# CHAPTER 8

## Host Habitat Assessment by a Parasitoid Using Fungal Volatiles

**Abstract** Fitness of parasitic wasps depends on the ability of females to assess the quality of host patches. Intense growth of mould following insect infestation of stored grain can lead to distinct sites of extreme environmental conditions (hot spots) causing an increased insect mortality. We studied the influence of mould on chemical orientation, host recognition behaviour and fitness of the parasitoid *L. distinguendus* (Förster), parasitising beetle larvae that develop in stored grain. Volatiles emitted from wheat infested by *Aspergillus sydowii* and *A. versicolor* repelled female parasitoids in an olfactometer bioassay. Foraging *L. distinguendus* females are known to orientate towards the odour of larval host faeces. We therefore studied the impact of mould on the attractiveness of larval faeces from the granary weevil *Sitophilus granarius*. Faeces from moulded weevil cultures remained attractive (*A. versicolor*) or elicited a neutral response (*A. sydowii*), but parasitoids clearly preferred the odour of non-moulded faeces when offered simultaneously. The common fungal volatile 1-octen-3-ol was detected as a major compound in larval faeces from moulded weevil cultures and repelled female parasitoids in the olfactometer when tested as synthetic chemical at different doses. Bioassays investigating the host recognition behaviour of *L. distinguendus* revealed that females spent less time on grains containing hosts from moulded weevil cultures and showed less drumming and drilling behaviour than on grains without mould infestation. *L. distinguendus* females had a clearly reduced fitness when offered hosts from moulded weevil cultures for oviposition. We conclude from these results that *L. distinguendus* females use 1-octen-3-ol for host habitat assessment to avoid negative fitness consequences resulting from secondary mould infestation of host patches. The fact that the response of females to the fungal volatile is innate, suggests that host-associated fungi played a crucial role in the evolution of host finding strategies of *L. distinguendus*.

**Key words** host finding, *Lariophagus distinguendus*, Pteromalidae, mould, semio-chemicals.

# Introduction

The reproductive success of parasitic Hymenoptera is not only determined by the number of eggs females lay but also by the survival and fecundity of their offspring. Since host organisms are the only source of nutrients for immature stages of parasitoids (Sequeira and Mackauer, 1992), parental fitness particularly depends on accurate assessment of the host sites in terms of their potential to sustain the development of their larvae. Thus, adaptation to reliable cues enabling the evaluation of the host patch quality is a selective advantage for females searching for oviposition sites (Meyling and Pell, 2006).

Successful parasitisation requires commonly a series of successive steps with host habitat location, host location and host recognition being the major elements (Vinson, 1976; Godfray, 1994; Quicke, 1997). Apart from physical cues (Schmidt 1991), volatile and non-volatile chemicals have been found to be of considerable importance at almost every level of this foraging process (Vinson, 1976; Vet and Dicke, 1992). Compounds involved may emanate from the host, host by-products, host food plants or organisms closely associated with the host (Vinson, 1981; Tumlinson et al., 1992; Steidle and van Loon, 2002).

Chemical cues can not only attract but also deter parasitoids from entering host sites or from laying eggs into unsuitable hosts. Foraging decisions of insects are affected by both extrinsic factors like the suitability of resources, presence of natural enemies or competitors and by intrinsic factors like experience and age of the ovipositing female (Vet and Dicke, 1992; Fransen and van Lenteren, 1993; Hoffmeister and Roitberg, 1997; Doumbia et al., 1998; Frechette et al., 2004). In contrast, little attention has been paid to the investigation of chemicals that reliably indicate unfavourable environmental conditions (e.g., temperature, humidity, $CO_2$ concentration) within the host habitat (Vinson,1980).

The solitary ectoparasitoid *Lariophagus distinguendus* (Förster) (Hymenoptera: Pteromalidae) parasitises immature stages of several stored-product infesting beetle species that develop inside grains and seeds (Steidle, 1998). The behaviour and chemical cues involved in the host finding process of this species have been extensively examined (Steidle and Schöller, 1997; Ruther and Steidle, 2000; Steidle, 2000; Steidle and Fischer, 2000; Steidle and Ruther, 2000; Steidle et al.,

2001a, b, 2003). Volatiles emitted by the larval faeces of the granary weevil *Sitophilus granarius* L. and other hosts mediate long-range orientation of the parasitoid during host habitat location (Steidle and Schöller, 1997; Steidle et al., 2001a). Once within the host habitat, females search for reliable cues indicating the presence of their hosts. Again, compounds originating from the larval faeces have been shown be crucial for host recognition (Steidle and Ruther, 2000). On grains infested with the host, *L. distinguendus* females perform a stereotypic sequence of behavioural elements including series of intense antennal drumming, tapping with the abdominal tip on the surface of the infested grain and finally drilling with the ovipositor into the grain (Steidle, 2000). After immobilising the host by injecting paralysing substances, females of *L. distinguendus* typically lay one single egg onto the surface of the host and the hatching larva develops inside the grain while feeding upon the host (Hase, 1924).

