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A Proposed Role for the Cuticular Fatty Amides of *Liposcelis bostrychophila* (Psocoptera: Liposcelidae) in Preventing Adhesion of Entomopathogenic Fungi with Dry-conidia

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Abstract

Maximum challenge exposure of *Liposcelis bostrychophila* to *Beauveria bassiana*, *Paecilomyces fumosoroseus*, *Aspergillus parasiticus* or *Metarhizium anisopliae* resulted in no more than 16% mortality. We investigated several of *L. bostrychophila*'s cuticular lipids for possible contributions to its tolerance for entomopathogenic fungi. Saturated C_{14} and C_{16} fatty acids did not reduce the germination rates of *B. bassiana* or *M. anisopliae* conidia. Saturated C_6 to C_{12} fatty acids that have not been identified in *L. bostrychophila* cuticular extracts significantly reduced germination, but the reduction was mitigated by the presence of stearamide. Cis-6-hexadecenal did not affect germination rates. Mycelial growth of either fungal species did not occur in the presence of caprylic acid, was reduced by the presence of lauric acid, and was not significantly affected by palmitic acid. *Liposcelis bostrychophila* is the only insect for which fatty acid amides have been identified as cuticular components. Stearamide, its major fatty amide, did not reduce germination of *B. bassiana* or *M. anisopliae* conidia or growth of their mycelia. Adhesion of conidia to stearamide preparations did not differ significantly from adhesion to the cuticle of *L. bostrychophila*. Pretreatment of a beetle known to be fungus-susceptible, larval *Oryzaephilus surinamensis*, with stearamide significantly decreased adhesion of *B. bassiana* or *M. anisopliae* conidia to their cuticles. This evidence indicates that cuticular fatty amides may contribute to *L. bostrychophila*'s tolerance for entomopathogenic fungi by decreasing hydrophobicity and static charge, thereby reducing conidial adhesion.

Key words: Psocoptera, cuticular lipids, entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, *Oryzaephilus surinamensis*, fungal adhesion

Introduction

The booklouse, *Liposcelis bostrychophila* (Psocoptera: Liposcelidae), was originally described from specimens that were collected from under tree bark in Mozambique [1], but it is now an abundant, cosmopolitan pest in households and various segments of the food industry. It has an affinity for grain products [2], and cereal shipments have been implicated in its dispersal [3]. It does not cause direct damage to stored products, but populations commonly build up to unacceptable levels [4].

We have observed in preliminary work that *L. bostrychophila* has a high tolerance for entomopathogenic fungi, as has been previously reported anecdotally [4]. Our earlier analysis of its cuticular lipids revealed a unique profile [5]. In addition, to saturated hydrocarbons and C_{16}–C_{18} free fatty acids and aldehydes, there are C_{16}–C_{22} amides, the major amide being stearamide. These amides are unknown among other insects. The purpose of the study described here was to determine if these cuticular lipids have a role in defense against fungal assault. In particular, we considered fatty amides, especially stearamide, which is used as a industrial slip additive [6], to be candidates for a role as compromising agents for hydrophobic interaction and charge attractions that are important for conidial attachment to host insects or plants [7, 8]

Materials and methods

Fungi

Beauveria bassiana (Hyphomycetes: Moniliales) isolate GHA was obtained as unformulated conidia from Emerald BioAgriculture, Butte MT. *Metarhizium anisopliae* (Hyphomycetes: Moniliales) isolate ESC1 was reisolated from BioBlast, a commercial mycoinsecticide of the defunct EcoScience Corp. *Paecilomyces fumosoroseus* (Hyphomycetes: Moniliales) isolate Mycotech 612, was obtained from Stephen Wraight, USDA-ARS, NY, and a transfer culture of *Aspergillus parasiticus* (Hyphomycetes: Moniliales) NRRL18786 was obtained from Joe Dorner USDA-ARS, GA. Conidia of the latter three species were produced on white rice. Rice in 250 g batches with 75 ml of water was autoclaved in polypropylene bags. It was inoculated with 10 ml of 2-day-old Sabouraud dextrose broth culture and incubated for 12 days at room temperature (ca. 26 °C). The conidia were then collected under a 40 mesh sieve and dried over anhydrous $CaSO_4$. Prior to assays, the germination rates were determined from conidia that were spread on Sabouraud dextrose agar (SDA) and incubated at 26 ± 1 °C for 18 hours. All germination rates were at least 90%.

