

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

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## Abstract

Host finding and parasitization by *Uscana lariophaga* Steffan, a potential biocontrol agent of the storage pest *Callosobruchus maculatus* (Fabricius), were investigated in stored cowpea. Host finding was shown to be a function of distance, time, host patch size and 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 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 after 2 h foraging time to 0.9 after 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 negative geotaxic response was shown not to be an artefact of the release method. The median net displacement rate in the direction of the host patch was two beans per hour ( $1.4 \text{ cm h}^{-1}$ ). The results suggest that *U. lariophaga* females start searching for hosts regardless of the quality of the olfactory information they receive. Additional observations indicated that *U. lariophaga* is adapted to a host with a patchy distribution, which implies that host finding over larger distances is relevant for *U. lariophaga*.

## Introduction

Cowpea, *Vigna unguiculata* Walp. (Fabaceae), is an important crop in Africa (Nwokolo & Ilechukwu, 1996). It is

attractive to subsistence farmers because it is relatively resistant to drought conditions (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. The 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

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Table 1. Experimental overview.

Expt	Effects tested	Container type	<i>Uscana</i> introduction method	Host patch size (approx. # eggs)	No. of host 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

loss of nutritional value (Modgil & Mehta, 1994, 1996), secondary infestation with toxin-producing fungi (Sinha & Wallace, 1966; Siwela, 1996), and in a reduced 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 *C. maculatus* (Caswell, 1981).

*Callosobruchus 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 days 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). In experimental cowpea stocks, a single *U. lariophaga* inoculation can suppress *C. maculatus* populations by up to 86% over at least three months compared to the controls (Van Huis *et al.*, 1998). Consequently, *U. lariophaga* has been proposed 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). *Callosobruchus 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 *et al.*, 1996a,b; Van Alebeek & Van Huis, 1997; Van Huis *et al.*, 1998, 2002). *Uscana 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

greater than 5–6 cm, and little attention has been paid to the displacement rate 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 at low densities early in the storage season (Stolk *et al.*, 2001). The current study was therefore devoted to investigating host patch finding capacity of *U. lariophaga* in stored cowpea.

In this paper, the effects of the following factors on host finding by *U. lariophaga* were studied: distance to the host patch, size of the host patch, time available for foraging and spatial orientation. In addition to these factors, two different methods of introducing the wasps into a cowpea stock were used: one which closely resembled the release method that was used in previous experiments (e.g. Van Alebeek & Van Huis, 1997), and another which was designed to minimize disturbance of *U. lariophaga*. These two methods were used in order to rule out disturbance as the main cause for negative geotaxis, since the method used in previous experiments may have affected normal behaviour and disturbance can induce negative geotaxis in insects (Surtees, 1963).

## Materials and methods

### General procedure

Host patch finding by *U. lariophaga* in stored cowpea was studied in six separate experiments (table 1). These experiments were necessary to study the effect of four interacting factors on host finding and to rule out any possible effect of the release method on the results. The general procedure is described first, followed by the individual experiments.

Experiments were carried out in tightly closed opaque containers, filled with cowpea and containing either one or two host patches. The containers were either horizontally-placed oblong boxes or vertical cylinders. The host patches consisted of small nylon gauze bags which contained beans carrying fresh *C. maculatus* eggs. *Uscana lariophaga* wasps were introduced into these containers at varying horizontal or vertical distances from the host patch or patches. Each experiment was completed 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 incubated in sealed Petri dishes at 30°C. After three days of incubation the numbers of parasitized and unparasitized

eggs in each host patch were counted. The host patch was considered found by *U. lariophaga* if it contained parasitized eggs or if a wasp was found in the host patch. All experiments were replicated over time.

Cowpea seeds, *V. unguiculata*, cv. 'Black Eye' were used in all experiments and for rearing. Before use, the beans were frozen at  $-18^{\circ}\text{C}$  and subsequently dried at  $45^{\circ}\text{C}$ , over a period of six days, to exclude any contamination with live 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, beans within a distance of 7.5 cm from the host patch in experiments 1 and 2 were not re-used. The remaining beans in experiment 1 and 2 were kept at  $45^{\circ}\text{C}$  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  $35 \pm 1^{\circ}\text{C}$  during photophase and at  $25 \pm 1^{\circ}\text{C}$  during scotophase (12L:12D). *Callosobruchus maculatus* eggs were obtained from 0- to 2-day-old females. If the eggs would not be used within 24 h after oviposition, they were stored at  $5 \pm 1^{\circ}\text{C}$  for up to one day prior to use. *Uscana lariophaga* was reared at  $30 \pm 1^{\circ}\text{C}$  and a photoperiod of 12L:12D. Female adult wasps used in the experiments were 2–17 h old and had been allowed to mate for about 2 h.