Since *L. distinguendus* attacks beetles that often lay their eggs in clumps within stored grain, intense infestation of the grain by a larger number of beetles can lead to a zoned increase of temperature and humidity within the microhabitat (Sinha and Wallace, 1966). These abiotic conditions promote the invasion of astigmatid mites and particularly the growth of mould (Shinha, 1961; Eighme, 1966). At a critical level of secondary infestation, however, environmental parameters in these hot spots deteriorate and cause an increased insect mortality within the patch. Thus, reproductive success of parasitoids attacking host larvae in areas of high secondary infestation should be lower than in areas of light or no secondary infestation. The ability to detect and avoid these suboptimal oviposition areas would therefore improve the fitness of *L. distinguendus.*

Fungi are well-known to release a multitude of volatile organic compounds originating from different metabolic pathways. The composition of these volatile profiles depends on the fungal species as well as on the growing medium and abiotic factors (Sunesson et al., 1995). However, a variety of chemicals is shared by several species and is reliably released under various conditions. Among these widespread fungal volatiles is a group of aliphatic eight-carbon compounds including 1-octen-3-ol, 3-octanone and 3-octanol (Kaminski et al., 1974) that are derived from enzymatic degradation of unsaturated fatty acids (Chitarra et al., 2004). In most species, 1-octen-3-ol occurs as major component and it has been suggested to use this chemical for the detection of mould infestation in stored grain

as well as in living and working environments (Samson, 1985; Tuma et al., 1989; Börjesson et al., 1992; Menetrez and Foarde, 2002).

In the present study, we investigated whether mould infestation of *S. granarius* cultures affects chemical orientation, host recognition behaviour and fitness of *L. distinguendus*. Mould species studied were *Aspergillus sydowii* (Bainier and Sartory) Thom and Church and *A. versicolor* (Vuillemin) Tiraboschi. Furthermore, we determined the amounts of typical eight-carbon fungal volatiles in the weevil cultures and tested the response of female parasitoids to synthetic 1-octen-3-ol. The possible function of fungal volatiles as indicators of suboptimal host patches is discussed.

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## Methods and materials

**Insect cultures** Insect cultures were kept at constant conditions of 25°C, 65% relative humidity and a daily light:dark cycle of 16:8 h. The *L. distinguendus* strain used in the experiments was collected on *Stegobium paniceum* L. in a flour mill in Uzwill, Switzerland. In our laboratory the parasitoid was reared on larvae (3$^{rd}$ and 4$^{th}$ instar) of the granary weevil *Sitophilus granarius* in wheat grains (*Triticum aestivum* L., variety Batis), as described by Steidle and Schöller (1997).

**Insects for experiments** For all experiments except for experiment 2, naïve *L. distinguendus* females were used, i.e., individuals without mating and oviposition experience. For this purpose, parasitoids were collected immediately after emergence and held in groups of at most 15 individuals in Petri dishes lined with moistened filter paper. To obtain experienced parasitoids, freshly emerged females were kept together with males in a Petri dish containing host-infested grains for parasitisation. After three days, females were removed and kept without hosts in Petri dishes on moistened filter paper. One hour before experiments, *L. distinguendus* were individualised in 1.5 ml microcentrifuge tubes for acclimation at room temperature. Behaviours shown by the parasitoids during experiments were recorded by using the computer software The Observer 3.0 (Noldus, Wageningen, The Netherlands).

**Fungal cultures** The mould species *A. versicolor* (Strain no.: BBA 72388) and *A. sydowii* (Strain no.: BBA 72389) used in the experiments were obtained from the culture collection of the Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA), Institute of Plant Virology, Microbiology and Biosafety, Berlin, Germany. The isolates originated from intense weevil- and fungi-infested grain cultures of our institute. Stock cultures of *A. sydowii* and *A. versicolor* were freeze-dried and stored at 4°C. Subcultures were cultivated on nutrient deficient agar SNA at room temperature for 1 to 2 weeks. A 10 ml portion of sterile water was added to the subculture and the surface was scraped off until conidia were suspended. The suspension was filtered through a folded filter paper (S&S 595, Schleicher & Schuell, Dassel, Germany) and the conidia concentrations of the *Aspergillus* species were determined by counting in a Bürker counting chamber and adjusted with sterilised water to $1.5 \times 10^6$ conidia per ml.

