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CUTICULAR LIPIDS OF THE BOOKLOUSE, *Liposcelis bostrychophila*: HYDROCARBONS, ALDEHYDES, FATTY ACIDS, AND FATTY ACID AMIDES

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Abstract—The booklouse, *Liposcelis bostrychophila*, is an increasingly common pest of stored food products worldwide. We report here the cuticular lipid composition of this pest (the first report of the hydrocarbons of any member of the Order Psocoptera and the first report of fatty acid amides as cuticular components for any insect). No unsaturated hydrocarbons were present. A homologous series of *n*-alkanes (C_{21}–C_{34}), monomethyl alkanes (3-, 4-, 5-, 7-, 9-, 11-, 12-, 13- and 15-methyl-) with a carbon chain range of C_{28}–C_{42}, and dimethyl alkanes (3, 7-; 9, 13-; 11, 15-; 13, 17-; 9, 21-; 11, 19-; and 13, 21-) with a carbon number range of C_{31}–C_{43} were identified. The relative abundances of these hydrocarbons were low, comprising approximately 0.0125% of total biomass. The amides were a homologous series (C_{16}–C_{22} in chain length), with the major amide being stearoyl amide. In addition to the amides, free fatty acids ($C_{16:1}$, $C_{16:0}$, $C_{18:2}$, $C_{18:1}$, and $C_{18:0}$ in chain length) and three straight chain aldehydes (C_{15}, C_{16}, and $C_{17:1}$ in chain length) also occurred as cuticular components. These findings are discussed in terms of the chemical and physiological ecology of this species.

Key Words—Psocoptera, chemical ecology, physiological ecology, mass spectra, fatty acid, fatty amide, aldehyde, hydrocarbon, cuticular lipids

INTRODUCTION

The booklouse, *Liposcelis bostrychophilia* Badonnel (Psocoptera: Liposcelididae), occurs worldwide (Lienhard, 1990) and is often considered a pest in households and various segments of the food industry (Turner, 1994). These are tiny (approximately 1 mm in length), wingless, light brown insects that are thelytokously

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parthenogenetic (hence no males have ever been found). They are capable of survival and reproduction on a wide variety of food items, although they are most often found on flour and other farinaceous products. The life cycle includes eggs, four nymphal stages, and adult females. The species does best at moderately high relative humidities (65–80%) and at temperatures between 20°C and 30°C (Turner, 1994). Other than these limited data on population parameters (reviewed in more detail in Turner, 1994), little is known of the biochemical and physiological properties of these insects (Ali, 1999), and their chemical ecology has not been studied. Liposcelidids are known, however, to be highly resistant to entomopathogenic fungi or bacteria (Turner, 1988; J.C.L. unpublished observations), suggesting that they might possess unusual cuticular lipids responsible for their relative immunity. Fungal infections are initiated by conidial attachment and germination on the cuticular surface. Accordingly, surface compounds are logical candidates in a search for antifungal defenses.

We report here the cuticular lipid composition of *L. bostrychophila* and discuss our findings with regard to the ecological parameters of its lifestyle. This is the first report of cuticular hydrocarbons in the order Psocoptera (common name: psocids) as well as the first report of fatty acid amides as cuticular lipid components of any insect.

METHODS AND MATERIALS

Insects. Cultures of *L. bostrychophila* were obtained from contaminated laboratory cultures of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), originally collected from Manhattan, Kansas, USA and maintained for several years. Psocid cultures were maintained at room temperature (ca. 28°C), ca. 80% relative humidity, under ambient light, and fed on insect-free wheat that had been previously damaged by rusty grain beetles (ensuring particles of wheat of a size most suitable for consumption by these tiny insects). Psocids to be used for experiments were collected from stock cultures using the method of Turner (1990).

