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MITES AS MATCHMAKERS: SEMIOCHEMICALS FROM HOST-ASSOCIATED MITES ATTRACT BOTH SEXES OF THE PARASITOID *Lariophagus distinguendus*

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Abstract—The role of volatile chemicals used for mate finding was studied for males of *Lariophagus distinguendus* (Först.), a parasitoid of the granary weevil *Sitophilus granarius* (L.). In bioassays that used a static four-chamber olfactometer, males were attracted by host feces, hexane extracts from host feces, and volatile extracts of the feces obtained by closed-loop stripping (CLS). On the other hand, volatiles emitted by unmated females did not elicit any preferences in males. Both sexes of *L. distinguendus* responded to a synthetic mixture of neral, geranial, neryl formate, and tridecane occurring in the investigated extracts. All compounds are common constituents of astigmatid mites that are often associated with possible hosts of *L. distinguendus*. In the system investigated, all main compounds found in CLS extracts from larval feces of *S. granarius* are due to the mold mite *Tyrophagus putrescentiae* (Schrank) that uses neral, geranial, and neryl formate as an alarm pheromone. The possible role of host-associated astigmatid mites in mate and host finding of *L. distinguendus* is discussed.

Key Words—Granary weevil, *Sitophilus granarius*, parasitoid, *Lariophagus distinguendus*, astigmatid mites, *Tyrophagus putrescentiae*, semiochemicals, host finding, mate finding, neral, geranial, neryl formate, tridecane.

INTRODUCTION

For successful reproduction, parasitoids have to be able to locate their mates and hosts. In most species, chemical cues are involved in mate- and host-finding mechanisms.

In many parasitic Hymenoptera, mate finding is mediated by female sex

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pheromones. Often more than one component is supposed to be involved in such pheromone systems, one serving for long-range attraction, others mediating close-range courtship behavior (Quicke, 1997). However, if long-range pheromones are missing, males have to rely on other chemical cues to locate females. One possible strategy for males is to use the same chemical cues as females use for host location because the probability of encounters with receptive females increases at possible oviposition sites.

For host location, female parasitoids often use chemical cues from their hosts, such as pheromones or feces (reviewed by Godfray, 1994; Quicke, 1997), or indirect cues emitted by infested host plants after feeding (e.g., Dicke and Vet, 1999; Steinberg et al., 1993; Turlings et al., 1991, 1995), or oviposition of the herbivorous host (Meiners and Hilker, 2000). Volatiles emitted by host-associated fungi also have been reported to attract female parasitoids (Greany et al., 1977; Madden, 1968).

Females of the polyphagous parasitoid *Lariophagus distinguendus* use volatiles emitted from larval feces of one of their hosts, the granary weevil, *Sitophilus granarius*, for host location (Steidle and Schöller, 1997). However, the chemical structures of the compounds responsible for this phenomenon have not been characterized.

In the present study, we investigate whether males of *L. distinguendus* use chemical cues from larval feces of *S. granarius* for chemical orientation, as females do. Furthermore, volatile constituents of the feces are identified, and individual synthetic compounds are tested for biological activity. Finally, we show that the volatile composition of the feces is due to the host-associated mite fauna.

METHODS AND MATERIALS

Insects. S. granarius were reared on 250 g of wheat grains of about 14% moisture content in 2-liter glass jars at a constant temperature of 25°C and 75% relative humidity. To obtain weevil larvae of known age, 30 ml of adult weevils were kept on 300 ml grain and removed seven days later. To rear *L. distinguendus*, newly emerged parasitoids were placed in Petri dishes with grains containing 15- to 30-day-old weevil larvae. To obtain experienced parasitoids, batches of 30 individuals (20 females and 10 males, 1–24 hr old) of *L. distinguendus* were allowed to mate and oviposit in Petri dishes containing 60 infested kernels and 60 mg of feces infested with *Tyrophagus putrescentiae*. One hour before the experiments, parasitoids were removed from the Petri dishes and kept individually in 1.5-ml polyethylene reaction tubes (Sarstedt, Nürnbrecht, Germany). Unmated females were collected immediately after hatching and kept isolated from males until the experiments. *Tyrophagus putrescentiae* mites were

reared in Petri dishes on larval feces from *S. granarius* at 25°C and 85% relative humidity.