**Preparation of moulded grain and weevil cultures** Three batches of wheat grain (750 g each) were autoclaved for 30 min. Two batches were treated with 45 ml conidia suspension of *A. sydowii* and *A. versicolor*, respectively. For control, the third batch of grain was treated with 45 ml sterilised water. The three batches of grain were incubated separately for four weeks in desiccators at 25°C. The humidity within the desiccators was adjusted to 65% by a saturated solution of ammonium nitrate (Winston and Bates, 1960). After this incubation period, mould was visible in the two treated batches of wheat but not in the control. Adult *S. granarius* (75 ml) were added to each batch of grain, allowed to lay eggs into the grains for seven days and subsequently removed. Larval faeces used for the olfactometer bioassays and the chemical analysis were obtained by sieving the grain of treated and control cultures 21 to 28 days after the adults had been removed. After removal of the larval faeces, weevil-infested grains of mould-treated and control cultures were used to investigate the influence of mould on the host recognition behaviour of *L. distinguendus* in experiment 2 and fitness of the parasitoids in experiment 3.

**Four-chamber olfactometer** The response of *L. distinguendus* females to different odour samples was examined using a static four-chamber olfactometer (Steidle and Schöller, 1997; Ruther and Steidle, 2000). The olfactometer consisted of a cylinder made of acrylic (4 cm height, diameter 19 cm) divided into four chambers by crosswise-arranged vertical plates. The top of the cylinder was covered by a plastic gauze (mesh 0.1 mm) functioning as a walking arena for the parasitoids. A lid

consisting of a plastic ring (4 mm height, 19 cm diameter) and glass plate was placed on top of the cylinder to prevent the parasitoids from escaping. Odour samples were placed in one of the chambers (test chamber) using a Petri dish (5.5 cm diameter). The opposite chamber was used as control chamber and the remaining two chambers adjacent to the test chamber were considered as buffer zones. A single female was released into the centre of the walking arena and the time the parasitoid spent in the field above the test or control chamber was recorded for 10 minutes. Parasitoids that rested more than 50% of the total observation time were assumed to be unmotivated and excluded from statistical analysis. To avoid biased results due to possible side preferences of the parasitoids, the position of the samples and the controls was rotated clockwise after each test. Walking arena and glass plate were regularly cleaned with ethanol and demineralised water. Odour sources were exchanged after five individuals had been tested.

**Experiment 1: Influence of mould infestation on host finding behaviour** The following odour samples were offered in the olfactometer: (a) 10 g grain infested with *A. sydowii* or *A. versicolor* vs. 10 g untreated grain ($N$ = 20 for each treatment), (b) 200 mg larval faeces from the non-moulded control culture vs. a piece of brown filter paper ($N$ = 21), (c) 200 mg larval faeces from the mould-infested weevil cultures (*A. sydowii*: $N$ = 21, *A. versicolor*: $N$ = 31) vs. brownish filter paper and (d) 200 mg larval faeces from the mould-infested weevil cultures (*A. sydowii*: $N$ = 21, *A. versicolor*: $N$ = 21) vs. 200 mg larval faeces from the non-moulded control culture.

**Experiment 2: Influence of mould infestation on host recognition behaviour** The host-recognition behaviour of experienced *L. distinguendus* females was examined in a bioassay chamber (10 mm diameter x 3 mm height) made from acrylic (Ruther et al., 2000). Single grains from weevil cultures infested with *A. sydowii* or *A. versicolor* or those from the control culture were presented to individual females. Arrestment time on the grains and characteristic elements of the host-recognition behaviour (number of drumming series and drillings) were recorded for 10 min using a stereo microscope under illumination of a microscope light. Grains were exchanged after every parasitoid tested ($N$ = 20 for each treatment).

