almost all eggs are parasitized (Van Alebeek *et al.*, 1996b), and that it can discriminate between parasitized and unparasitized eggs, thus reducing the

probability of superparasitization (Chapter 5). Imperfect discrimination ability might lead to wasps leaving patches early to avoid the risk of self-superparasitism (Rosenheim & Mangel, 1994), but this is not the case in *U. lariophaga*. I therefore speculate that the main factors influencing giving up time are encounters with a healthy host (which should cause a decrease in leaving tendency) and encounters with parasitized hosts (which should cause an increase in leaving tendency). These factors were also the most important ones for another trichogrammatid whose host has a clumped distribution, namely *Trichogramma brassicae* (Wajnberg *et al.*, 2000).

If an *U. lariophaga* female reaches a host patch which appears to contain parasitized eggs, its behaviour can be described by a simple rule: superparasitize as long as no unparasitized eggs have been encountered, and avoid superparasitism after the first unparasitized egg has been encountered (Chapter 5). This behaviour is adaptive if the following two conditions are both satisfied: (1) *U. lariophaga* cannot distinhuish between self- and conspecific-parasitized eggs, and (2) it can recognize patches that it has visited earlier, or the probability of visiting a patch more than once is small.

The current knowledge of the foraging behaviour of *U. lariophaga* can be used to simulate its functional response in stored cowpea using an individual-based approach. In the following two sections and in the Appendix I propose a basic layout for such a model and I discuss its possible uses.

Blueprint for a simulation model

The movement of an *U. lariophaga* female through a cowpea stock could be simulated using an imaginary, three dimensional grid consisting of 'cells' or layers of *e.g.* hexagonals (Figure 2). Each hexagonal in the grid stands for an individually recognized bean. For any bean, it can be calculated which other beans border it, making use of the dimensions of the grid and of appropriate algorithms. Host clusters are collections of individual beans with their corresponding numbers of unparasitized and parasitized eggs. The spatial distribution of infested beans can be based on the spatial egg distributions presented in Chapter 2, but more information is needed on the location of clusters in storage. An imaginary *U. lariophaga* female can then navigate through this space according to a set of behavioural rules (see Appendix). Model output can be the number of found host patches, the number of parasitized eggs, the parasitoid's egg load, the spatial position of the parasitized eggs (represented by x,y,z-coordinates), and the three-dimensional walking trajectory, represented by the spatial position of the beans that were visited. The model could be evaluated first by visually comparing the walking trajectories in a single layer of beans with those found in Chapter 3, and then by comparing model predictions with the results of published and unpublished functional response experiments (Van Alebeek *et al.*,

1996a,b; S.B. Slumpa & F.A.N. van Alebeek, unpublished data) and host finding experiments (Van Alebeek & Van Huis, 1997; Chapter 4).

A flow diagram showing the basic framework of behavioural rules is shown in Figure 3. Two key behavioural processes, the determination of residence times per bean and the choice of new beans to move to, are elaborated in Figures 4 and 5 (discussed in the Appendix). Stochasticity can be simulated using a random number generator and appropriate probability density functions for relevant behaviours. The program should end after either a predetermined amount of time or when the parasitoid is dead (not shown in Figure 3). The proposed model is discussed in more detail in the Appendix.

Utility of a simulation model

What would be the utility of such a model? First and foremost, it summarizes our current knowledge and best guesses, and it identifies gaps in our understanding of *U. lariophaga* foraging behaviour. The conceptual model as developed above and in the Appendix already draws the attention to some of these gaps, such as the spatial distribution of host clusters, the duration of the influence of a host encounter on residence time and turning angles, and the effect of host encounters and the

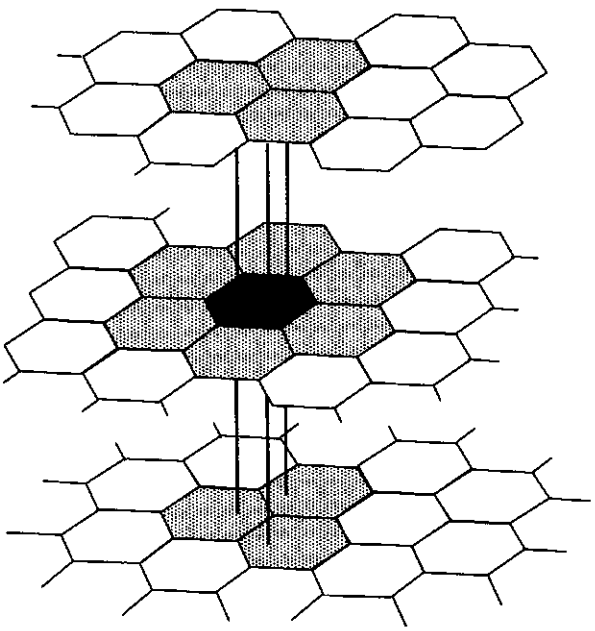

Figure 2. Section of an imaginary three dimensional grid composed of layers of hexagonals. Each hexagonal represents a single bean. Each 'bean' is in touch with twelve other 'beans'.

presence of hosts on vertical movement. A model can also help in directing further research because analyses can show the sensitivity of model output (*e.g.* the number of found host patches) to changes in the model and in model parameters.

The model could be used to compare the plausibility of different searching algorithms, similar to the work of Hoffmeister *et al.* (2000) on *Halticoptera rosae*, an egg-larval parasitoid of rose-hip flies. They used a spatially explicit model to show that out of three possible searching strategies (random search, systematic search, and following of the fly's marking pheromone trail), only trail following produced results that were compatible with experimental findings. In our case, this approach could be used for aspects of the foraging behaviour that are still unclear or that are difficult to investigate. For instance, if the negative geotaxis of *U. lariophaga* is implemented as shown in Figure 5, this will probably result in parasitoids that spend a lot of time in the top layers of the bean grid. It is questionable whether this also occurs in reality. For instance, in a traditional granary in Niger, Van Alebeek (unpublished data) found high numbers of parasitized eggs accross all depths of an up to 45 cm deep layer of cowpea pods.