Preparation of Extracts. Larval feces from *S. granarius* were obtained by sieving grain infested by 19- to 30-day-old weevil larvae. Hexane extracts were prepared by treating 20 g feces for 15 min with 40 ml hexane (Biomol, Hamburg, Germany) at room temperature in an ultrasonic bath. After filtration, 500 μl of the extract were used in the bioassay. Headspace extracts were obtained by use of the closed-loop stripping (CLS) technique (Brechbühler AG, Zürich, Switzerland). For this purpose, the volatiles from 5 g feces were trapped for 4 hr at room temperature on the charcoal layer (5 mg) of the adsorption tube. The volatiles were eluted twice with 25 μl dichloromethane each (Biomol). The combined eluates were used in the bioassay. For quantification of the volatiles, the adsorption tube was eluted twice with 25 μl dichloromethane containing 1-dodecene at a concentration of 10 ng/μl as an internal standard. The combined eluates were analyzed by GC-MS.

Mite extracts were obtained by rinsing 20 *Tyrophagus putrescentiae* mites with 20 μl hexane for 3 min. After removal of the mites, the extract was used for chemical analysis.

Synthetic Mixture of Mite Semiochemicals. Based on the quantitative chemical analysis of the CLS extracts, a mixture of synthetic mite semiochemicals was prepared containing tridecane (Aldrich, Steinheim, Germany) at 60 μg/ml, citral (mixture of neral and geranial; Roth, Karlsruhe, Germany) at 10 μg/ml, and neryl formate at 5 μg/ml. Neryl formate was synthesized by esterification of nerol (Aldrich) with formic acid (Aldrich) by use of 4-dimethylamino pyridine as catalyst and dicyclohexyl carbodiimide as condensation reactant, as has been described in detail elsewhere for similar esters (Ruther et al., 1998).

Bioassays. Experiments were performed in a static four-chamber olfactometer (Figure 1) made of acrylic glass, consisting of a cylinder (4 cm high × 19 cm diam.) divided by vertical plates into four chambers. In one chamber, an odor sample was placed in a Petri dish (5.5 cm diam.) laid out with brown filter paper (4 cm diam.). The remaining chambers contained Petri dishes with filter paper as controls. A walking arena (19 cm diam. × 1 cm high) consisting of plastic gauze (mesh 0.5 mm), a rim of acrylic glass, and covered with a glass plate was placed on the cylinder. The olfactometer was placed on the bottom of a white bucket (29 cm diam. × 36 cm high) and illuminated from above resulting in 2050 lux in the olfactometer. To avoid biased results due to side preferences of the parasitoids, the position of the samples and the controls was changed clockwise after each test. To avoid contamination of the walking arena with sample odors or by possible pheromones of the parasitoids, walking arenas and glass plates were cleaned regularly with ethanol and dimineralized water. The parasitoids were exposed to the following samples: (1) 10 unmated females—in these experiments, the sample container was sealed by gauze (mesh

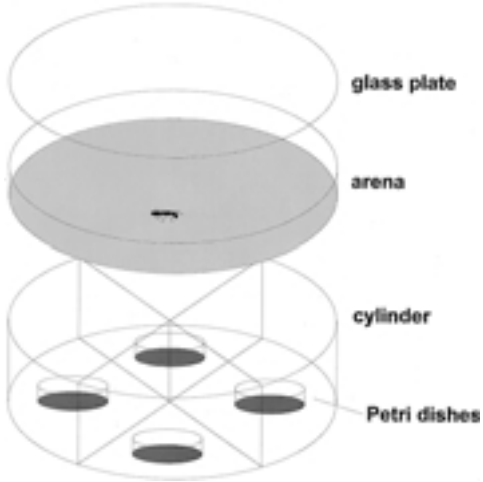

FIG. 1. Static four-chamber-olfactometer used for bioassays. For details see text.

0.5 mm), and thus, semiochemicals were able to diffuse to the walking arena and the females were prevented from escaping, (2) 150 mg larval feces from *S. granarius*; (3) 500 μl hexane extract from larval feces; (4) 50 μl CLS extract from larval feces; and (5) 50 μl of the synthetic mixture of mite semiochemicals. Samples 1–4 were tested with male parasitoids exclusively, sample 5 was tested with both sexes.

When extracts were tested, the control chambers contained filter papers treated with the pure solvents. Before the bioassays, the solvents were allowed to evaporate for 15 min. For all experiments, samples were renewed after five parasitoids each. The parasitoids were released individually in the center of the walking arena and their behavior (walking, resting) and position in the four sectors above the chambers were registered for 10 min by using the computer software The Observer 3.0 (Noldus, Wageningen, The Netherlands, 1989). When testing *L. distinguendus* in the described bioassay, about 20% of the parasitoids rested more than 50% of the observation time. Those parasitoids were assumed to be unmotivated and, therefore, were not included in the statistical analysis. The time the parasitoids spent within the four sectors was analyzed for homogeneity by using the repeated measures ANOVA for dependent data (Bortz, 1993). In case of significant deviation from homogeneity, the Scheffé-test for multiple comparisons was used. In cases when data were not distributed equally, statistical analysis was done by a nonparametric Friedman test, followed by Wilcox-Wilcoxon tests for separation of means. Statistical analyses were done by using Statistica scientific software (StatSoft, Hamburg, Germany).