#### *Methods of introducing U. lariophaga into the cowpea stocks*

In four experiments (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 approx. 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. In order to release the parasitoid, each vial could be opened from the outside of the container by pulling strings that were attached to the cotton wool plugs. 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, beans carrying parasitized eggs about to emerge were inserted at one location into the cowpea stock. This method is more natural and minimizes disturbance for *U. lariophaga*.

#### *Host finding over long horizontal distances*

In the first experiment, host finding of *U. lariophaga* was investigated over a series of horizontal distances of up to 75 cm. The containers used measured  $95 \times 20 \times 20$  cm ( $l \times w \times h$ ). The host patch contained 20 beans carrying  $565 \pm 146$  (SD) fresh *C. maculatus* eggs and was placed 10 cm from one end of the container, at the centre of the bean mass. This large number of eggs in the host patch was chosen with the intention that each wasp that reached the host patch could lay all her eggs. At 5, 15, 25, 40 or 75 cm from the 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 5 cm treatment, which had four replicates.

As the experiment was carried out with parasitized eggs rather than individual female wasps (in order to avoid disturbance), separate observations were made to monitor the emergence of female wasps from the parasitized eggs. 50–100 parasitized eggs, from the same batch as those in the release bag, were incubated in darkened Petri dishes. Wasps that emerged from these eggs were counted and sexed every day. The 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 two days after emergence (Van Huis *et al.*, 1991a).

In experiment 2, host finding was compared at two horizontal distances, using host patches of two different sizes. The containers used measured  $94 \times 18$  cm at the top,  $91 \times 14$  cm at the bottom, and 16.5 cm in height. The host patch was placed 7.5 cm from one end of the container and comprised either 40 beans carrying  $562 \pm 108$  (SD) fresh *C. maculatus* eggs or 10 beans carrying  $29 \pm 4$  (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 (Stolk *et al.*, 2001; Boeke *et al.*, 2004). 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.

#### *Host finding over short horizontal distances*

In experiment 3, host finding was investigated for three horizontal distances and three time intervals. Horizontally placed cylindrical containers (length 14.5 cm, diameter 11 cm) were used, containing one host patch at 2.3 cm from one end of the container. Each host patch contained  $26 \pm 4$  (SD) eggs on 10 beans. One *U. lariophaga* female was released from a glass vial at 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.

#### *Vertical host finding*

In experiment 4, up- and downward host finding was investigated 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  $387 \pm 182$  (SD) eggs on 10 beans. A few beans with a total of 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 at this low wasp density, the parasitized eggs were accompanied by an open gel capsule containing three *U. lariophaga* males. This was done because mating might influence host finding behaviour. After 72 h the experiment was ended. The experiment was replicated 11 times.

In experiment 5, up- and downward host finding was investigated in upright cylinders with one host patch. The

host patch contained  $26 \pm 4$  (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). One *U. lariophaga* female was released from a glass vial at the centre of the container, 5 cm away 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 an analysis of this experiment together with the '5 cm, 4 h' 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 both top and bottom host patches were used in one container. Experiment 6 was thus a 'choice' version of the 'no-choice' experiment 5. The experiment was replicated 20 times.

#### Statistical analysis

The probability that the host patch was found was analysed as a function of distance for experiments 1 and 2, and as a function of distance and time for experiment 3, using binary (logistic) regression. Cochran's test was used to investigate 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. Distance and time were tested for their effect on the probability that a *U. lariophaga* wasp was found still at or near the release point in experiment 3, using binary regression.