The model can help answer some of the questions that arose from experiments. The model can for instance be used to estimate the probability of non-directed searching behaviour producing more or less straight walking trajectories in the vicinity of the host patch (see Chapter 3). It could also be used to investigate the adaptiveness of increased walking behaviour at low host densities if this also implies a reduced longevity (see Chapter 6). The model could show for which spatial distributions and host densities this behaviour is optimal, which provides a clue as to whether the behaviour observed in Chapter 6 can be an adaptation to natural conditions or not.

Biological control using *U. lariophaga*

What does the information on foraging behaviour of *U. lariophaga*, presented in this thesis, tell us about the possibilities of biological control of *C. maculatus* in stored cowpea? Van Alebeek (1996a) discussed eight criteria for the evaluation of *U. lariophaga* as a biocontrol agent, including compatibility with the storage environment, synchronization of the relevant life stages of host and parasitoid, and parasitoid searching efficiency. He pointed out that synchronization is one of the most critical issues in this system, since the developmental time and longevity of *U. lariophaga* are both much shorter than those of *C. maculatus*. Long periods with no or low numbers of parasitizable host eggs cannot be overcome by a wasp with comparatively short developmental time and adult longevity. Chapter 6 shows that the

situation may be even worse: at low host densities, *U. lariophaga* lives even shorter than at high host densities. Thus, if a *C. maculatus* infestation in storage is significantly suppressed by *U. lariophaga*, biological control might still fail because the *C. maculatus* population can resurge after such a period of low bruchid numbers. Providing honey in such a way that it is inaccessible to the bruchids but available to *U. lariophaga* (Van Huis *et al.*, 1991a), or inoculation of granaries with *U. lariophaga* could solve this problem, but at the moment it is unlikely that this will fit into the socio-economic reality of West Africa.

Parasitoid searching efficiency is one of the other evaluation criteria used by Van Alebeek (1996a). Van Huis *et al.* (1998) suggested that host finding at low bruchid densities may be a problem for *U. lariophaga*. This is confirmed by results presented in Chapter 4. The probability that a host patch was found decreased rapidly as the distance between release point and host patch increased. In addition, the host finding probability was smaller for small patches than for large patches, implying that small patches might escape parasitism.

Van Huis *et al.* (1990) mentioned bruchid kairomones in the context of improving biological control of *C. maculatus*. It seems now, however, that the practical use of the kairomones associated with bruchid eggs will be limited, especially in tradi-

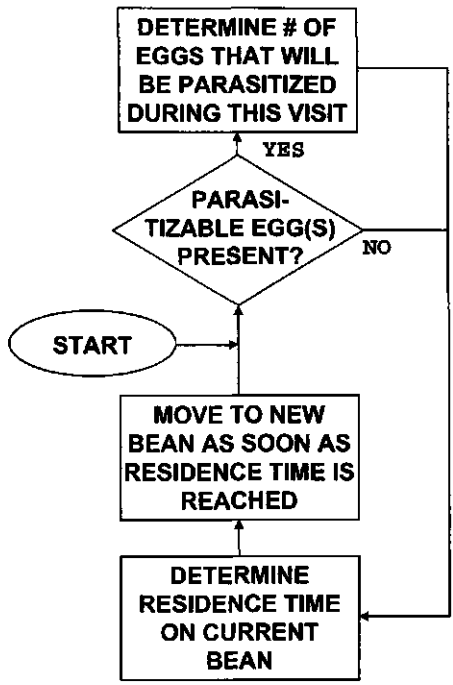

Figure 3. Flow diagram of a model proposed for simulating *U. lariophaga* foraging behaviour.

tional storage in developing countries. The potential benefits of kairomones to improve biological control are: (1) deterring oviposition of *C. maculatus*; (2) attracting natural enemies; (3) triggering or initiating searching behaviour of natural enemies; (4) decreasing the leaving tendency of natural enemies present in storage; and (5) guiding natural enemies towards hosts. The oviposition deterrent produced by *C. maculatus* is not, however, an absolute deterrent, but is only used by females to choose between beans with different egg loads (Credland & Wright, 1990). Furthermore, Van Alebeek (1996b) argued that cowpea granaries are 'ecological islands', with little migration between granaries. This implies that the repulsion of bruchids and the attraction of natural enemies through the use of kairomones (possibilities 1 and 2) will not be easy to obtain, leaving only the role of kairomones in searching behaviour of natural enemies already inside storage. Observations on the searching behaviour of *U. lariophaga* (Chapter 3) and analysis of host finding experiments (Chapter 4) both suggest that, inside cowpea stocks, *U. lariophaga* starts searching regardless the presence or concentration of bruchid kairomones (possibility 3). Indiscriminate application of kairomones to stored cowpea might decrease the tendency of natural enemies to leave the cowpea stock, resulting in more available searching time (possibility 4), but it will not make searching more effective because those additional kairomones will not guide natural enemies towards host patches (possibility 5).