GC-MS Analysis. Analytical separations were performed on a Fisons 8060 GC. Mass spectra were obtained on a Fisons MD800 quadrupole mass spectrometer (Thermoquest, Egelsbach, Germany). Analyses were carried out by using a 30-m × 0.32-mm-ID DB-5ms fused silica column, film thickness 0.25 μm (J&W/Fisher Scientific, Wiesbaden, Germany), with helium as carrier gas (pressure 10 kPa). The temperature program started at 40°C, temperature was held for 1 min, and raised 4°C/min to 280°C. The column effluent was ionized by electron impact ionization (EI) at 70 eV. Injection volumes were 1 μl (splitless) in the case of the CLS extracts and 2 μl (splitless) in the case of mite extracts. Kovats indices were estimated according to van den Dool and Kratz (1963) by coinjection of a hydrocarbon mixture (C_7–C_{30}; Aldrich). Location of the double bonds in the alkenes occurring in the extracts was determined by iodine catalyzed methylthiolation that used dimethyl disulfide (Aldrich) and GC-MS analysis of the formed derivatives (Francis and Veland, 1981; Howard et al., 1988). Quantitative analysis ($N = 2$) of the volatiles in the CLS extracts was done by comparing the peak areas of individual compounds with the peak area resulting from the coinjection of 10 ng of the internal standard (1-dodecene).

RESULTS

Bioassays. When *L. distinguendus* males were exposed to 10 unmated females in the olfactometer, they did not prefer the test sector over the control sectors (Figure 2). On the other hand, they strongly preferred the test sector when larval feces from *S. granarius* were presented. The chemicals responsible for this behavior are extractable with hexane, as extracts from larval feces elicited strong preference of the test sector by males. Selective enrichment of volatile compounds from larval feces by use of CLS led to highly active extracts as well.

A synthetic mixture containing neral, geranial, neryl formate, and tridecane—compounds occurring in both CLS extracts from larval feces and astigmatid mites living in the feces (see below)—was tested towards males and females of *L. distinguendus*. Both sexes preferred the test sector over the control sectors when the synthetic mixture was presented in the olfactometer (Figure 3).

Astigmatid Mites. The behaviorally active larval feces from *S. granarius* contained a high number of astigmatid mites, with one acarid species predominating, the mold mite, *Tyrophagus putrescentiae* (Schrank).

Chemical Analyses. Ten compounds were identified by GC-MS in the behaviorally active CLS extracts from larval feces of *S. granarius* (Table 1): four terpenoids, five hydrocarbons, and one aromatic compound. The identity of another aromatic is still unknown. The amounts per 50 μl, i.e., the volume used in the bioassay, were between 50 ng for geranial and 3000 ng for tridecane (Table 1). All compounds identified in the CLS extracts also occurred in hex-