The factors involved in experiments 1, 2, 3 and 5 were tested for an effect on the number of parasitized eggs. This was done using the Kruskal-Wallis test for experiments 1 and 5 and Friedman's test with multiple observations per cell, corrected for ties, for experiments 2 and 3. Additional tests were carried out to test for a possible effect on the number of parasitized eggs for those host patches that were found (thus leaving out patches without parasitism). This was done using the Bernard and Van Elteren test for experiment 3 (Bernard & Van Elteren, 1953) and the Kruskal-Wallis test for experiment 5. The Bernard and Van Elteren test is a generalized form of Friedman's test which allows for unequal numbers of observations per cell (missing values). Non-parametric tests were used in all these cases because 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 those cases in experiments 4 and 6 where only one host patch was found by *U. lariophaga*, the probability of the top and the bottom cluster being found was compared 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 the number of beans with parasitized eggs was compared for the top and bottom clusters using the same test.

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

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

with  $t_{arrival}$  = time needed for the wasp to reach the host patch (s),  $t_{total}$  = total time available (s) and  $t_{par}$  = time spent on parasitization (s).

The total time available was 2, 4 or 8 h, depending on the treatment. The time spent on parasitization consisted of the time spent on oviposition and the time in between, searching for new hosts. The time needed for an oviposition was assumed to be constant (156 s), whereas the time in between subsequent ovipositions was assumed to increase as more eggs in a cluster are parasitized (based on unpublished data, C. Stolk *et al.*). Thus,

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

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:

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

with  $f_{unp,i}$  = the fraction of unparasitized eggs in the host cluster after the  $i$ -th parasitization + 0.001 to avoid zeroes (based on unpublished data, C. Stolk *et al.*).

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

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

The estimated arrival times were analysed using Cox regression analysis. Cox regression analysis is a type of survival analysis which can be used to analyse time spans until a certain event or 'failure' occurs (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 the current 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

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

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 from a distance of 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 5 cm away 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

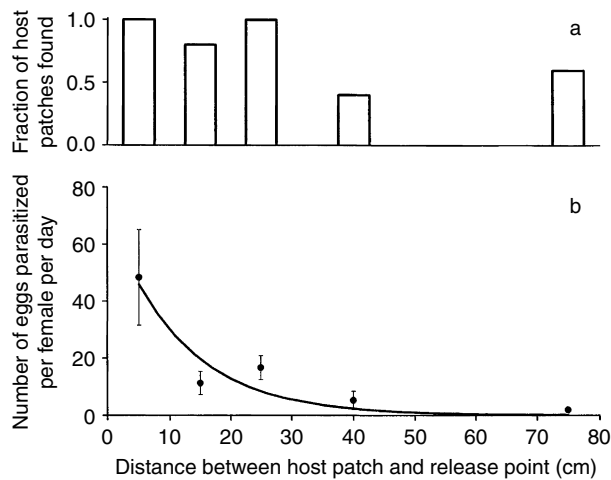


Fig. 1. (a) The fraction of host patches that were found by *Uscana lariophaga* as a function of distance between the host patch and the *Uscana* release bag and (b) the number of parasitized *Callosobruchus maculatus* eggs per female per day as a function of distance between the host patch and the *Uscana* 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).

calculating the probability of the observed sex ratio for each week, using 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

### 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 by *U. lariophaga* ( $P = 0.49$ ; fig. 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 ( $P < 0.01$ ) (fig. 1).

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

In experiment 2, both host patch size and distance had a significant effect on the probability of finding a host patch ( $P = 0.025$  and  $P = 0.010$ , respectively). The effect of host patch size was positive and the effect of distance negative (fig. 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) (fig. 2b).

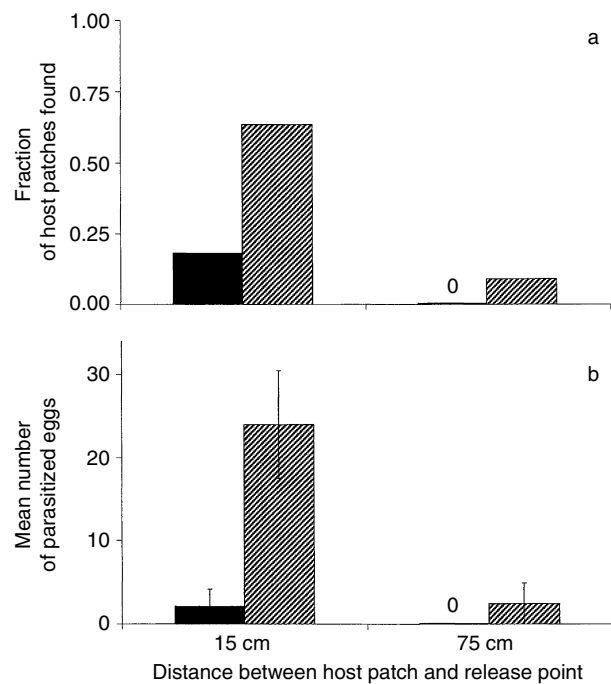


Fig. 2. (a) The fraction of small (■) and large (▨) host patches found and (b) the mean number of eggs parasitized by individual *Uscana lariophaga* females during one day at two distances from the release site (experiment 2). Error bars indicate the standard error (N = 11 for each treatment).