**Experiment 3: Influence of mould infestation on parasitoid fitness** This experiment was done to estimate the offspring production of *L. distinguendus* females in a no-choice situation with hosts from moulded and non-moulded weevil cultures. Virgin females were allowed to copulate with a male and subsequently placed individually in Petri dishes containing weevil-infested grains from the moulded weevil-cultures (*A. sydowii*: *N* = 25, *A. versicolor: N* = 23) or the control culture (*N* = 25). Weevil-infestation of grains was equal in moulded (*A. sydowii*: 82%, *A.versicolor*: 76%) and non-moulded (78%) weevil cultures. Parasitoids were allowed to oviposit until their death. Offspring was assessed by counting the emerged parasitoids after 30 days. In addition, hind tibia length (e.g., Kazmer and Luck, 1995; Ellers et al., 1998) was used to measure the body size of randomly selected individuals from the offspring of the three treatments (*A. sydowii: N* = 102, *A. versicolor: N* = 91, control: *N* = 143).

**Experiment 4: Response to synthetic 1-octen-3-ol** The response of *L. distinguendus* females to the typical fungal volatile 1-octen-3-ol was investigated in another olfactometer experiment. The chemical was applied at different doses [1500 ng (*N* = 24), 300 ng (*N* = 25) and 30 ng (*N* = 19)] to a filter paper disc (diameter 4 cm, Melitta, Germany) and offered in the test chamber of the olfactometer. Paper discs treated with the pure solvent (30 µl dichloromethane) were used as control.

**Volatile collection** Volatiles emitted from the larval faeces of the three different weevil cultures (*N* = 3 for each treatment) were collected using the closed-loop stripping (CLS) technique as described elsewhere (Ruther and Steidle, 2000). Volatiles from 5 g larval faeces were collected for 4 h on a 1 mm charcoal layer (5 mg) of a CLS-adsorption tube (65 mm length x 5 mm diameter) (Gränicher & Quartero, Daumazan, France). The volatiles were eluted with 25 µl dichloromethane containing 5 ng/µl methyl undecanoate as an internal standard and used for chemical analysis by coupled gas chromatography-mass spectrometry (GC-MS).

**GC-MS analysis** Volatile extracts were analysed by GC-MS using a Fisons 8060 GC (Fisons Instruments) equipped with a 30 m x 0.32 mm ID x 0.25 µm film thickness DB-5ms column (J & W Scientific, Folsom, CA, USA) and coupled to a

Fisons MD 800 quadrupole MS operated in the electron impact (EI) mode at 70 eV. Helium was used as carrier gas at a head pressure of 10 kPa. The oven temperature was 40°C for 4 min and then rose to 280°C at a rate of 5°C/min. The final temperature was maintained for 10 min for thermal cleaning of the column. Peaks were identified by comparison of mass spectra and retention times with those of synthetic reference compounds. For quantification of selected compounds, the peak area of each volatile was related to the peak area of the internal standard.

**Statistical analysis** The arrestment times of female parasitoids spent in test and control field of the olfactometer were compared by the Wilcoxon-matched pairs test (experiments 1 and 4). The arrestment time on the weevil-infested grains and the number of drumming series and drilling in experiment 2 were analysed by the Kruskal-Wallis *H* test followed by multiple Bonferroni-corrected Mann-Whitney *U* tests for individual comparisons. Number of offspring (experiment 3) was compared by a one-way ANOVA and subsequent least significant difference (LSD) tests for post hoc comparison. Tibia lengths were analysed by a two-way ANOVA (factor 1: treatment, factor 2: sex). Statistical analyses were done using Statistica 4.5 scientific software (StatSoft, Hamburg, Germany).

## Results

**Experiment 1: Influence of mould infestation on host finding behaviour** *L. distinguendus* females avoided the odour of moulded wheat irrespective of the *Aspergillus* species used to inoculate the grain. In both *Aspergillus* species, the field above the uninfested wheat was significantly preferred over inoculated grain (Fig. 1a). Parasitoids were strongly arrested by the odour of larval faeces from weevil cultures without mould (Fig. 1b). When given the choice between larval faeces from moulded weevil cultures and filter paper as control, *L. distinguendus* females significantly preferred the odour of faeces from the *A. versicolor* cultures whereas the odour of faeces from the *A. sydowii* cultures was neither preferred nor avoided ($P$ = 0.64) (Fig. 1c). However, when faeces from moulded and non-moulded cultures were offered simultaneously, females clearly preferred the faeces from the non-moulded cultures in both cases (Fig. 1d).