Chemical Analyses. Insects were frozen at −5°C for 24 hr, and gently sieved through stacked 40 and 50 mesh sieves to remove most culture contaminants. The larger psocids collected on the 50 mesh sieve were used for extractions. Cuticular lipids were extracted by immersing the insects in three successive 0.25-ml portions of hexane for 10 sec each time. The combined extracts from each sample were concentrated under a stream of N_2, and the total extract was analyzed by mass spectrometry as described below. Hydrocarbons were then isolated by chromatography on a 3-cm-long silica gel column (BioSil A, Bio-Rad Laboratories, Richmond, California, USA) as described earlier (Howard et al., 1978). The psocids are very

small insects (individual psocids weigh ca. 60 μg), containing insufficient hydrocarbon for single insect analyses, so samples consisted of ca. 2 mg of insects per replicate (ca. 333 psocids), and three replicates were analyzed. Compositional data were converted to percent values of the total volatile portion of the extract. An estimate of the total quantity of extractable hydrocarbons was calculated by calibration with an internal standard (eicosane, 57 ng/μl). This estimate was then used to calculate the hydrocarbons as a fraction of the total mass of psocids extracted.

Electron impact mass spectral analyses were conducted using a Hewlett-Packard 5890A gas chromatograph (GC) (Hewlett-Packard, Inc., San Fernando, California, USA) fitted with a DB-5 column (15 m long $\times$ 0.25 mm ID; J & W Scientific, Folsom, California, USA) interfaced to a Hewlett-Packard 5971 mass selective detector (MSD) and a Hewlett-Packard HP G1034C data system. Helium was the carrier gas, with a column head pressure of 0.75 kg/cm^2. Electron impact mass spectra were obtained at 70 eV. The GC was programmed at 100°C/min, 5°C/min to 320°C, 320°C for 20 min. The splitless injector was set at 275°C and the GC-MSD interface was at 280°C. Retention times of each hydrocarbon component and equivalent chain length values (ECL) were obtained by comparison with known *n*-alkane standards (Howard et al., 1978). Individual components were identified from their EI-MS fragmentation patterns (Jackson and Blomquist, 1976; Nelson, 1978) in conjunction with ECL values. Fatty acids and amides were tentatively identified from their characteristic mass spectra (Budzikiewicz et al., 1967). An authentic sample of stearoyl amide was synthesized from stearoyl chloride and ammonium acetate (Fieser and Fieser, 1967), and fatty acids were converted to their methyl esters by reaction with (1,1)-*N*,*N*-dimethylhydrazine (McDaniel and Howard, 1985). GC-MS analysis of the synthetic amide and fatty acid esters (Ryhage and Stenhagen, 1963) were then conducted to verify preliminary identifications. Aldehydes were detected by their conversion to *N*,*N*-dimethyl hydrazones and identified as such from their characteristic EI mass spectra. Comparisons to authentic standards were not made.

Verification that the lipids obtained were indeed of cuticular origin was obtained by gently wiping several insects with a 7-μm polydimethylsiloxane bonded-phase fiber in a Supelco (Bellafonte, Pennsylvania, USA) solid-phase microextraction (SPME) holder and then analyzing the absorbed lipids by GC-MS using the parameters listed above, with the exception that the fiber was desorbed for 2 min at 280°C with the septum purge closed before beginning the temperature program.

Identification and Voucher Specimens. Verification of species identity was obtained by submitting specimens to Dr. Edward L. Mockford, Illinois State University, Normal, Illinois, USA. Voucher specimens are in Dr. Mockford's personal collection, accession number ELM6168.

RESULTS

An unusual assortment of cuticular lipids was found in *L. bostrychophila*, including hydrocarbons, fatty acids, fatty primary amides, and aliphatic aldehydes. Direct evidence that these components were all cuticular in origin was obtained from the SPME analysis: all components seen by hexane extraction were also seen in the SPME sample, albeit in greatly reduced quantities. A homologous series of *n*-alkanes (C_{21}–C_{34}), monomethyl alkanes (3-, 4-, 5-, 7-, 9-, 11-, 12-, 13- and 15-methyl-) with a carbon chain range of C_{28}–C_{42}, and dimethyl alkanes (3, 7-; 9, 13-; 11, 15-; 13, 17-; 9, 21-; 11, 19-; and 13, 21-) with a carbon number range of C_{31}–C_{43} were identified (Table 1). The amount of extracted hydrocarbons was low, comprising approximately 0.0125% of total biomass. Most components occurred as less than 3% of the total volatile portion of the extract, with only two peaks each representing more than 10% of the total (C_{29} and the isomeric mixture of *X*-methyl-C_{31}). No unsaturated hydrocarbons were found.