So far, *U. lariophaga* has mainly been considered for a conservation strategy of biological control in traditional cowpea storage. The application of biological control in traditional storage in West Africa will at present, however, be difficult to achieve, for reasons mentioned above. We may also consider large scale storage of beans in either developing or industrialized countries (*e.g.* cowpeas in Nigeria, or organic soy beans in the United States). If there would be a niche for inundative releases of natural enemies in stored beans, *U. lariophaga* would be a good candidate. It compares favourably with larval parasitoids in the following respects: (1) it kills the pest in the egg stage, before the bruchid larva has damaged the bean; and (2) per parasitoid individual, far fewer beans are needed for rearing. The latter can be illustrated by the following calculation:

One cowpea seed of the variety 'Black Eyes' can support the development of about eight *C. maculatus* individuals. The larval parasitoids *Dinarmus basalis* and *Eupelmus vuilleti* are both solitary parasitoids. Recorded sex ratios of these parasitoids vary from 13 to 75% females (Gauthier *et al.*, 1997; Terrasse *et al.*, 1996), but if we assume a sex ratio of 70% females, this implies that on average about ($0.7 \times 8 =$) 5.6 females can emerge from a single cowpea seed. For *U. lariophaga*, on the other hand, the four *C. maculatus* females that can on average emerge from a single seed are of interest. Based on an average lifetime fecundity of 75 eggs, these

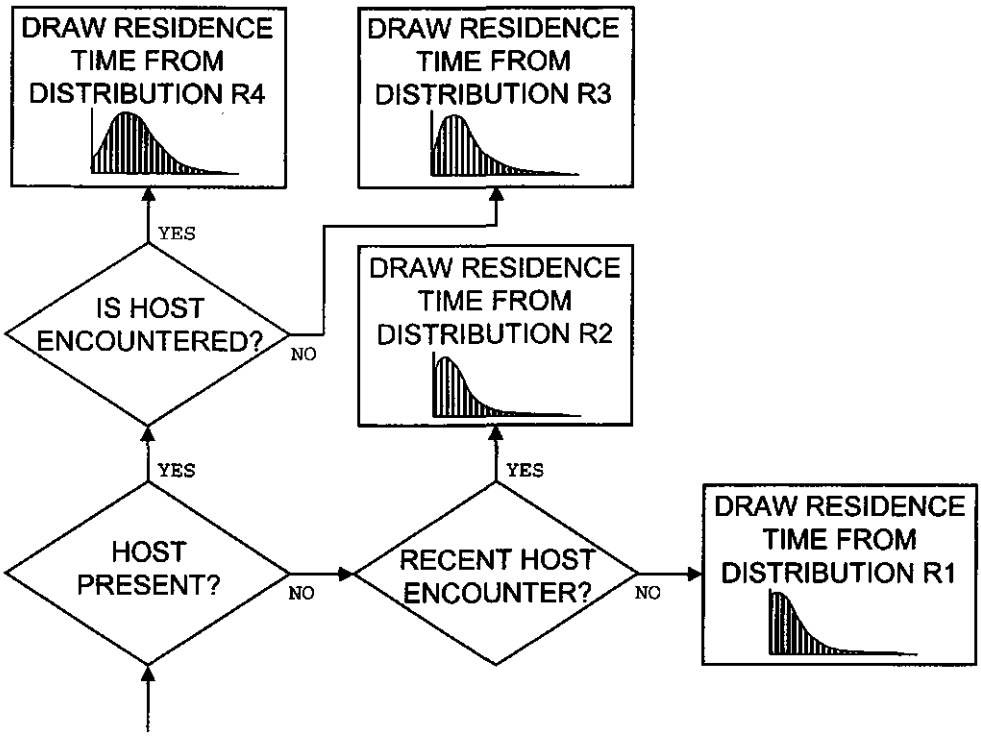

Figure 4. Flow diagram of the determination of residence time per bean in the proposed model of *U. lariophaga* foraging behaviour. Distributions of residence times R1 - R4 are represented by rough sketches.

females produce $4 \times 75 = 300$ eggs. Note that these eggs need not be oviposited on beans; *C. maculatus* oviposits almost on anything that is smooth and preferably round (Credland & Wright, 1988). Even if only 50% of these eggs would be parasitized in a rearing, and assuming a sex ratio of 60% females, 90 *U. lariophaga* females would be produced from a single cowpea seed.

Uscana lariophaga can also be reared on the eggs of *Acanthoscelides obtectus* Say (Col.: Bruchidae), another pest of stored beans (A. den Dikken & K. van Huis, unpublished results). This bruchid does not glue its eggs onto beans; the eggs are dropped in between seeds instead. These eggs can be gently sieved off before the hatching larvae enter a seed, and they can be glued onto paper cards in the same fashion as is done with *Ephestia* spp. eggs for *Trichogramma* spp. rearings (F. Wäckers, personal communication). If the eggs are thoroughly sieved off every day, the oviposition substrate (*i.e.*, the beans) need hardly be renewed because they remain free of infestation. The *A. obtectus* eggs would have to be sterilized by *e.g.* UV or gamma radiation before the egg cards are introduced into storage. *Uscana lariopha-*

ga can develop in UV-sterilized *C. maculatus* eggs (A. den Dikken & K. van Huis, unpublished results); it would have to be investigated whether the same is true for sterilized *A. obtectus* eggs.

It should be borne in mind that, if *U. lariophaga* is constantly reared at high host densities, it might become less adapted to searching at low host densities (see Chapter 6 for a discussion). This could be overcome if the parasitoid is forced to move through a layer of beans before it reaches the host eggs. If the females have to travel downward before finding hosts, such a rearing setup might even select for *U. lariophaga* females that do not display negative geotaxis any more. This could make them more useful for release on top of stored beans. Otherwise, the strong negative geotaxis in *U. lariophaga* implies that it would best be released at the bottom of bean stocks.