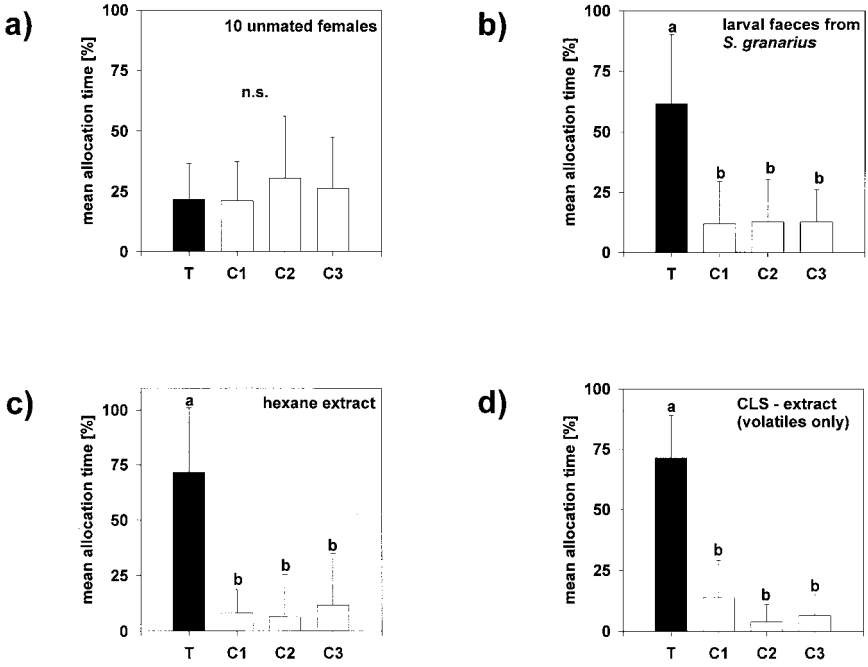

FIG. 2. Mean allocation times (±SD, $N = 20$) of *L. distinguendus* males in the sectors of a four-chamber-olfactometer (T = test, C1–C3 = controls). The test field contained 10 unmated females (a), larval feces from the host *S. granarius* (b), hexane extracts from feces (c), or extracts from CLS of the feces (d). Means with different letters are significantly different at $P < 0.001$ (a,d: repeated measures ANOVA for dependent data/Scheffé-test for multiple comparisons; b,c: Friedman test/Wilcox-Wilcoxon test).

ane extracts from 20 *T. putrescentiae* mites (Figure 4). Thus, the composition of volatiles in the larval feces from *S. granarius* was essentially determined by coexisting astigmatid mites.

DISCUSSION

L. distinguendus males showed no preferences when 10 receptive females were presented in a static four-chamber olfactometer. As we did not find any evidence for the existence of a female-produced attractant, we expect that males have to use other volatile sources for female location. Females of *L. distinguendus* are strongly attracted to volatiles emitted by larval feces of their host, the granary weevil *S. granarius* (Steidle and Schöller, 1997). This study shows that males are using the same host related cues for chemical orientation as females.

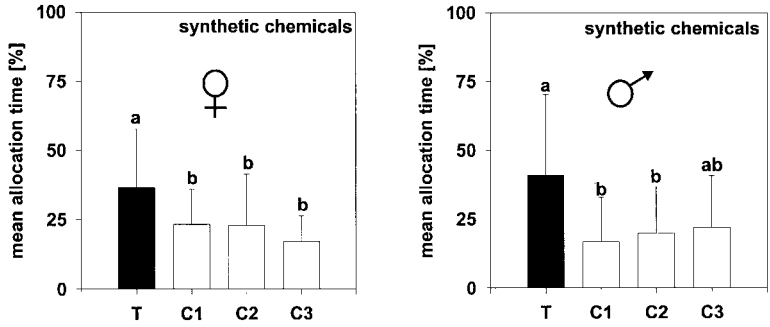

FIG. 3. Mean allocation times (±SD, $N = 25$) of *L. distinguendus* females and males in the sectors of a four-chamber-olfactometer (T = test, C1–C3 = controls). The test field contained a mixture of synthetic chemicals occurring in extracts from feces of *S. granarius* (see text for concentration). Means with different letters are significantly different at $P < 0.05$ (repeated measures ANOVA for dependent data/Scheffé-test for multiple comparisons).

Females of *L. distinguendus* are receptive at the moment of emergence. Thus, males that rely on the same chemical cues increase the probability of mating not only with host-locating females but also with hatching females at the oviposition sites. Chemical analyses of CLS extracts from larval feces of *S. granarius* led

TABLE 1. COMPOUNDS IN EXTRACTS FROM CLOSED-LOOP STRIPPING (CLS) OF LARVAL FECES FROM GRANARY WEEVIL, *S. granarius*[a]

No.	Compound	Concentration in the CLS extract (ng/50 μl)	Identification
1	2-hydroxy-6-methylbenzaldehyde	200	Leal et al. (1988)
2	neral	450	MS/RI
3	geranial	50	MS/RI
4	neryl formate	250	MS/RI
5	unknown (mw 150)	200	
6	tridecane	3000	MS/RI
7	β-acaridial	150	Leal et al. (1989)
8	(*Z*,*Z*)-6,9-pentadecadiene	620	DMDS
9	(*Z*)-7-pentadecene	350	DMDS
10	(*Z*)-6-pentadecene	630	DMDS
11	pentadecane	130	MS/RI

[a]Identification of the compounds is based on comparison of mass spectra and linear retention indices with those of authentic reference compounds (MS/RI), on interpretation of the mass spectra of the reaction products after derivatization with dimethyl disulfide (DMDS), or on comparison of mass spectra with data from the literature.