### Host finding over short horizontal distances

Both distance and available time had a significant effect on the probability of *U. lariophaga* 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 (fig. 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 ( $P < 0.001$ ) (fig. 3b). If, however, the replicates in which the parasitoid failed to find the host patch were omitted, distance had no effect on the number of parasitized eggs ( $P = 0.25$ ) while available time had a positive effect ( $P < 0.001$ ) (fig. 3c).

Altogether 39 females were found 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 a 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 *U. lariophaga* were found back at the release site; after 8 h this figure had shrunk to 12% ( $P = 0.032$ ).

Figure 4a shows cumulative distributions of estimated times of arrival in the host patch (equation 1) for individual wasps released at 2.5, 5 and 10 cm distance. Data from the 8 h treatments were omitted from the calculation for this figure since the wasps had parasitized almost all the eggs, rendering them unfit for a reliable 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

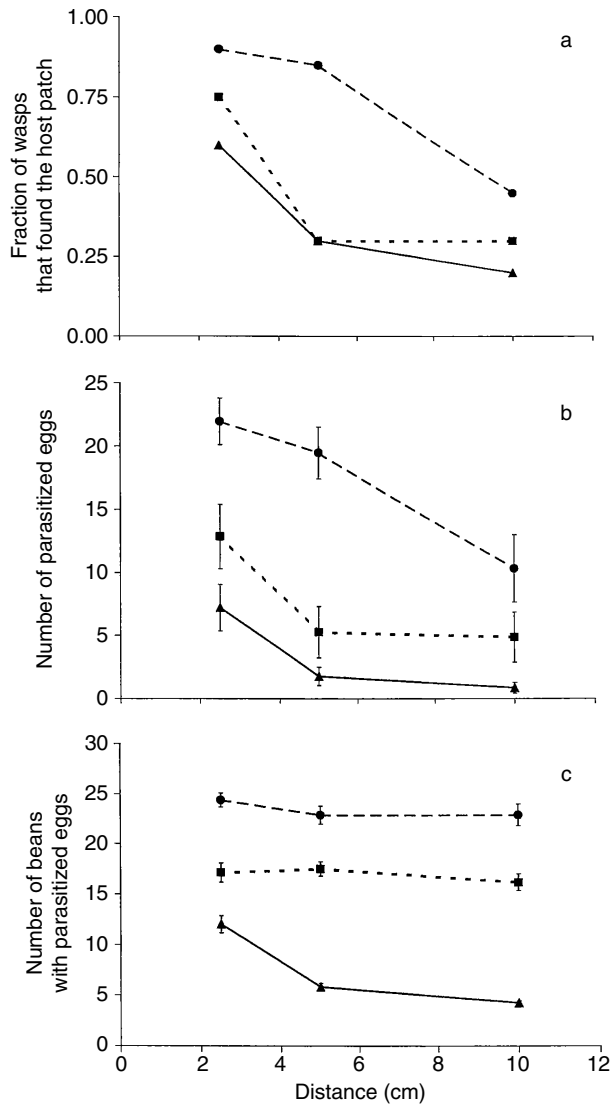


Fig. 3. The effect of distance and time ( $\blacktriangle$ , 2 h;  $\blacksquare$ , 4 h;  $\bullet$ , 8 h) on finding probability and parasitization of *Callosobruchus maculatus* eggs by *Uscana lariophaga*: (a) the fraction of host patches found, (b) the mean number of parasitized eggs per host patch, and (c) the mean number of parasitized eggs for host patches that were found. Error bars indicate the standard error ( $N=20$  for each data point).

the other hand, were very similar for each respective distance (data not shown). This indicates that these estimates were fairly accurate, and arrival times from the 2 and 4 h treatments were pooled in fig. 4a.