The research described in this thesis provides insight into processes, such as host finding, that determine success or failure of biological control of bruchids in stored cowpea by *U. lariophaga*. No 'quick' solution to the problem of bruchids in stored cowpea in West Africa is available; but it is clear that *U. lariophaga* already plays an important part in the natural suppression of bruchid populations. This suppressive effect of *U. lariophaga* can in principle be further exploited, especially in situations where *U. lariophaga* can be mass reared and released for the control of low density bruchid populations in storage.

Appendix

The conceptual model that was briefly introduced in this chapter is explained in more detail in this Appendix on the basis of two key behavioural processes: the determination of residence times per bean, and the choice of new beans to move to.

Residence times and parasitizations

Each time a wasp visits a bean, a residence time is drawn from a distribution. Which distribution is used depends on the presence or absence of host eggs on that bean, and on encounters with hosts (Figure 4). Based on the empirical distributions presented in Figure 2 of Chapter 3, four distributions for residence time, R1-R4, can be determined. If the bean does not contain a host and if no unparasitized host has recently[2] been encountered, the residence time is drawn from distribution R1; if the bean does not contain a host but if an unparasitized host has recently been encountered, distribution R2 is used; if the bean does contain one or more hosts but if no host is encountered, distribution R3 is used; and if at least one host is parasitized during this visit, distribution R4 is used. In the latter case, the residence

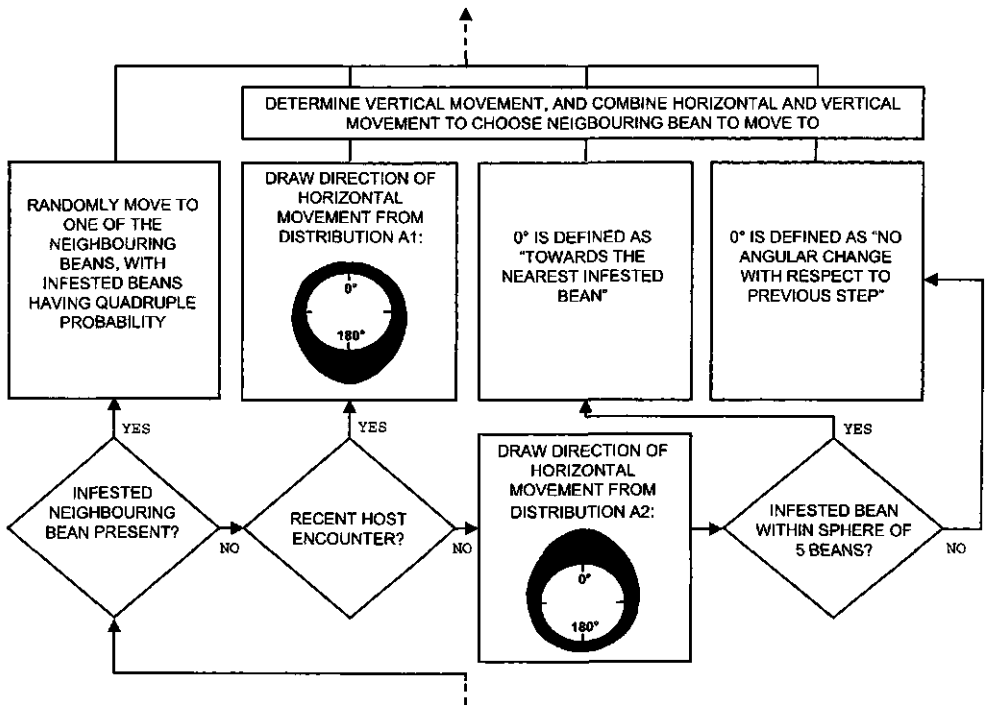

Figure 5. Flow diagram representing the choice of the next bean in the proposed model of *U. lariophaga* foraging behaviour. Angular distributions A1 and A2 are roughly sketched on a circular axis.

time is increased with an additional 163 s for each parasitization (based on Chapter 5).

If the current bean contains n ($n = 1,2,3,...$) unparasitized host eggs, then k ($k = 0,1,2,3...$) eggs are parasitized during this visit. The probability of k out of n hosts being parasitized should be based on Table 5 in Chapter 3, although additional data can be obtained from analysis of the Observer-files that were used in Chapter 5. Because the program simulates only one single parasitoid, and because self-superparasitism is a rare event (Chapter 5), superparasitism does not need to be included in the model. At each parasitization, the parasitoid's egg load decreases by one egg. In addition, new eggs are continuously matured at a rate of 0.8 eggs·h^{-1} (based on Van Huis *et al.*, 1991b, and Van Alebeek *et al.*, 1996b). For simplicity, egg load dynamics are not included in Figure 3.

Figure 3 shows that the residence time per bean is determined after it has been determined whether a host will be parasitized during this visit. This calculation order is chosen because the residence time is prolonged once an unparasitized host is encountered and parasitized (Chapter 3). The reverse order of first calculating

residence time, and then determining the number of parasitized hosts could also be chosen, but this would require determination of the relationship between residence time and the number of hosts found during the visit. The Observer-data presented in Chapter 5 can be used to establish this relationship.

Figure 2 in Chapter 6 suggests that a circadian rhythm may be present in *U. lariophaga*. Circadian rhythms in locomotory activity are common in parasitic hymenoptera, including Trichogrammatidae (Fleury *et al.*, 1991). If necessary, the algorithm proposed in Figure 4 could be extended to provide for a circadian rhythm.

Moving to next bean

As soon as the predetermined residence time of a visit has been reached, the wasp moves to one of the neighbouring beans (Figure 5). This involves three-dimensional movement. The available experimental data concerning the choice of a next bean were, however, measured in a two-dimensional horizontal plane (Chapter 3). Behavioural rules based on observations in two dimensions cannot be indiscriminately be scaled-up from two to three dimensions, since *U. lariophaga* shows strong negative geotaxis (Chapter 5). As a starting point, I therefore propose to treat horizontal and vertical movement separately in a number of cases (see below).