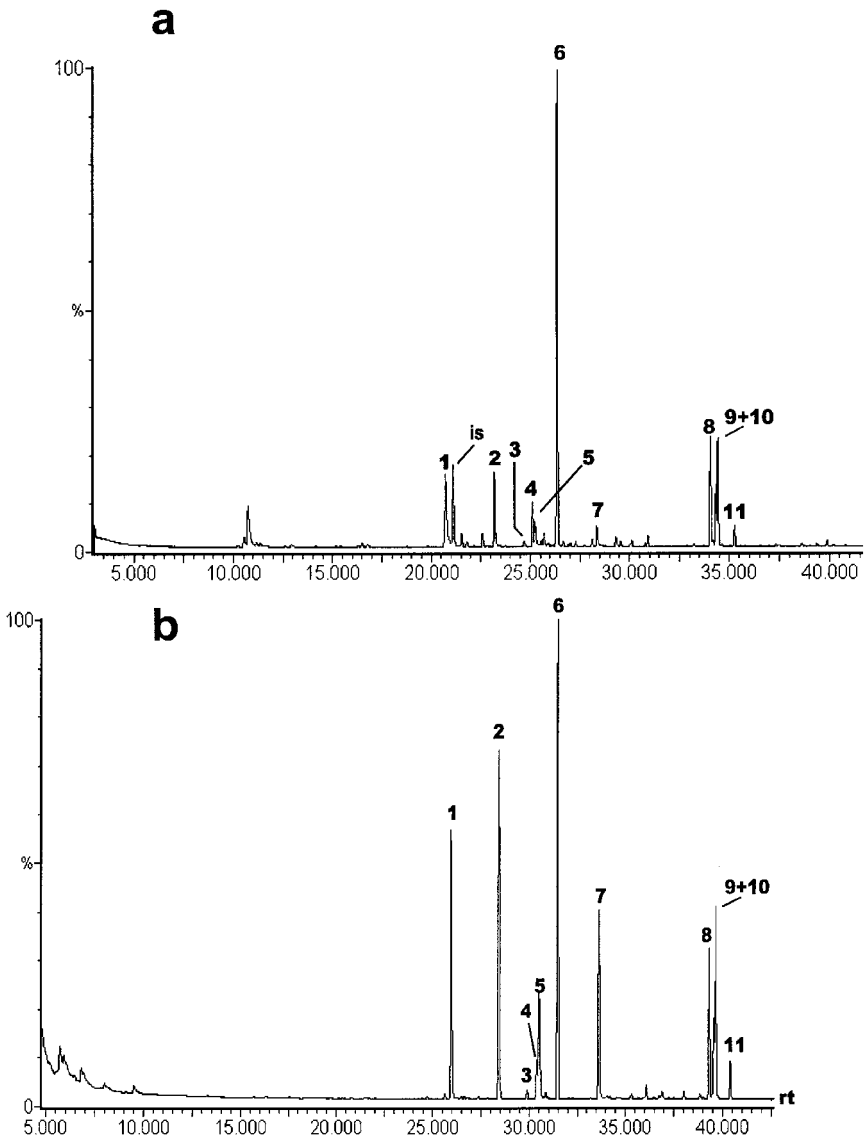

FIG. 4. Total ion current chromatograms of a CLS extract from larval feces of *S. granarius* (a) and a hexane extract of *T. putrescentiae* mites (b). The numbers correspond to those in Table 1, is = internal standard.

to the identification of 10 main compounds. A synthetic mixture containing four of these constituents, neral (**2**), geranial (**3**), neryl formate (**4**), and tridecane (**6**) attracted both sexes of *L. distinguendus* (Figure 4).

An interesting aspect of this study is the fact that the biologically active compounds do not originate from the host feces themselves but from astigmatid mites living in the feces. In the system investigated, all main compounds found in the CLS extracts from the feces are due to the predominating mite species, the mold mite *T. putrescentiae*. Numerous species of astigmatid mites have been shown to communicate via pheromones. The compounds involved act as alarm, sex, or aggregation pheromones (Kuwahara, 1991). *T. putrescentiae* has been intensively investigated with respect to its chemical ecology. Neral (**2**), geranial (**3**), and neryl formate (**4**) have been identified as alarm pheromones in this species (Kuwahara et al., 1975, 1979; My-Yen et al., 1980). These compounds and tridecane (**6**) were selected for bioassays because they not only occur in *T. putrescentiae* but are widespread among mites infesting stored products. Neral and geranial have been found in at least 14 species and, neryl formate in 9 (Kuwahara, 1991). Tridecane is also a common compound found in solvent extracts from many astigmatid mites, often occurring as the major compound (Curtis et al., 1981; Howard et al., 1988; Tuma et al., 1990). The function of this compound is still unknown. Possibly it serves as a solvent for the actual semiochemicals. Tridecane has been suggested as an indicator substance to monitor mite infestation in stored grain (Tuma et al., 1990).