Net displacement rates in the direction of the host patch were calculated from the data shown in fig. 4a. Most adult *U. lariophaga* progressed towards the host patch at net rates of about  $1\text{--}2\text{ cm h}^{-1}$ , as shown by the representative frequency distribution of net displacement rates for the '2.5 cm and 4 h' treatment (fig. 4b). The median net displacement rate for the 2.5 cm data was  $1.4\text{ cm h}^{-1}$ . The median net displacement rate could not be calculated for the other distances, because the percentage of wasps that reached the host patch was

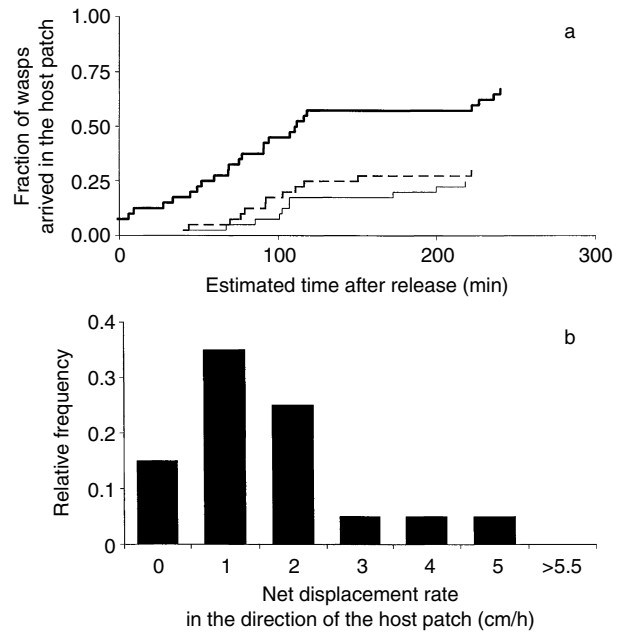


Fig. 4. Estimated arrival times and corresponding net displacement rates of *Uscana lariophaga*. (a) The fraction of wasps arriving at the host patch, after being released 2.5 ( $\blacktriangle$ ), 5 ( $\blacksquare$ ) or 10 ( $\bullet$ ) cm from the host patch, as a function of time. Arrival times were 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 were 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 was based on the number of wasps found at or near the release site. Two wasps, which were not found at the release site and had not reached the host patch, were not included in this distribution. Their net displacement rate in the direction of the host patch was lower than  $2.5/4 = 0.63\text{ cm h}^{-1}$ .

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 (fig. 4a). The corresponding 'quartile' net displacement rates were almost equal at  $2.9$ ,  $2.6$  and  $2.8\text{ cm h}^{-1}$ , respectively.

The effect terms 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 was found at 5 cm, for instance, is 0.32 times the rate at which the host patch was 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,  $df = 2$ ).

#### Vertical host finding

In seven out of the 12 replicates of experiment 4, both host patches were found by *U. lariophaga*, 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,