If one or more of the neighbouring beans contain a host egg (*i.e.*, if the wasp is inside or near a host patch), the wasp randomly moves to one of the neighbouring beans. The infested beans, however, have quadruple 'weight' in terms of probability of receiving the wasp, compared to any of the uninfested beans. For instance, if four out of the 12 neighbouring beans contain eggs, then the wasp will move to one of these four beans with probability $\frac{4 \cdot 4}{12+3 \cdot 4} \approx 0.67$. This is double the normal probability of $\frac{4}{12} \approx 0.33$ (compare Figure 6 in Chapter 3).

If none of the neighbouring seeds contain a host egg, the decision of which bean to move to consists of two steps: (1) determining the movement in the horizontal plane; and (2) determining the vertical movement. For the first step, an angle is drawn from a distribution ranging from -180° to +180°. The shape of the distribution depends on two factors: whether a host has recently been encountered (*i.e.*, during the last 15 minutes); and whether a host egg is present within a sphere of five beans around the current position of the wasp. If a host egg has recently been encountered, an angle is drawn from distribution A1; if no host has recently been

[2] It is unknown how long the influence of a host encounter on residence time and tortuosity lasts. Provisionally, "recently" could be interpreted and implemented as "during the last 15 minutes" (see Discussion in Chapter 3).

encountered, distribution A2 is used (Figure 5; distributions based on Figure 4b in Chapter 3). If no host egg is in the vicinity, 0° in distribution A2 stands for an angular change of 0° with respect to the previous step; but if one or more host eggs occur within a sphere of 5 beans, 0° is interpreted as moving on a straight line towards the nearest infested bean, or towards one of them if several infested beans are equally near.

In the second step, namely deciding the vertical movement, there are three possible outcomes. Movement can be either downward or upward, or there can be no vertical movement. These outcomes occur with the following probabilities: 0.14 for downward movement, 0.29 for no movement, and 0.57 for upward movement (based on the 1:2:4 ratio found in Chapter 4). Vertical movement might also be influenced by the presence of host eggs in the vicinity and by recent host encounters; but at present not enough is known to incorporate this in a model. Finally, the two directions of movement, vertical and horizontal, are combined to form a three-dimensional vector. The wasp moves to the neighbouring bean (out of 12) towards which the vector points. Of course, if one of the grid boundaries (*e.g.*, the top layer) has been reached, movement in that direction will be blocked (not included in Figure 5).

As mentioned earlier, this proposed separation of movement into a horizontal and vertical component serves only as a starting point. I do not think that horizontal and vertical movement are independent processes; but linking horizontal and vertical movement in a more realistic way would at present involve much speculation. The model would, however, be a suitable tool to test such 'speculative' assumptions regarding movement of *U. lariophaga* through a cowpea stock (see section 'Utility of a simulation model').

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Summary

A niche for biological control in stored products exists in both industrialized and developing countries (**Chapter 1**). In industrialized countries, the attention for biological control is mainly inspired by environmental and health concerns, whereas in developing countries effective and safe methods of stored product protection are often altogether lacking to subsistence farmers. The latter is particularly true in the case of cowpea (*Vigna unguiculata* Walpers) which is often infested with the destructive stored-product beetle *Callosobruchus maculatus* Fabricius (Col.: Bruchidae). This thesis reports on studies of the behaviour of a natural enemy and potential biocontrol agent of this beetle, the egg parasitoid *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae). The continuous thread that runs through the thesis is foraging behaviour of *U. lariophaga* at low densities of its host. At low densities, control of the beetle is most useful (at high beetle densities the stored product is already lost); yet, *U. lariophaga* performs less well at low than at high beetle densities.

In **Chapter 2**, the storage environment is described from the perspective of *U. lariophaga* females foraging for *C. maculatus* eggs. The three-dimensional spatial oviposition pattern of *C. maculatus* in cowpea stocks was characterized using point pattern analyses. Individual *C. maculatus* females oviposited in single clusters, variable in shape, and containing on average 70 eggs. The egg density was highest at the center of a cluster and decreased towards the periphery. The spatial oviposition pattern of beetles which emerged from egg clusters such as those produced by individual females was not confined to one specific area but was scattered throughout the bean mass. No effect of the density of the 'parent' cluster on the the spatial egg pattern produced by emerging females could be detected. The data are used to argue that the probability p of encountering at least one other bean with eggs after a parasitization is a function of the number n of beans that are visited: $p = 1-0.42\cdot(0.37)^{(n-1)}$.

In **Chapter 3**, I describe the foraging behaviour of *U. lariophaga* females in artificial arenas with a single, horizontal layer of cowpea seeds. Search trajectories were recorded at a spatial resolution of single beans, while behavioral components were recorded at a temporal scale of seconds. This allows for a meaningful interpretation at the level of individual parasitoids. The most important factor influencing the behaviour of *U. lariophaga* was an encounter with a host egg: this changed the walking trajectory from 'straight' to 'tortuous' and it increased the residence time per bean. *Uscana lariophaga* seemed attracted to host eggs from a distance of about 4-6 beans, and it showed a preference to move onto beans with an

egg. Once it was on a bean with an egg, however, it often failed to find the egg during one visit.