2-Hydroxy-6-methylbenzaldehyde (**1**) is a well-known constituent of several astigmatid mite species, among them *T. putrescentiae* (Kuwahara, 1991). However, biological functions have only been shown for other species. It has been described as an alarm pheromone in *T. perniciosus* (Leal et al., 1988), whereas in *Acarus immobilis* and *Aleuroglyphus ovatus*, it mediates sexual behavior (Kuwahara, 1991; Kuwahara et al., 1992). The terpenoid dialdehyde β-acaridial (**7**) was identified for the first time in *T. putrescentiae* (Leal et al., 1989), and again, biological activity has been shown only for another species, the acarid mite *Caloglyphus polyphyllae* (Leal and Kuwahara, 1989). The C_{15} hydrocarbons identified in this study have been described previously from *T. putrescentiae* and *T. neiswanderi* (Howard et al., 1988). In the latter species, (*Z*)-7-pentadecene (**9**) and (*Z*)-6-pentadecene (**10**) have been shown to be components of the alarm pheromone (Kuwahara et al., 1989).

To our knowledge, this study is the first to demonstrate that both sexes of a parasitoid respond to pheromones of a host-associated nonmicroorganism. Until now only host-associated microorganisms have been shown to produce volatile metabolites that attract parasitoids. Thibout et al. (1993) found that volatile dialkyl disulfides attracting the parasitoid *Diadromus pulchellus* are produced by bacteria living in the feces of the host, *Acrolepiopsis assectella*. Greany et al. (1977) have shown that females of the fruit fly parasitoid *Biosteres longicaudatus*

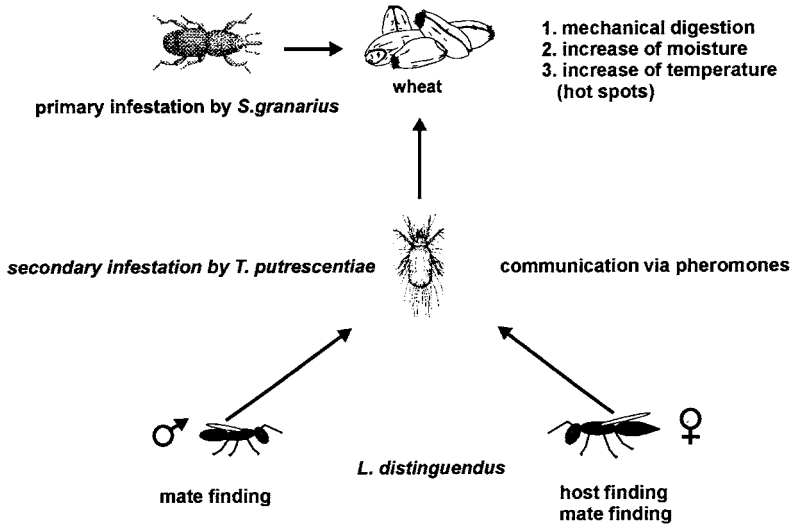

FIG. 5. Possible scenario for mate and host finding of *L. distinguendus* in the system wheat (*Triticum aestivum*)/*S. granarius*/*T. putrescentiae* (for details see text).

are attracted by fermentation products of host associated fungi such as ethanol, acetaldehyde, and acetic acid. Madden et al. (1968) investigated host finding in *Ibalia leucospoides*, a parasitoid of the woodwasp *Sirex noctilio*, and found that female parasitoids respond to the volatiles emitted by the fungus *Amylostereum* sp. This fungus is symbiotically associated with the host and is inoculated into coniferous trees during oviposition by *Sirex* females.

From the results of the present paper, a fascinating scenario of host and mate finding in *L. distinguendus* can be deduced (Figure 5). Intense infestation of stored wheat by the primary pest *S. granarius* causes mechanical digestion of the substrate and an increase of moisture and temperature, resulting in so-called hot spots (Sinha and Wallace, 1966). These conditions favor secondary infestation by moisture-sensitive astigmatid mites that are often associated with beetle-induced hot spots (Sinha, 1961; Eighme, 1966). Consequently, volatiles emitted by these mites should reliably indicate the presence of potential hosts. Thus, both sexes of *L. distinguendus* responding to these volatiles benefit from this behavior, as it facilitates the location of areas of high host density as well as mates. The fact that *L. distinguendus* responded to compounds that are common for many stored-product-infesting mite species increases the ecological significance of the observed phenomenon. Categorization of the bioactive chemicals as kairomones appears equivocal at first glance, as the emitters of the compounds, i.e., the mites, are not the actual target of the parasitoids. However, as beetle

infestation improves the living conditions for astigmatid mites in stored grain, we conclude that attraction of beetle parasitoids causes disadvantages for the mites as well and, thus, mite volatiles can be categorized as kairomones.