Insects

Liposcelis bostrychophila (Psocoptera: Liposcelidae) was colonized ca. one year prior to the assays from an infested culture of *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) on wheat grains. Its identity was confirmed by Dr. Edward Mockford, Illinois State University. The *L. bostrychophila* colony was maintained on 10:1:1 wheat flour, dried skimmed milk, brewer's yeast overlaid with puffed rice, a modification of the method of Leong and Ho [9]. The saw-toothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae), were from our locally-derived colony that is maintained on rolled oats with 1% brewers yeast. Insects were maintained at 30 °C and 60% RH in darkness.

Fungus-insect bioassay

Ten mg of conidia or 5 mg of conidia with 5 mg of diet were placed in 1 oz. (30 ml) plastic cups with 1 g of rinsed wheat kernels. Ten adult *L. bostrychophila* of mixed age were collected by allowing them to crawl out of the diet and across a glass watch plate into each treatment cup. There were three replicates per trial, and the experiment was carried out three times. Mortality was scored after 12 days of incubation in darkness at 26 ± 1 °C and 85 ± 1% RH over a saturated solution of KCl.

Germination

Conidia were suspended in 0.25% glucose in sterile water to a concentration of 10^7/ml, and 0.1 ml was spread on water agar. Fifty μl of 20 nM lipid solutions in hexane were pipetted onto nitrocellulose membranes. After hexane evaporation, the membranes were inverted onto the agar which was incubated for 20 h at 26 ± 1 °C. Germination rates were taken on 100 conidia in each of three replicates. The criterion for germination was the presence of a visibly detectable germ tube at 400×.

Radial growth

Media were prepared by dissolving the test compounds in dimethyl sulfoxide (DMSO), then mixing with 0.25% strength SDA to achieve 2 mM of test compound and 1% DMSO. Control agar also contained DMSO. The agar was inoculated with 2 mm disks of 2-day-old fungus cultures on SDA. Radial growth at 26 ± 1 °C was measured at 24 h intervals.

Adhesion

Adhesion of conidia to *L. bostrychophila*, stearamide, and other hydrophobic surfaces was compared to their adhesion to glass slides. Ten adult *L. bostrychophila* were attached to each slide with double-stick tape. Polystyrene slides (cut from petri dishes) and glass slides were cleaned with 95% ethanol. Household paraffin (Gulfwax, ChevronTexaco, San Ramon, CA) and stearamide (TCI, Tokyo) were melted onto glass slides. Silanized glass coverslips were prepared as described by Terhune and Hoch [8]: the coverslips were cleaned with 1:1 concentrated HCl: methanol for 45 min, given three water rinses, placed in concentrated H_2SO_4 for 45 min, water rinsed until neutral pH, placed in boiling water for 10 min, and given three rinses in CH_3Cl. They were then immersed in diphenyldichlorosilane (DPS), dimethyldichlorosilane (DMS), or (tridecafluoro-1,1,2,2,-tetrahydrooctyl)-1-trichlorosilane (TDF) (all from Sigma-Aldrich, St. Louis, MO), rinsed in CH_3Cl three times and baked at 100 °C for one hour.

Conidia were applied to the test substrates including the psocids by rapid vacuum release that dispersed the conidia in a fine aerosol that settled onto the surfaces. Conidia were stained for this purpose by immersion for 1 h at room temperature in 1 mg/ml fluorescein isothiocyanate (FITC) in 0.05 M carbonate-bicarbonate buffer with 0.1% Tween 80. The conidia were then washed with the buffer and air-dried. Five slides or coverslips with each treatment were placed on a support in a vacuum desiccator. A small weigh boat with 20 mg of sifted, stained conidia were placed in the center of the desiccator bottom. A vacuum was drawn to 10 psi then rapidly released. After 20 min, the slides were removed and incubated for one hour at room temperature. They were then washed with 250 ml of deionized water released from a separatory funnel at a height of 15 cm. The number of attached conidia was scored on four 4,000 μm^2 areas under the corner squares of an ocular grid with epifluorescence at 400×. There were three counts/slide of random areas of artificial substrate or mid-ventral abdomen on psocids. One slide was prepared and scored for each treatment, and the experiment was carried out 5 times on separate days. The hydrophobicity or wettability of each artificial substrate was determined by the methanol solution spreading method of Gerhart and co-workers [10].