In **Chapter 4**, finding of host patches by *U. lariophaga* females in stored cowpea is analyzed. Host finding is shown to be a function of distance, of time, of host patch size and of the spatial position of *U. lariophaga* relative to the host patch. *Uscana lariophaga* females were able to find hosts up to 75 cm horizontal distance from the release point, which was the largest distance tested. The probability that a host patch was found when an individual *U. lariophaga* female was released at 2.5 cm horizontal distance from the host patch ranged from 0.6 at 2 h foraging time to 0.9 at 8 h foraging time. At 10 cm from the host patch, host finding probability ranged from 0.2 to 0.45 at these respective foraging times. Finding probabilities doubled compared to horizontal distances when *U. lariophaga* was released below the host patch, and halved when it was released above the host patch. The median net displacement rate in the direction of the host patch was estimated at two beans per hour (1.4 $cm \cdot h^{-1}$) when *U. lariophaga* was releas ed at 2.5 cm from the host patch.

What happens if *U. lariophaga* finds a host patch that has already been found and exploited by another female? This situation is likely to occur when the density of *C. maculatus* is low relative to the density of *U. lariophaga*, and it is studied in **Chapter 5**. Experienced *U. lariophaga* females were individually released into an arena containing 15 host eggs. The arena initially contained only unparasitized eggs, but gradually, as parasitoids were released, more eggs were parasitized. Two treatments were used: a 'self' treatment, in which females encountered eggs that had been parasitized by themselves, and a 'conspecific' treatment, in which females encountered eggs that had been parasitized by themselves and eggs that had been parasitized by others. An encounter with an unparasitized egg in the same arena significantly reduced the probability that a parasitized host egg would subsequently be superparasitized. As a result, self superparasitism occurred only twice, whereas conspecific superparasitism was observed 40 times (out of a total of 556 parasitizations). In another experiment, in which experienced females were confronted with a single host egg, self- and conspecific superparasitism were both equally rare, and occurred in 6% of these no-choice tests. Superparasitism was not a result of failure in host discrimination, but possibly adaptive behaviour. This appears from the fact that females, when superparasitizing, adapted the sex ratio of their offspring in the direction of the sex with the highest survival probability. Since superparasitism was a rare event, its effect on biological control using *U. lariophaga* is probably limited.

At low host densities, *U. lariophaga* will generally find few hosts per unit of time. Many insects live longer when they can produce little or no offspring, due to plas-

ticity in resource allocation. In **Chapter 6**, it is studied whether such a trade-off between reproduction and survival also exists in *U. lariophaga*. The opposite, however, was found: females that had access to 98 hosts lived significantly longer than females that had access to 0 or 10 host eggs (45 versus 33 h on average). This reduced longevity at zero or low host density may have been a consequence of intense searching, since females at low density displayed significantly more walking activity than females at high host density. Based on the results presented in this chapter it cannot be decided whether this increased walking behaviour and reduced longevity at low host densities is an adaptation to natural host distributions, or to rearing conditions.

In **Chapter 7**, current understanding of *U. lariophaga* foraging behaviour is summarized using a conceptual model, and implications of *U. lariophaga* foraging behaviour for biological control of *C. maculatus* are discussed. The conceptual model shows which aspects of the behaviour are still unknown. A simulation model, based on the conceptual model, could be used to study the consequences of the foraging behaviour of *U. lariophaga* for its functional response in stored cowpea. As for biological control, difficulty in host finding, due to large distances between host clusters, may be one of the main causes of the poor performance of *U. lariophaga* at low host densities. In addition, the reduced longevity of *U. lariophaga* at low host densities does not allow it to 'wait' until hosts are more abundant. (Note that this reduced longevity may be caused precisely by the fact that *U. lariophaga*, as a parasitoid of sessile hosts, does not seem to practice a 'sit and wait' strategy). *Uscana lariophaga* appears to be attracted by kairomones associated with host eggs; but these kairomones cannot easily be applied to improve biological control. Additional releases of *U. lariophaga* could improve biological control at low host densities; but mass rearing and releasing *U. lariophaga* is currently not feasible for rural areas in West Africa. *Uscana lariophaga* would, however, be a good candidate for inundative biological control of bruchids, since its rearing requires the use of only small amounts of beans and because large numbers of parasitoids can be produced on a small surface area.

Samenvatting

In zowel geïndustrialiseerde als in ontwikkelingslanden bestaat een niche voor biologische bestrijding in opgeslagen producten (**hoofdstuk 1**). In geïndustrialiseerde landen is biologische bestrijding vaak een alternatief voor chemische bestrijding en wordt de aandacht voor biologische bestrijding vooral ingegeven door zorgen over milieu en gezondheid; in ontwikkelingslanden daarentegen beschikken zelfvoorzienende boeren veelal over geen enkele effectieve en veilige manier om opgeslagen producten te beschermen tegen plaaginsecten. Een voorbeeld van dit laatste is het gewas *cowpea* (ogenboon, *Vigna unguiculata* Walpers), dat in opgeslagen toestand vaak aangetast wordt door de kever *Callosobruchus maculatus* Fabricius (Col.: Bruchidae). Dit proefschrift behandelt het gedrag van een natuurlijke vijand en potentiële biologische bestrijder van deze kever, de eiparasiet *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae). De rode draad in dit proefschrift is het foerageergedrag van *U. lariophaga* bij lage dichtheden van de gastheer. Bij lage dichtheden heeft bestrijding van de kever nog zin (bij hoge dichtheden zijn de opgeslagen bonen immers al verloren); maar bij lage gastheren verloopt de bestrijding van de kever door de sluipwesp juist minder voorspoedig dan bij hoge keverdichtheden.