As mentioned before, the compounds tested in this study are known to act as alarm pheromones in astigmatid mites. The investigated system not only contained astigmatid mites but also predatory mites as possible antagonists. Therefore, these organisms might be involved in the observed phenomena as elicitors of the alarm pheromone emission in the astigmatid mites. This aspect needs further investigation.

The parasitoids tested were experienced. Like numerous other insects, *L. distinguendus* has been shown to learn from experience (Steidle, 1998). Thus, the observed behavior may be due to associative learning. This aspect is currently under investigation.

The synthetic mixtures of volatiles tested in this study did not elicit the same degree of attractiveness towards *L. distinguendus* as extracts from larval feces. This might be due to other compounds that have not yet been tested. However, since *L. distinguendus* should also be able to locate hosts and mates in those habitats where secondary infestation by astigmatid mites has not yet occurred, there may be other chemicals that are involved in host and mate finding in this species.

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REFERENCES

BORTZ, J. 1993. Statistik für Sozialwissenschaftler, 4th ed. Springer-Verlag, Berlin.

CURTIS, R. F., HOBSON-FROHOCK, A., FENWICK, G. R., and BEREEN, J. M. 1981. Volatile compounds from the mite *Acarus siro* in food. *J. Stored Prod. Res.* 17:197–203.

DICKE, M., and VET, L. E. M. 1999. Plant–carnivore interactions: Evolutionary and ecological consequences for plant herbivore and carnivore, pp. 483–520, *in* H. Olff, V. K. Brown, and R. H. Drent (eds.). Herbivores, Plants and Predators. Proc. of the 33rd Symposium of the British Ecology Society, 1998. In press.

EIGHME, L. E. 1966. Relationships of insects to hot spots in stored wheat. *J. Econ. Entomol.* 59:564–569.

FRANCIS, G. W., and VELAND, K. 1981. Alkylthiolation for the determination of double-bond position in linear alkenes. *J. Chromatogr.* 219:379–384.

GODFRAY, H. C. J. 1994. Parasitoids. Princeton University Press, Chichester, UK.

GREANY, P. D., TUMLINSON, J. H., CHAMBERS, D. L., and BOUSH, G. M. 1977. Chemically mediated host finding by *Biosteres (Opius) longicaudatus*, a parasitoid of the tephritid fruit fly larvae. *J. Chem. Ecol.* 3:189–195.

HOWARD, R. W., KUWAHARA, Y., SUZUKI, H., and SUZUKI, T. 1988. Pheromone study on acarid mites. XII. Characterization of the hydrocarbons and external gland morphology of the opisthonotal gland of six species of mites (Acari: Astigmata). *Appl. Entomol. Zool.* 23:58–66.

KUWAHARA, Y. 1991. Pheromonal communication of mites and ticks, pp. 43–52, *in* F. Dusbábek and V. Bukva (eds.). Modern Acarology. Academia, Prague.

KUWAHARA, Y., ISHII, S., and FUKAMI, H. 1975. Neryl formate: Alarm pheromone of the cheese mite, *Tyrophagus putrescentiae* (Schrank) (Acarina, Acaridae). *Experientia* 31:1115–1116.

KUWAHARA, Y., FUKAMI, H., ISHII, S., MATSUMOTO, K., and WADA, Y. 1979. Pheromone study on acarid mites II. Presence of the alarm pheromone in the mold mite, *Tyrophagus putrescentiae* (Schrank) (Acarina: Acaridae) and the site of its production. *Jpn. J. Sanit. Zool.* 30:309–314.

KUWAHARA, Y., LEAL, W. S., NAKANO, Y., KANEKO, Y., NAKAO, H., and SUZUKI, T. 1989. Pheromone study on astigmatid mites XXIII. Identification of the alarm pheromone of the acarid mite, *Tyrophagus neiswanderi* and species specificities of alarm pheromones among four species of the same genus. *Appl. Entomol. Zool.* 24:424–429.