Free fatty acids accounted for ca. 10–40% of total volatile lipids and were tentatively identified from their characteristic mass spectra (Budzikiewicz et al., 1967). Molecular ions were present, and *m*/*z* 60 was also always present. Conversion of the fatty acids to methyl esters produced diagnostic mass spectra (Ryhage and Stenhagen, 1963) that allowed tentative identifications of the common fatty acids, *Z*-9-$C_{16:1}$, $C_{16:0}$, $C_{17:0}$, *Z*,*Z*-6,9-$C_{18:2}$, *Z*-9-$C_{18:1}$, $C_{19:0}$, and $C_{20:0}$. Comparison of the retention times of the derivatized acids to that of a standard methyl ester mixture allowed tentative assignment of double bond locations. In all cases, the psocid methyl esters had identical retention times and mass spectra to those of known standards.

The primary fatty acid amides were clearly detectable in the crude extracts. They too have characteristic mass spectra, with a base peak at *m*/*z* 59, an intense ion, at *m*/*z* 72, an M-43 ion, and weak, but usually detectable, molecular ions (Table 2, Figure 1) (Budzikiewicz et al., 1967). These amides occurred as a homologous series from C_{16} to C_{22}, with the even carbon number components being the major compounds. Synthetic stearoyl amide had identical properties to the C_{18} psocid amide. The remaining amides had ECL values differing by integer values from that of stearoyl amide, indicating that their carbon chains were not branched.

The aliphatic aldehydes were discovered serendipitously as unexpected *N*,*N*-dimethyl hydrazone peaks in the crude reaction mixture of the methyl esters (McDaniel and Howard, 1985). The C_{15} and C_{16} aldehydes were saturated, with ECL values differing by one unit, whereas the C_{17} aldehyde had a double bond in an undetermined location. Figure 2 illustrates the mass spectra of the *N*,*N*-dimethyl hydrazones of each of these aldehydes, with a prominent molecular ion and an *m*/*z* 86 McLafferty rearrangement ion as the base peak. The free aldehydes were buried under other peaks and hence were not detected in the initial analysis of the crude extract.

TABLE 1. CUTICULAR HYDROCARBONS OF *Liposcelis bostrychophila*

Compound	ECL[a]	CN[b]	Diagnostic EI-MS ions (m/z)	Percent (mean ± SD)[c]
C_{21}	21.00	21	296	0.2 ± 0.1
C_{22}	22.00	22	310	0.5 ± 0.3
C_{23}	23.00	23	324	0.5 ± 0.3
C_{24}	24.00	24	338	1.5 ± 1.0
C_{25}	25.00	25	352	3.1 ± 2.4
C_{26}	26.00	26	366	4.4 ± 3.5
C_{27}	27.00	27	380	5.6 ± 3.7
3-MeC_{27}	27.72	28	365, 379(M-15)	1.6 ± 1.5
C_{28}	28.00	28	394	0.9 ± 0.3
3-MeC_{28}	28.72	29	379	0.1 ± 0.1
C_{29}	29.00	29	408	10.4 ± 1.8
9-;11-MeC_{29}	29.28	30	140, 308, 407 (M-15); 168, 280, 407 (M-15)	2.1 ± 1.0
7-MeC_{29}	29.38	30	112, 336, 407 (M-15)	0.8 ± 0.4
5-MeC_{29}	29.48	30	85, 365, 407 (M-15)	2.2 ± 1.6
3-MeC_{29}	29.72	30	393, 407 (M-15)	2.6 ± 1.9
C_{30}	30.00	30	422	3.6 ± 3.1
3, 7-DiMeC_{29}	30.09	31	127	0.3 ± 0.2
9-;11-;12-MeC_{30}	30.25 to 30.32	31	140, 323;168, 295; 183, 281	1.0 ± 0.3
4-MeC_{30}	30.54	31	71, 393	0.3 ± 0.2
3-MeC_{30}	30.72	31	407	0.3 ± 0.2
C_{31}	31.00	31	436	6.8 ± 3.3
9-;11-;13-;15-MeC_{31}	31.22 to 31.32	32	140, 337, 435 (M-15); 169, 309, 435 (M-15); 196, 280, 435 (M-15); 224, 252, 435 (M-15)	10.5 ± 3.6
7-MeC_{31}	31.38	32	112, 365, 435 (M-15)	3.6 ± 1.9
5-MeC_{31}	31.48	32	85, 393, 435 (M-15)	1.3 ± 0.8
11,15-;13, 17-DiMeC_{31}	31.52 to 31.54	33	168, 323, 239, 252, 449 (M-15); 196, 295, 267, 224, 449 (M-15)	3.4 ± 1.1