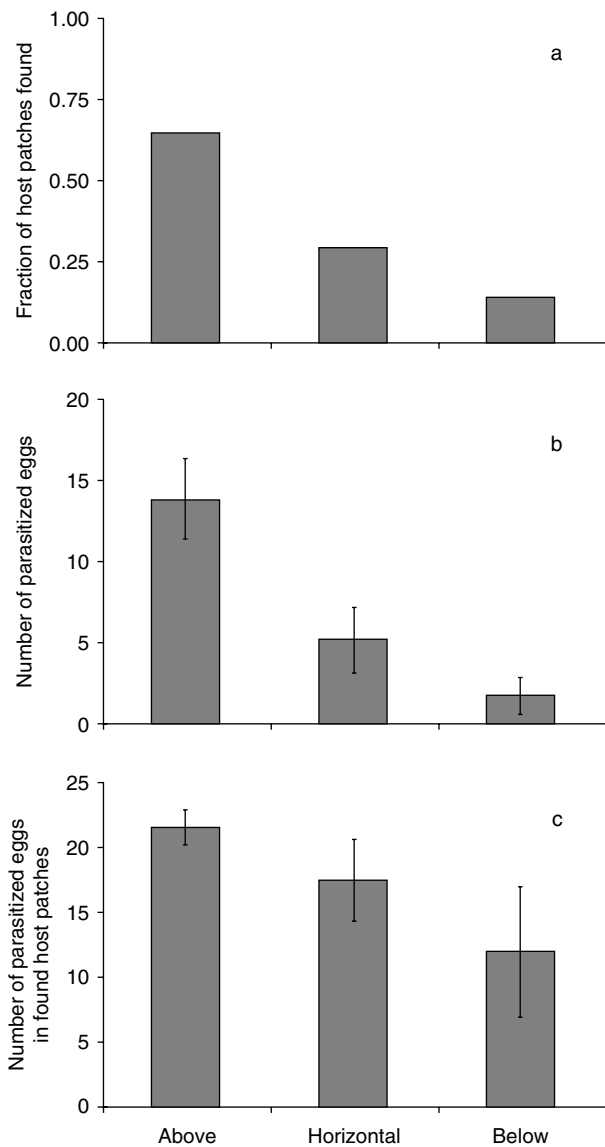


Fig. 5. (a) The fraction of host patches found, (b) the mean number of parasitized eggs per host patch, and (c) the mean number of parasitized eggs for host patches that were found, as a function of the position of the host patch with respect to the site of release of *Uscana lariophaga*. The 'horizontal' data were taken from experiment 3 and the other data from experiment 5. Error bars indicate the standard error (N=20 for each treatment).

but this difference was not significant (60 vs. 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 vs. 4.4,  $P=0.012$ ).

In experiment 5, 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 (fig. 5a). The position of the host cluster also had a significant effect on the number of parasitized eggs ( $P < 0.01$ ) (fig. 5b). For host

patches that were found, there was no effect of orientation on the number of parasitized eggs ( $P=0.15$ ) (fig. 5c).

In experiment 6, only one of the two host patches was found in 14 out of the 20 replicates; 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 showed that *U. lariophaga* can find host patches over distances up to 75 cm. So far, host patch finding by *U. lariophaga* in stored cowpea has only been measured for distances of 5 and 6 cm, although in a dispersal study the wasp had been shown to travel at least 20 cm through stored cowpea (Van Alebeek, 1996a; Van Alebeek *et al.* 1996a,b; Van Alebeek & Van Huis, 1997). The results of the current study, however, suggest that it takes *U. lariophaga* a long time to cover 75 cm. This appears from the fact that very few eggs were parasitized, even though a substantial fraction of host patches was found at this distance. It seems therefore that, when the wasps finally arrived at this host patch, they had little time or energy left before they died or before the experiment ended, and/or most eggs were not parasitizable any more. At 30°C, *U. lariophaga* lives for about two days (Van Huis *et al.*, 1991a, 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<sup>-1</sup> 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, fig. 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 a 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. From a short distance from a host patch *U. lariophaga* may show directed search, mediated by odours related to *C. maculatus* eggs (Van Huis *et al.*, 1994b; Ormel *et al.*, 1995).

The probability of finding a host patch doubles if the host patch is above rather than level with the release site, and this probability halves if the host patch is underneath the release site (experiment 5; fig. 5a). This result, and experiment 6, confirm the findings of Van Alebeek & Van Huis (1997) and F.A.N. van Alebeek & A. Conteh (unpublished results) who showed that *U. lariophaga* has a strong negative geotaxis response. Experiment 4 shows that this negative geotaxis is also present when the parasitoid is released by emergence from the host (i.e. naturally), instead of from a vial or a gel capsule. A negative geotaxis response has also been reported

for other parasitoids of stored product pests, such as *Trichogramma* spp. (Quednau, 1958), *Eupelmis vuilleti* (Crawford) (Hymenoptera: Eupelmidae) (Cortesero *et al.*, 1997) and *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) (Press, 1988). An exception is *Lariophagus distinguendus* (Förster) (Hymenoptera: Pteromalidae), which does not show a clear geotaxis response and which is able to find hosts in stored grain up to a depth of 4 m (Steidle & Schoeller, 2002).

#### Net displacement rates

In experiment 4, the median net replacement rate of *U. lariophaga* was estimated to be  $1.4 \text{ cm h}^{-1}$ . Van Alebeek (1996a) estimated the median net displacement rate in stored cowpea seeds at  $0.62 \cdot 10^{-5} \text{ m s}^{-1}$ , which corresponds to  $2.2 \text{ cm h}^{-1}$ . 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 travelled at least 50 cm in all directions in 24 h ( $2.1 \text{ cm h}^{-1}$ ).