In order to test the effect of stearamide on conidial attachment to a fungus-susceptible insect, third instar *O. surinamensis* were allowed to accumulate stearamide on their exteriors by confinement with no other absorbable material. The larvae were held without food for 18 h at 26 ± 1 °C in 3 oz. plastic cup with or without 1 g of stearamide. All larvae were then cleaned by collection on a 50 mesh sieve and washing by three successive submersions in 100 ml of water that was drawn off by suction through a Buchner funnel. The stearamide-treated and untreated *O. surinamensis* larvae and *L. bostrychophila* that had not been exposed to exogenous stearamide were placed in screen-covered cups within a vacuum desiccator and dusted with FITC-stained *B. bassiana* or *M. anisopliae* conidia as above. After one hour, the insects were placed on slides with double stick tape and washed with 500 ml of deionized water. Adhesion was scored by counting the conidia as described above on 0.0625 mm^2 areas of the ventral center of the abdomen on 10 insects per treatment. The experiment was carried out three times.

Table 1. Mortality of *Lipocelis bostrychophilus* after 10 days of exposure to conidia of four fungi with or without diet.

Fungus	Diet	% Mortality ± SE
Metarhizium anisopliae	−	16.0 ± 5.0a
	+	11.3 ± 4.2ab
Paecilomyces fumosoroseus	−	6.0 ± 3.7ab
	+	7.2 ± 4.0ab
Beauveria bassiana	−	2.2 ± 2.2ab
	+	2.2 ± 1.5ab
Aspergillus parasiticus	−	1.4 ± 1.4b
	+	2.2 ± 1.5ab
None	−	4.6 ± 3.4ab
	+	3.6 ± 1.8ab

Means (overall) followed by the same letter are not significantly different by the Student–Neuman–Keuls test (α = 0.05).

Data analysis

Correlation analysis and ANOVA with Student–Newman–Keuls post hoc procedure were preformed with InStat software [11].

Results

Liposcelis bostrychophila *bioassay*

Mortality of the insects was not significantly greater with any of the fungi present than without fungi added, and the presence of diet had no significant effect (Table 1, F = 2.26; d.f. = 9,80; $P > 0.05$). The only significant difference was lower mortality for diet-free *A. parasiticus* than for diet-free *M. anisopliae*.

Germination

None of the lipids that were previously identified in *L. bostrychophila* cuticular extracts by Howard and Lord [5] significantly reduced the germination rates of either *B. bassiana* or *M. anisopliae* (Figure 1). The germination rates were significantly lower than controls only with C_6-C_{12} fatty acids (F = 89.0; d.f. = 15,47; $P < 0.05$). Germination of both fungi in contact with the inhibitory fatty acids was significantly greater when they were applied to the conidia in combination with stearamide ($P < 0.01$). Germination of conidia treated with cis-6-hexadecenal was not significantly different from control germination.