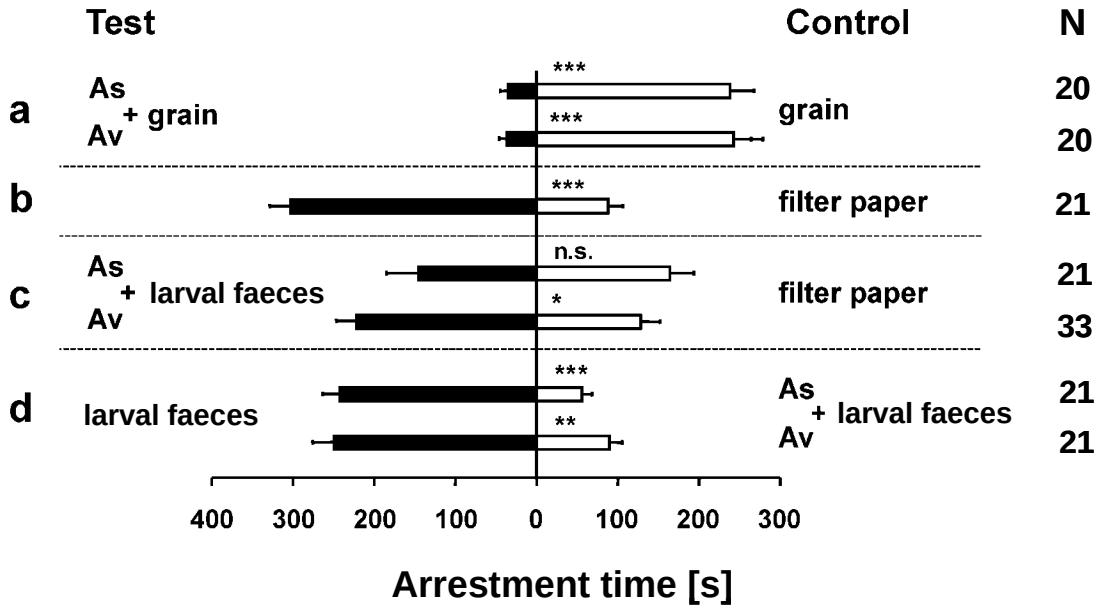


**Fig. 1** Mean arrestment time (± SE) of *L. distinguendus* females in the odour fields above test and control chamber of a two choice olfactometer during a 10 min observation period. N.s. = not significant; asterisks indicate significant differences at $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) (Wilcoxon-matched pairs test).

**Experiment 2: Influence of mould infestation on host recognition behaviour** Arrestment time of female parasitoids was significantly decreased on wheat grains from *A. sydowii*- and *A. versicolor*-infested weevil cultures when compared to the non-moulded control grains (Fig. 2a). Moreover, characteristic elements of the host recognition behaviour (drumming and drilling) were shown less often on grains from the moulded weevil cultures than on control grains (Fig. 2b-c). No significant differences were found between grains from weevil cultures infested by *A. sydowii* and *A. versicolor*, respectively (arrestment time: $U = 145.5$, $P < 0.14$; drilling: $U = 153$, $P = 0.20$; drumming: $U = 138$, $P = 0.09$).

**Experiment 3: Influence of mould infestation on parasitoid fitness** Females of *L. distinguendus* parasitised hosts from both moulded and non-moulded weevil-cultures. However, the number of offspring was significantly lower when host larvae originated from moulded weevil cultures infested with either *A. sydowii* or *A. versicolor* as compared to non-moulded cultures (Fig. 3). There was no significant difference in the number of offspring between the two mould species ($P = 0.43$, LSD). However, body size as measured by the length of the tibiae was

significantly affected by the infestation with mould. Parasitoids that had developed on hosts from non-moulded weevil cultures had longer tibiae (female: 0.579 mm ± 0.003; male: 0.5058 mm ± 0.005) than individuals from *A. sydowii*- (female: 0.571 mm ± 0.003; male: 0.5058 mm ± 0.003) or *A. versicolor*-moulded cultures (female: 0.573 mm ± 0.003; male: 0.500 mm ± 0.003). Moreover, females of *L. distinguendus* had generally longer tibiae than males. There was no interaction observed between treatment and sex (Table 1).