The average diameter of the cowpea seeds used in the experiments was 0.7 cm (Stolk *et al.*, 2001). If this is taken into account, the median net displacement rate of  $1.4 \text{ cm h}^{-1}$  can also be expressed as two beans per hour. 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* (C. Stolk *et al.*, unpublished).

#### 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 a wasp was found back at the release site at the end of the experiment. Distance did, however, have an effect on the probability that a wasp found the host patch. It might be assumed 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, which excludes ventilation, and convection within the small containers can equally be ruled out). If this assumption is true, the current 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 slightly earlier than females (Van Huis & Appiah, 1995; see also experiment 1). These observations indicate that *U. lariophaga* males usually mate with wasps that emerge at the same location. This type of 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 (Stolk *et al.*, 2001).

#### Other parasitoids in stored products

In this paper, host finding ability or displacement of *U. lariophaga* was studied. 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 eggs of *Caryedon serratus* (Olivier) (Coleoptera: Bruchidae) in stored groundnut by *Uscana caryedoni* (Viggiani) (Hymenoptera: Trichogrammatidae) decreased rapidly with depth: no parasitization was found below the fourth 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 *A. calandrae* and *Pteromalus* sp. were found at the centre of commercial corn bins than near the walls. Flinn *et al.* (1992) mention finding unidentified hymenopteran parasitoids in the central regions and apparently not in the periphery of a  $351 \text{ m}^3$  grain bin. Howe (1943) investigated a spontaneously heating bulk of stored grain, and showed that most of the *L. distinguendus* individuals were found in the top 25 cm of the grain bulk. Based on this limited amount of information, it might seem that parasitoids of stored product insects tend to occur at the centre and near the surface of stored produce. 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 in the central regions of stored wheat.

Host finding capacity in stored produce has been studied for several parasitoid species (Press, 1988, 1992; Brower, 1990; Verma, 1990; Schoeller *et al.*, 1994, 1996; Cortesero *et al.*, 1997; Schoeller, 2000). In most cases, however, parasitoids were released on top of the stored product and only vertical penetration into the stored product was measured. It has, for instance, been shown that *E. vuilleti* females can find bruchid larvae down to at least 77 cm depth when they are released on top of stored cowpea (Cortesero *et al.*, 1997), and that *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) is able to find host eggs up to at least 55 cm depth (Schoeller *et al.*, 1994). However, Steidle & Schoeller (2002) showed that *L. distinguendus* can find hosts in stored

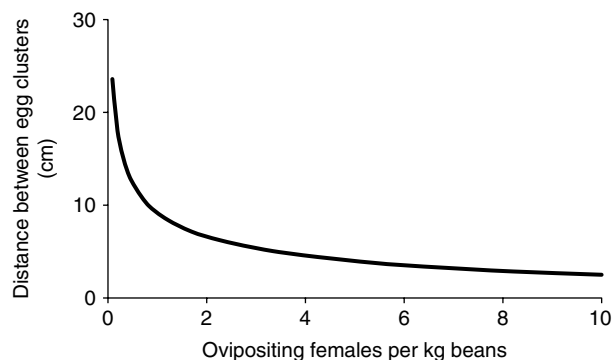


Fig. 6. Distance between egg clusters if they are homogeneously distributed through a cowpea mass, as a function of *Callosobruchus maculatus* female density. The specific weight of cowpeas was set at  $700 \text{ kg m}^{-3}$ .



grain at a depth of 50 cm and at a horizontal distance of 4 m (but not at 6 m) from the release point. *Trichogramma 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 (Schoeller, 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 paper, 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 are expected to 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 location 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. A model proposed by McCoy & Powelson (1974) can be used to estimate the distances between ovipositing *C. maculatus* females under the assumption that they are uniformly distributed in a cowpea mass:

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

where  $V$  = volume ( $\text{cm}^3$ ),  $N$  = the number of beetles in volume  $V$ , and  $D$  = distance between beetles (cm). If it is also assumed that each female oviposits in a single, spherical cluster with a volume of  $19.1 \text{ cm}^3$  (Stolk *et al.*, 2001) and with a resulting diameter of 3.3 cm, the distance between fresh egg clusters is

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

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 disproportionately 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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