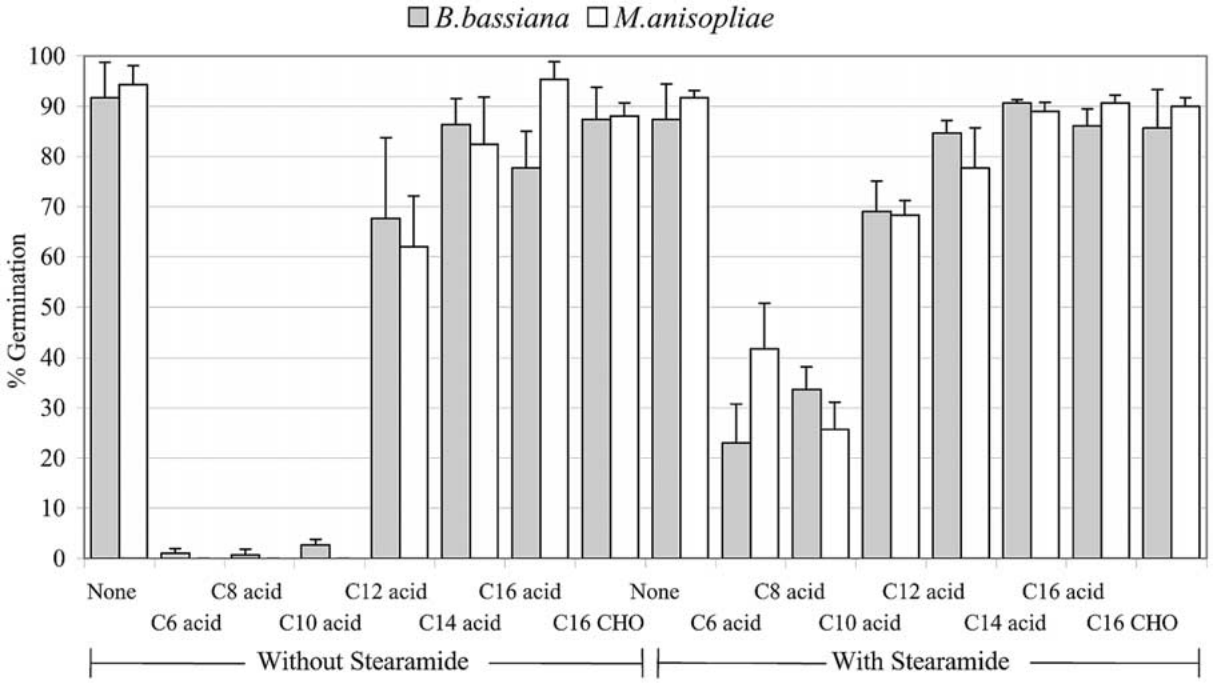

Figure 1. The effect of fatty acids (C8-C16 acid), cis-6-hexadecenal (C16 CHO), and stearamide on germination of *Beauveria bassiana* and *Metarhizium anisopliae* conidia. Bars represent standard errors.

Radial growth

Mycelial growth on agar with lauric acid was significantly less than on control agar for *M. anisopliae* (F = 98.5; d.f. = 4,15; $P < 0.01$) but not for *B. bassiana* (F = 10.0; d.f. = 4,15; $P > 0.05$) (Figure 2). Neither palmitic acid nor stearamide significantly affected growth of either fungus. Neither *B. bassiana* nor *M. anisopliae* grew on agar with caprylic acid.

Adhesion

Adhesion of *B. bassiana* conidia to the test surfaces relative to glass did not differ significantly among paraffin, stearamide, and *L. bostrychophila* cuticles, but adhesion to all three was significantly less than to polystyrene (Table 2, F = 6.91; d.f. = 3,96; $P < 0.01$). In the case of *M. anisopliae* conidia, adhesion did not differ significantly among paraffin, stearamide, and *L. bostrychophila* cuticles, but the adhesion to stearamide and *L. bostrychophila* cuticles were significantly less than to polystyrene (F = 5.8; d.f. = 3,96; $P < 0.01$). In a separate series of experiments, *B. bassiana* conidial adhesion to polystyrene, DPS, and DMS did not differ significantly from one another, but adhesion to all three was significantly less than adhesion to TDF (F = 8.2; d.f. = 3,96; $P < 0.01$). *Metarhizium anisopliae* adhesion to TDF was significantly greater than adhesion to polystyrene, that in turn was significantly greater than adhesion to DPS or DMS (F = 14.6; d.f. = 3,96; $P < 0.01$). The wettabilities of the artificial surfaces did not correlate with the adhesion ratios for either *B. bassiana* ($r^2 = 0.013$, $P = 0.80$) or *M. anisopliae* ($r^2 = 0.024$, $P = 0.77$).

Significantly fewer *B. bassiana* (F = 51.7; d.f. = 2,86; $P < 0.01$) and *M. anisopliae* (F = 26.7; d.f. = 2,87; $P < 0.01$) conidia were attached to *O. surinamensis* larvae that had been exposed to stearamide for 18 h prior to application than to untreated beetle larvae. There was no significant difference between the number of conidia of either fungus adhered to stearamide-treated *O. surinamensis* larvae or *L. bostrychophila* ($P > 0.05$, Figure 3).

Discussion

Liposcelis bostrychophila requires relative humidities above approximately 60% to maintain water balance [12], and consequently it is most abundant in fungus-rich environments. Indeed, Mills and coworkers [13] reported that *L. bostrychophila* feeds on fungal con-