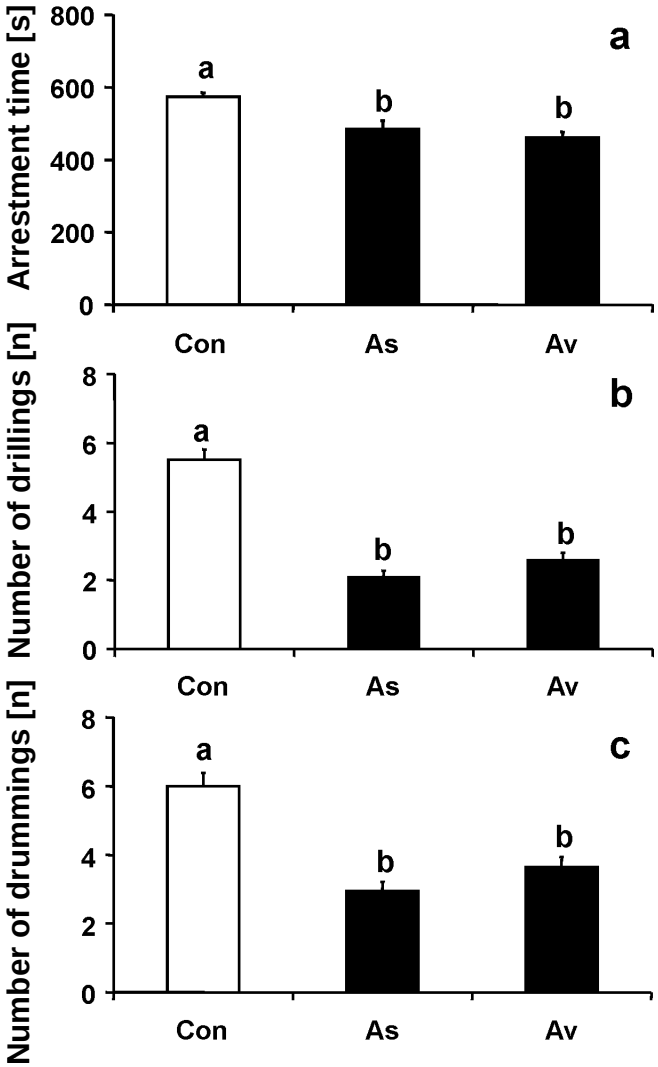


**Fig. 2** Response of *L. distinguendus* females to host-infested grains originating from non-moulded weevil cultures (Con) and those infested by *A. sydowii* (As) or *A. versicolor* (Av). (a) Mean arrestment time on the grain (± SE), (b) number of drumming series (± SE) and (c) number of drillings (± SE) during a 10 min observation period. Bars with different lowercase letters are significantly different at $P < 0.001$ (Kruskal-Wallis $H$ test followed by Bonferroni-corrected Whitney-Mann $U$ tests for multiple comparisons; $N = 20$).

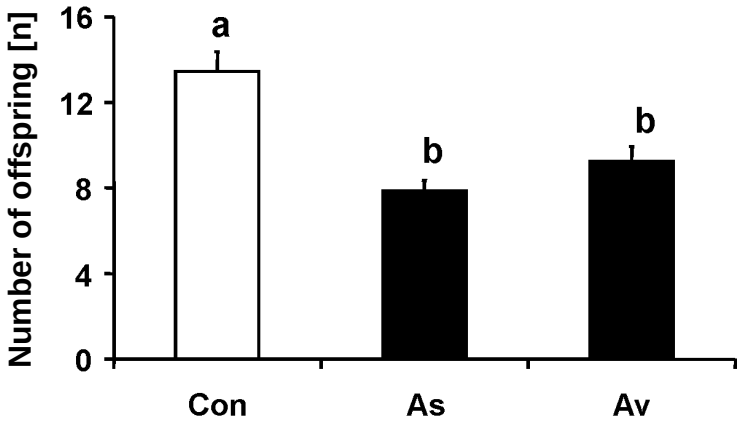


**Fig. 3** Mean number of offspring (± SE) of *L. distinguendus* females that parasitised host-infested grains from non-moulded weevil cultures (Con) and those infested by *A. sydowii* (As) or *A. versicolor* (Av). Bars with different lower caseletters are significantly different at $P < 0.01$ (one-way ANOVA; $N = 24$-$26$).

**Experiment 4: Response to synthetic 1-octen-3-ol** *L. distinguendus* females avoided significantly synthetic 1-octen-3-ol at doses between 1500 to 300 ng (Fig. 4). At a dose of 30 ng, however, parasitoids were neither repelled nor attracted to the synthetic chemical ($P = 0.75$).

**GC-MS analysis** The major eight-carbon fungal volatile detected in larval faeces from moulded weevil cultures was 1-octen-3-ol accompanied by lower amounts of 3-octanone and 3-octanol (Table 2). In larval faeces from non-moulded host cultures all compounds were found only in traces.