In **hoofdstuk 2** wordt de opslagomgeving beschreven vanuit het perspectief van *U. lariophaga*-vrouwtjes die naar *C. maculatus*-eieren zoeken. Het drie-dimensionale eilegpatroon van *C. maculatus* in opgeslagen cowpea wordt hier gekarakteriseerd met 'punt-patroon' analyses. Individuele *C. maculatus*-vrouwtjes legden hun eieren elk in één cluster. Deze clusters waren variabel van vorm en bevatten gemiddeld 70 eieren. De eidichtheid was het hoogst in het midden van een cluster en nam af in de richting van de rand van het cluster. Het ruimtelijk eilegpatroon van kevers die zelf uit zo'n eicluster kwamen was niet beperkt tot één bepaald gebied in de opgeslagen cowpea; hun eileg was verspreid door de gehele bonenmassa. Er kon geen effect worden aangetoond van de eidichtheid in het 'oudercluster' op het ruimtelijk eilegpatroon van de kevers die uit zo'n cluster kwamen. De verzamelde gegevens worden gebruikt om te beredeneren dat de kans p om na een parasitering tenminste één andere boon met eieren te ontmoeten een functie is van het aantal bonen n dat bezocht wordt: $p = 1-0.42\cdot(0.37)^{(n-1)}$.

In **hoofdstuk 3** beschrijf ik het foerageergedrag van *U. lariophaga*-vrouwtjes in kunstmatige arena's met één enkele, horizontale laag cowpea-bonen. Zoektrajecten werden vastgelegd met een ruimtelijke resolutie van individuele bonen, terwijl gedragscomponenten werden waargenomen op een tijdsschaal van seconden. Dit maakt een betekenisvolle interpretatie van de waarnemingen op het niveau van

individuele sluipwespen mogelijk. De belangrijkste factor die het gedrag van *U. lariophaga* beïnvloedde was een ontmoeting met een gastheer-ei: dit veranderde het looptraject van 'recht' in 'kronkelig' en het verhoogde de verblijfsduur per boon. *Uscana lariophaga* leek aangetrokken te worden door gastheereieren vanaf een afstand van ongeveer 4-6 bonen, en bij het overstappen van de ene boon naar de andere vertoonde ze voorkeur voor bonen met een ei. Als ze eenmaal was aangekomen op een boon met een ei slaagde ze er echter meestal niet in om het ei binnen de tijdsduur van één boonbezoek te vinden.

In **hoofdstuk 4** wordt het vinden van eiclusters, door *U. lariophaga*-vrouwtjes in opgeslagen cowpea, geanalyseerd. Het vinden van eiclusters is een functie van afstand, tijd, grootte van het eicluster, en van de ruimtelijke positie van *U. lariophaga* ten opzichte van het eicluster. *Uscana lariophaga*-vrouwtjes waren in staat om gastheren te vinden tot op een horizontale afstand van 75 cm van de loslaatplek. Dit is tevens de grootste afstand die getest is. De kans dat een eicluster werd gevonden wanneer een individueel *U. lariophaga*-vrouwtje werd losgelaten op 2.5 cm horizontale afstand vanaf het eicluster variëerde van 0.6 bij 2 uur zoektijd tot 0.9 bij 8 uur zoektijd. Vanaf een afstand van 10 cm vanaf het eicluster variëerde de kans dat het eicluster gevonden werd van 0.2 tot 0.45 bij 2 respectievelijk 8 uur zoektijd. De kans dat het eicluster gevonden werd verdubbelde wanneer *U. lariophaga* werd losgelaten ónder in plaats van naast het eicluster, en halveerde wanneer de sluipwesp werd losgelaten bóven in plaats van naast het eicluster. De mediane netto verplaatsingssnelheid in de richting van het eicluster werd geschat op twee bonen per uur (1.4 $cm \cdot h^{-1}$) wanneer *U. lariophaga* werd losgelaten op een horizontale afstand van 2.5 cm vanaf het eicluster.

Wat gebeurt er als *U. lariophaga* een eicluster vindt dat al eerder was gevonden en grotendeels geparasiteerd door een ander vrouwtje? Deze situatie zal zich waarschijnlijk voordoen als de dichtheid van *C. maculatus* laag is ten opzichte van de dichtheid van *U. lariophaga*, en dit wordt bestudeerd in **hoofdstuk 5**. *Uscana lariophaga*-vrouwtjes met een parasiteringservaring in een ongeparasiteerde gastheer werden individueel losgelaten in een arena met 15 gastheereieren. Aanvankelijk bevatte de arena alleen ongeparasiteerde eieren, maar geleidelijk aan werden meer eieren geparasiteerd. Twee behandelingen werden ingezet: een behandeling waarin vrouwtjes eieren tegenkwamen die ze zelf eerder hadden geparasiteerd; en een behandeling waarin vrouwtjes eieren tegenkwamen die door henzelf waren geparasiteerd én eieren die door andere vrouwtjes waren geparasiteerd. Een ontmoeting met een ongeparasiteerd ei in de arena zorgde voor een significante daling van de kans op superparasitering (d.w.z. de kans dat een reeds geparasiteerd ei opnieuw zou worden geparasiteerd) door het betreffende vrouwtje. Dit had tot gevolg dat 'zelf'-superparasitisme (waarbij een geparasiteerd ei door hetzelfde

vrouwtje opnieuw wordt geparasiteerd) slechts twee maal voorkwam, terwijl 'soortgenoten'-superparasitisme (waarbij een geparasiteerd ei door een ánder vrouwtje opnieuw wordt geparasiteerd) 40 keer werd waargenomen (uit een totaal aantal van 556 parasiteringen). In een tweede experiment, waarin vrouwtjes met een parasiteringservaring één enkel gastheerei kregen aangeboden, waren 'zelf'- en 'soortgenoten'-superparasitisme beide even zeldzaam: beide kwamen voor met een frequentie van 6%. Superparasitisme was niet het gevolg van een gebrekkig onderscheid tussen geparasiteerde en ongeparasiteerde eieren, maar was mogelijk adaptief gedrag. Dit blijkt uit het feit dat vrouwtjes, wanneer ze superparasiteerden, de sexratio van hun nakomelingen aanpasten in de richting van het geslacht met de hoogste overlevingskans. Omdat superparasitisme een zeldzaam verschijnsel was, is het effect ervan op de biologische bestrijding door *U. lariophaga* waarschijnlijk beperkt.