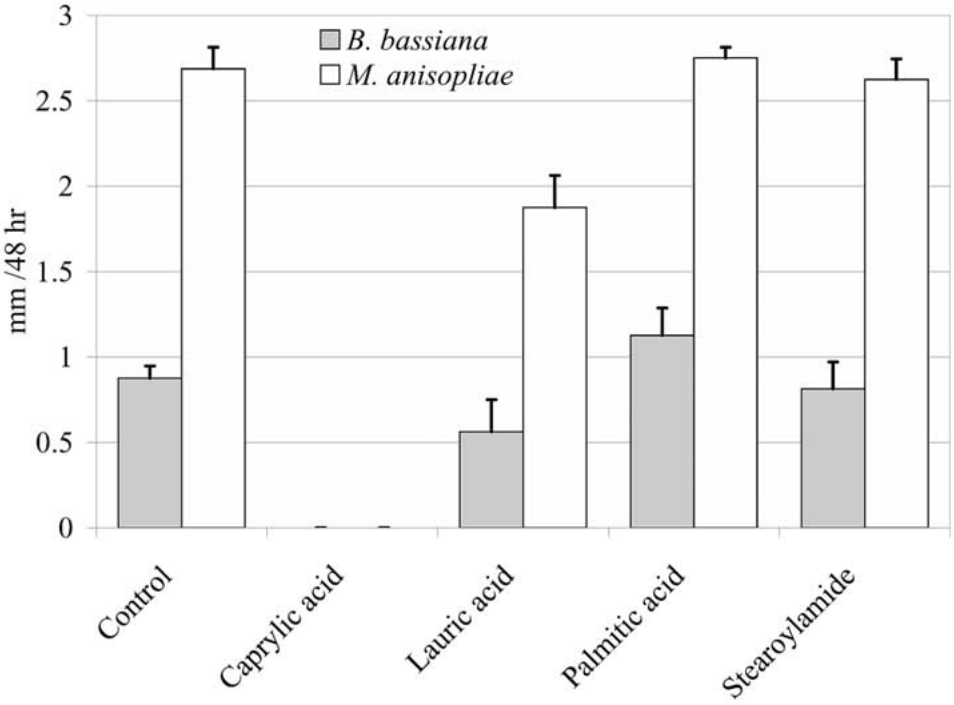

Figure 2. Radial growth of *Beauveria bassiana* and *Metarhizium anisopliae* on agar with fatty acids or stearamide. Bars represent standard errors.

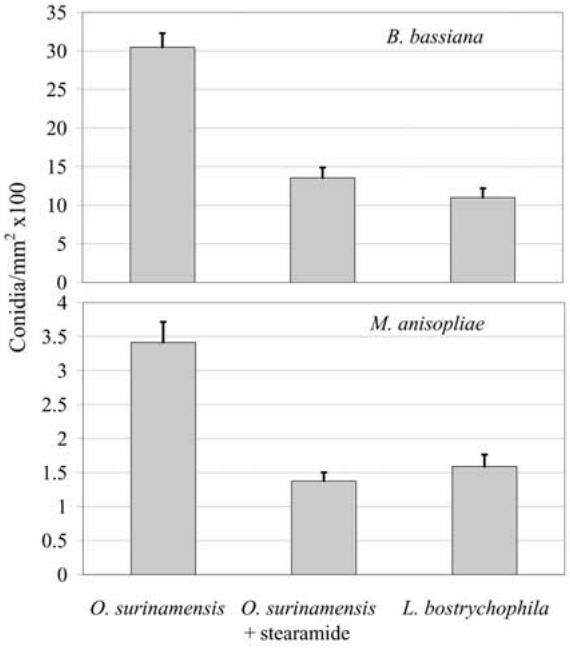

Figure 3. Adhesion of *Beauveria bassiana* and *Metarhizium anisopliae* to cuticles of *Liposcelis bostrychophila* and *Oryzaephilus surinamensis* with and without exposure to stearamide. Bars represent standard errors.

Table 2. Adhesion of *Beauveria bassiana* and *Metarhizium anisopliae* conidia to hydrophobic substrates and *Liposcelis bostrychophila* cuticles (ratio of conidia/unit area adhered to conidia/unit area adhered to glass ± SE)

Substrate (Wettability)	Adhesion ratio	
	M. anisopliae	*B. bassiana*
Experiment 1		
Polystyrene (21)	46.5 ± 8.5a	33.9 ± 6.1a
Paraffin (9)	31.9 ± 6.3ab	15.6 ± 3.1b
Stearamide (6)	20.0 ± 4.1b	14.0 ± 2.8b
Cuticle (N/A)	15.0 ± 2.6b	11.7 ± 2.5b
Experiment 2		
Polystyrene (21)	50.4 ± 4.8b	35.1 ± 3.5b
TDF (6)	68.6 ± 5.2a	57.2 ± 5.1a
DMS (14)	34.5 ± 3.5c	33.7 ± 3.4b
DPS (28)	31.3 ± 4.1c	32.8 ± 4.2b