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## Discussion

To study the ability of parasitoids to use chemical cues for the assessment of host habitat quality, the response of *L. distinguendus* was tested to grains and host faeces contaminated with the two mould species *A. sydowii* or *A. versicolor*. Both species co-occur with the parasitoid's hosts in its natural stored grain habitat under humid and warm conditions. The olfactometer experiments clearly demonstrate that naïve females of *L. distinguendus* prefer the odour of non-moulded grains when offered simultaneously with grains infested by one of the two mould species. Likewise, odour from non-moulded host faeces is strongly preferred over odour

from moulded host faeces. Obviously, *A. sydowii* and *A. versicolor* release volatiles that are repellent for females of *L. distinguendus*. As shown in experiments in which non-moulded and moulded faeces were tested against filter paper only, the repellent effect of volatiles released by *A. sydowii* is even strong enough to neutralise the attractive effect of the odour released by non-moulded host faeces. In agreement with these results, host recognition behaviour of wasps was much more intense on weevil-infested grains from non-moulded cultures when compared to those from moulded ones. Thus, in a bulk of weevil-infested stored grain containing patches with and without mould, parasitoids would most likely orientate towards non-moulded areas during the host finding process and prefer hosts in non-moulded grains for oviposition.

Chemical analyses and further olfactometer tests point to 1-octen-3-ol as chemical cue that might be responsible for the avoidance response of the parasitoid. This compound is emitted from moulded faeces in amounts that repel *L. distinguendus* females. Lower concentrations of 1-octen-3-ol, comparable to the amounts released by faeces from cultures with no visible mould, had no effect on the wasps. Interestingly, 1-octen-3-ol is released as a major volatile not only by the two *Aspergillus* species studied here but also by numerous fungi from other taxa (Kaminski et al., 1974; Börjesson et al., 1992; Sunesson et al., 1995; Jelen and Wasowicz, 1998; Fischer et al., 1999). Thus, 1-octen-3-ol reliably indicates fungal growth in stored grain.

**Table 1** Two-way ANOVA of tibia lengths of *L. distinguendus* males (m) and females (f) developing in non-moulded weevil cultures (Con) and weevil cultures infested by *A. sydowii* (As) or *A. versicolor* (Av) (*df:* degrees of freedom; *MS*: mean squares).

| **Source** | ***df*** | ***MS*** | ***F*** | ***P*** |
|---|---|---|---|---|
| Treatment (Con, As, Av) | 1 | 0.5429 | 722.706 | < 0.001 |
| Sex (m, f) | 2 | 0.0030 | 3.950 | 0.020 |
| Treatment x Sex | 2 | 0.0001 | 0.154 | 0.857 |
| Error | 391 | 0.00075 | | |

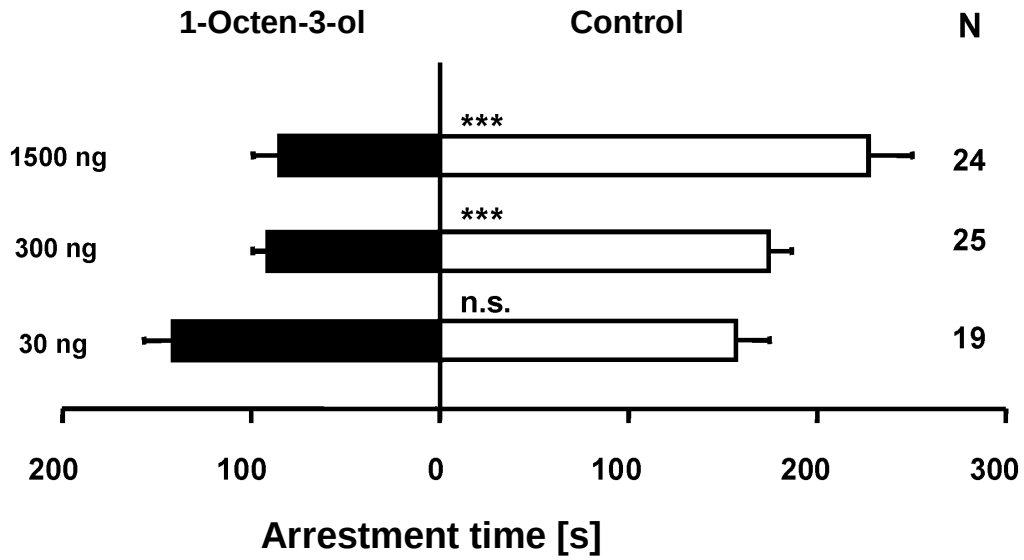


**Fig. 4** Mean arrestment time (± SE) of *L. distinguendus* females in the odour fields above test and control chamber of a two choice olfactometer during a 10 min observation period. Test fields were treated with synthetic 1-octen-3-ol at different doses; control fields were treated with pure solvent. N.s. = not significant; *** indicates significant differences at $P < 0.001$ (Wilcoxon-matched pairs test).