KUWAHARA, Y., SATO, M., KOSHII, T., and SUZUKI, T. 1992. Chemical ecology of astigmatid mites XXXII. 2-Hydroxy-6-methyl-benzaldehyde, the sex pheromone of the brown-legged grain mite, *Aleuroglyphus ovatus* (Troupeau) (Acarina: Acaridae). *Appl. Entomol. Zool.* 27:253–260.

LEAL, W. S., and KUWAHARA, Y. 1989. β-Acaridial, the sex pheromone of the acarid mite *Caloglyphus polyphyllae. Naturwissenschaften* 76:332–333.

LEAL, W. S., NAKANO, Y., KUWAHARA, Y., NAKAO, H., and SUZUKI, T. 1988. Pheromone study of acarid mites XVII. Identification of 2-hydroxy-6-methyl-benzaldehyde as the alarm pheromone of the acarid mite *Tyrophagus perniciosus* (Acarina: Acaridae), and its distribution among related mites. *Appl. Entomol. Zool.* 23:422–427.

LEAL, W. S., KUWAHARA, Y., and SUZUKI, T. 1989. 2-(*E*)-(4-Methyl-3-pentylidene)-butanedial, β-acaridial: A new type of monoterpene from the mold mite *Tyrophagus putrescentiae* (Acarina, Acaridae). *Agric. Biol. Chem.* 53:875–878.

MADDEN, J. L. 1968. Behavioral response of parasites to the symbiotic fungus associated with *Sirex noctilio* F. *Nature* 218:189–191.

MEINERS, T., and HILKER, M. 2000. Induction of plant synomones by oviposition of a phytophagous insect. *J. Chem. Ecol.* 26:221–232.

MY-YEN, L. T., MATSUMOTO, K., WADA, T., and KUWAHARA, Y. 1980. Pheromone study on acarid mites V. Presence of citral as a minor component of the alarm pheromone in the mold mite, *Tyrophagus putrescentiae* (Schrank, 1781) (Acarina: Acaridae). *Appl. Entomol. Zool.* 15:474–477.

QUICKE, D. L. J. 1997. Parasitic Wasps. Chapman & Hall, London.

RUTHER, J., SIEBEN, S., and SCHRICKER, B. 1998. Role of cuticular lipids in nestmate recognition in the European hornet *Vespa crabro* L. (Hymenoptera, Vespidae). *Insectes Soc.* 45:169–179.

SINHA, R. N. 1961. Insects and mites associated with hot spots in farm stored grain. *Can. Entomol.* 93:609–621.

SINHA, R. N., and WALLACE, H. A. H. 1966. Ecology of insect-induced hot spots in stored grain in western Canada. *Res. Popul. Ecol.* 8:107–132.

STEIDLE, J. L. M. 1998. Learning pays off: Influence of experience on host finding and parasitism in *Lariophagus distinguendus. Ecol. Entomol.* 23:451–456.

STEIDLE, J. L. M., and SCHÖLLER, M. 1997. Olfactor host location and learning in the granary weevil parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae). *J. Ins. Behav.* 10:331–342.

STEINBERG, S., DICKE, M., and VET, L. E. M. 1993. Relative importance of infochemicals from first and second tropic level in long-range host location by the larval parasitoid *Cotesia glomerata. J. Chem. Ecol.* 19:47–59.

THIBOUT, E., GUILLOT, J. F., and AUGER, J. 1993. Microorganisms are involved in the production of volatile kairomones affecting the host seeking behavior of *Diadromus pulchellus*, a parasitoid of *Acrolepiopsis assectella. Physiol. Entomol.* 18:176–182.

TUMA, D., SINHA, R. N., MUIR, W. E., and ABRAMSON, D. 1990. Odor volatiles associated with mite-infested bin-stored wheat. *J. Chem. Ecol.* 16:713–724.

TURLINGS, T. C. J., TUMLINSON, J. H., HEATH, R. R., PROVEAUX, A. T., and DOOLITTLE, R. E. 1991. Isolation and identification of the allelochemicals that attract the larval parasitoid, *Cotesia marginiventris* (Cresson), to the microhabitat of one of its hosts. *J. Chem. Ecol.* 17:2235–2251.

TURLINGS, T. C. J., LOUGHGRIN, J. H., MCCALL, P. J., RÖSE, U. S. R., LEWIS, W. J., and TUMLINSON, J. H. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proc. Natl. Acad. Sci. U.S.A.* 92:4169–4174.

VAN DEN DOOL, J., and KRATZ, P. D. 1963. A generalization of the retention index system including linear programmed gas–liquid partition chromatography. *J. Chromatogr.* 11:463.