Means within Experiment and column followed by the same letter are not significantly different by the Student–Neuman–Keuls test ($\alpha = 0.05$).

taminants of wheat and flour. Turner [4], however, states that fungal development in *L. bostrychophila* cultures reduces growth rates and may lead to colony loss. The results of the maximum challenge exposure (Table 1) indicate that the psocid is tolerant of some

of the most common entomopathogenic species. *Aspergillus parasiticus*, routinely found in grains, was included as a check for possible physical effects of immersion in a mass of dry conidia. None was apparent, since the mortalities with *A. parasiticus* were not significantly different from the fungus-free controls. Our finding of no mortality greater than the 16% with *M. anisopliae* is an indication of unusual tolerance. *Liposcelis bostrychophila*'s unique profile of cuticular lipids [5] raised the question of their possible role as an initial point of antifungal defense.

The cuticle is an insect's first line of defense against fungal assault. In addition to providing a physical barrier, the cuticles of some insects bear compounds with fungicidal and fungistatic properties. Fatty acids of ten or fewer carbons present on the cuticles of several larval Lepidoptera are inhibitory to germination and growth of several fungi including *B. bassiana*, but inhibition is mitigated by nutrients [14–16]. Here we confirm those effects and report that lauric acid also has mild inhibitory effects. Similarly, short chain aldehydes detected among the cuticular lipids of the hemipteran *Nezara viridula* were inhibitory to *M. anisopliae* germination [17]. No fatty acids or aldehydes of fewer than 16 carbons were in the *L. bostrychophila* cuticular extracts that we analyzed in previous work [5], and we detected no *B. bassiana* or *M. anisopliae* germination or growth inhibition by any of the longer chain lipids that we tested. Indeed, stearamide mitigated the effects of C_6–C_{12} saturated fatty acids and appears to be nutritive for the fungi. We have found no evidence of adverse effects on fungal development among the cuticular components that we have tested.

Adhesion of conidia to the cuticle is the initial step in the infection process and is an appropriate target for a first line of antifungal defense. The conidia of some terrestrial entomopathogenic fungi, e.g., *Verticillium lecanii* and *Hirsutella thompsonii*, have mucilaginous coatings, but the dry aerial conidia of *B. bassiana* and *M. anisopliae* depend on passive physical phenomena for initial adhesion to their hosts [7, 18]. Several surface properties, including charge, texture, and hydrophobicity affect passive adhesion. Hydrophobic interaction has been implicated in the adhesion of both phytopathogenic [8] and entomopathogenic [7] fungi to host surfaces. Our results did not show a correlation between substrate wettability and adhesion, but surface charges, particularly on polystyrene, were not controlled and may have affected adhesion.

Adhesion of neither *B. bassiana* nor *M. anisopliae* to stearamide was significantly different from adhesion to *L. bostrychophila* cuticles (Table 2). Stearamide is an amphiphilic compound, and its orientation after being melted onto a glass surface may not be an accurate reflection of its alignment on the surface of an insect. Larval *O. surinamensis* are susceptible to *B. bassiana* [19], and its cuticular lipids do not include amides [20]. When larvae were allowed to take up exogenous stearamide, conidial adhesion to their cuticles was significantly reduced relative to adhesion to untreated larvae and did not differ significantly from adhesion to *L. bostrychophila* cuticles (Figure 3). Fatty acid amides of 15–21 carbons occur on *L. bostrychophila* cuticles with stearamide accounting for 76% [5]. Although our experiments were conducted with stearamide, which was commercially available, we feel confident that the results are relevant for the related amides. Fatty acid amides are used as slip additives in plastic formulations where they tend to migrate to the surface imparting lubrication and reduction of static charge [6]. Those properties would make it an impediment to conidial adhesion. *Liposcelis bostrychophila* is the only insect for which the presence of cuticular fatty amides has been reported, and they are abundant [5]. We suggest that this insect may have evolved secretion of amphiphilic cuticular compounds as a heretofore-unreported antifungal defense as an adaptation to its fungus-rich habitats.

Note

This article reports the results of research only. Mention of a proprietary product does not constitute a recommendation or endorsement by the U.S. Department of Agriculture.

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