Bij lage gastheerdichtheden zal *U. lariophaga* in het algemeen minder gastheren per tijdseenheid vinden. Veel insecten leven langer als ze weinig of geen nakomelingen kunnen produceren, dankzij re-allocatie van energievoorraden. In **hoofdstuk 6** wordt bestudeerd of zo'n uitwisseling (*trade-off*) tussen reproductie en overleving ook voorkomt bij *U. lariophaga*. Het tegenovergestelde werd echter gevonden: vrouwtjes die toegang hadden tot 98 gastheereieren leefden significant langer dan vrouwtjes die toegang hadden tot 0 of 10 gastheereieren (gemiddeld 45 versus 33 uur; bij deze dichtheden werden gemiddeld respectievelijk 61 en 17 eieren geparasiteerd). Deze verkorte levensduur bij geen of weinig gastheren kan het gevolg zijn geweest van intensief zoekgedrag, omdat vrouwtjes bij lage gastheerdichtheid meer loopactiviteit vertoonden dan vrouwtjes bij hoge gastheerdichtheid. Op grond van de gegevens die in dit hoofdstuk zijn gepresenteerd kan niet worden vastgesteld of deze toename in loopactiviteit en de verkorte levensduur bij lage gastheerdichtheid een aanpassing zijn aan een natuurlijke verdeling van gastheren, of aan kweekomstandigheden.

In **hoofdstuk 7** wordt het verkregen inzicht in het foerageergedrag van *U. lariophaga* samengevat door het formuleren van een conceptueel model, en worden de gevolgen van het zoekgedrag van *U. lariophaga* voor de biologische bestrijding van *C. maculatus* besproken. Het conceptuele model laat zien welke aspecten van het gedrag nog onbekend zijn. Een simulatiemodel, gebaseerd op het conceptuele model, zou gebruikt kunnen worden om de gevolgen te bestuderen van het foerageergedrag van *U. lariophaga* voor haar functionele respons in opgeslagen cowpea. Wat de biologische bestrijding betreft: problemen met het vinden van gastheren vanwege de grote afstanden tussen eiclusters is mogelijk één van de belangrijkste oorzaken voor de matige resultaten die geboekt worden met *U. lariophaga* bij lage gastheerdichtheden. Bovendien staat de verkorte levensduur van *U. lari-*

ophaga bij lage gastheerdichtheden de sluipwesp niet toe om te 'wachten' tot de gastheren meer talrijk zijn. (Merk op dat deze verkorte levensduur mogelijk juist veroorzaakt wordt doordát *U. lariophaga*, als een parasitoïd van vastzittende gastheereieren, geen strategie van 'stilzitten en wachten' hanteert). *Uscana lariophaga* blijkt aangetrokken te worden door kairomonen die gerelateerd zijn aan gastheereieren; maar het is niet duidelijk hoe deze kairomonen kunnen worden ingezet om de biologische bestrijding te verbeteren. Aanvullende loslatingen van *U. lariophaga* zouden de biologische bestrijding bij lage gastheerdichtheden kunnen verbeteren; maar het massaal kweken en loslaten van *U. lariophaga* in opgeslagen bonen is momenteel niet haalbaar op het West-Afrikaanse platteland. In principe zou *Uscana lariophaga* echter een goede kandidaat zijn voor inundatieve biologische bestrijding van zaadkevers: voor de kweek zijn slechts kleine hoeveelheden bonen nodig en grote aantallen sluipwespen kunnen worden gekweekt op een klein oppervlak.

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Clemens Stolk

De auteur

Clemens Stolk werd geboren op 26 augustus 1972 te Bergschenhoek. Het VWO doorliep hij op de scholengemeenschappen Jacobus Revius (1984-1988) en Guido de Brès (1988-1990), beide te Rotterdam. In 1990 begon hij in Wageningen aan de studie Planteziektenkunde. Tijdens deze studie bestudeerde hij in een afstudeervak in de entomologie het effect van een cytoplasmatische incompatibiliteit-inducerende *Wolbachia*-bacterie op de fitness van de sluipwesp *Nasonia vitripennis*. In een ander afstudeervak onderzocht hij de eigenschappen van een analytisch model dat de interactie beschrijft tussen een sclerotiën-vormende bodemschimmel en een parasitaire schimmel. Zijn stage bracht hij door bij de Agricultural Research Organization 'Volcani Center' in Israël. Daar deed hij onder meer onderzoek naar het effect van verschillende stoffen op de kieming van conidieën van *Trichoderma harzianum*, een commercieel gebruikte antagonist van de plant-pathogene schimmel *Botrytis cinerea*. In 1996 studeerde hij af (cum laude). Van 1997 tot 2002 voerde hij het in dit proefschrift beschreven onderzoek uit aan het Laboratorium voor Entomologie van de Wageningen Universiteit. Zijn aanstelling als aio onderbrak hij in 2001 voor twee maanden om te assisteren bij de coördinatie van het vak Ecologie. Sinds augustus 2002 is hij werkzaam als beleidsmedewerker onderzoek bij de branche-organisatie voor bedrijven in plantaardig uitgangsmateriaal, Plantum NL, te Gouda.

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