The ultimate reason for the avoidance of fungal odour by the parasitoid is probably a reduced fitness, as shown here by a lower number of offspring on hosts from moulded weevil cultures. This might be due to one or several of the following mechanisms: (1) Female parasitoids laid a reduced number of eggs on hosts from moulded cultures. This hypothesis is supported by the fact that important elements of the host recognition behaviour (arrestment, drumming and drilling) were shown to a significantly lesser extend when host-infested grains from moulded weevil cultures were offered. Thus, moulded grains might be less attractive for oviposition by *L. distinguendus*. (2) Parasitoids had a higher pre-emergence mortality due to mycotoxins. Numerous mould species including the genus *Aspergillus* are known to synthesise toxic metabolites (Atalla et al., 2003; El-Shanawany et al., 2005). These might have decreased the parasitoid's and/or the host's survival rate. (3) Competition between mould and host larvae for wheat nutrients decreased host performance allowing a lower number of parasitoid larvae to develop into adults. It is known from many studies that parasitoid larvae developing on suboptimal hosts may be subjected to an increased pre-emergence mortality as well as a reduced longevity or fecundity (Vinson, 1980 and references therein). Parasitoids from moulded weevil cultures were significantly smaller supporting the hypothesis that

**Table 2** Mean amounts (ng per sampling ± SE, *N* = 3) of typical eight-carbon fungal volatiles from the headspace of larval faeces from non-moulded weevil cultures (Con) and weevil cultures infested by *A. sydowii* (As) or *A. versicolor* (Av).

| Compound | Con | As | Av |
|---|---|---|---|
| 1-octen-3-ol | 17 ± 4 | 516 ± 5 | 730 ± 46 |
| 3-octanone | 19 ± 4 | 116 ± 14 | 272 ± 47 |
| 3-octanol | 5 ± 1 | 14 ± 2 | 120 ± 7 |

the hosts were not of equal quality. Van den Assem et al. (1989) reported for *L. distinguendus* a correlation between the body size and the reproductive success of the parasitoids. Larger females were shown to produce more offspring than smaller individuals because they lived longer and were more fertile. In contrast, size was less important for males. Thus, mould infestation might also influence the number of offspring in the second generation resulting in a decreased inclusive fitness. However, it has to be tested whether the subtle differences in body size observed in this study can actually influence fitness of the parasitoids. (4) Secondary infestation by *Aspergillus* mould caused unfavourable environmental conditions for parasitoid and/or host development. Local mass breeding of insects in stored grain and subsequent mould infestation can lead to distinct areas with extreme abiotic conditions. The development of such hot-spots has been described in detail by Sinha and Wallace (1966). Initially, the metabolic activity of the primary pest leads to a moderate increase of temperature and humidity providing optimal conditions for moisture-sensitive secondary pests like astigmatid mites and mould. With increasing growth of fungi, however, moisture and temperature as well as the concentration of carbon dioxide may reach levels that cause a decline of insect populations and finally a breakdown of the hot spot (Sinha and Wallace, 1966). *L. distinguendus* and some of its hosts clearly prefer lower humidities over those commonly found in hot spots (Perttunen, 1972; Steidle and Reinhard, 2003). Parasitoid females may recognise at an early stage the development of such unfavourable environmental conditions by avoiding fungal volatiles like 1-octen-3-ol.

In conclusion, *L. distinguendus* females innately use the common fungal volatile 1-octen-3-ol and possibly other components of the fungal odour bouquet for host habitat assessment. Thereby, they avoid negative fitness consequences resulting

from secondary mould infestation of host patches. Nevertheless, parasitoids are able to develop in hosts from moulded weevil cultures. Therefore, it makes sense that females preferred the odour of larval faeces from moulded weevil cultures (*A. versicolor*) in absence of the more attractive alternative, i.e., faeces from non-moulded host cultures. The fact that the response of females to the fungal volatile is innate, suggests that host-associated fungi played a crucial role in the evolution of host finding strategies in *L. distinguendus*.

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