TABLE 1. CONTINUED

Compound	ECL[a]	CN[b]	Diagnostic EI-MS ions (m/z)	Percent (mean ± SD)[c]
9, 21-DiMeC$_{31}$	31.64	33	140, 351, 168, 323, 449 (M-15)	1.9 ± 0.9
3-MeC$_{31}$	31.72	32	421, 435 (M-15)	3.6 ± 3.1
C$_{32}$	32.00	32	57	3.2 ± 1.9
3, 7-DiMeC$_{31}$	32.09	33	435, 127, 365, 449 (M-15)	2.6 ± 3.3
11-MeC$_{32}$	32.28	33	168, 323	1.0 ± 0.3
11, 15-DiMeC$_{32}$	32.54	34	168, 323, 239, 267	0.7 ± 0.3
Unknown 1	32.87	–	141, 364	0.3 ± 0.2
C$_{33}$	33.00	33	57	1.5 ± 1.8
11-;13-;15-;17-MeC$_{33}$	33.20 to 33.28	34	168, 336, 463 (M-15); 196, 308, 463 (M-15); 224, 280, 463 (M-15); 252, 463 (M-15)	3.9 ± 0.7
11,15-;13, 17-DiMeC$_{33}$	33.52 to 33.54	35	168, 351, 239, 281, 477 (M-15); 196, 323, 252, 267, 477 (M-15)	5.1 ± 0.8
3-MeC$_{33}$	33.72	34	449	0.3 ± 0.1
C$_{34}$	34.00	34	57	1.0 ± 1.1
3, 7-DiMeC$_{33}$	34.09	35	463, 127, 393	0.5 ± 0.4
17-MeC$_{35}$	35.20	36	252, 281	0.8 ± 0.1
13, 17-DiMeC$_{35}$	35.52	37	196, 351, 267, 280	1.1 ± 0.3
11, 15-DiMeC$_{37}$	37.54	39	168, 379, 267, 309	0.7 ± 0.1
13-MeC$_{39}$	39.23	40	196, 393	0.2 ± 0.2
11,19-;13, 21-DiMeC$_{39}$	39.40 to 39.50	41	168, 435, 295, 309; 196, 407, 323, 280	2.2 ± 0.4
Unknown 2	39.60	–	196	0.2 ± 0.1
13-MeC$_{41}$	41.23	42	196, 421	0.2 ± 0.1
11,23-;13, 21-DiMeC$_{41}$	43.40 to 43.50	43	168, 463, 280, 351; 196, 435, 308, 323	1.0 ± 0.5

[a] ECL = equivalent chain length.
[b] CN = carbon number.
[c] $N = 3$.

TABLE 2. FATTY ACID AMIDES OF *Liposcelis bostrychophila*

Amide	% of total amides (mean ± SD)[a]	Diagnostic EI-MS ions (*m/z*)
$C_{15}H_{31}CONH_2$	5.8 ± 1.8	59, 72
$C_{16}H_{33}CONH_2$	0.6 ± 0.1	59, 72
$C_{17}H_{35}CONH_2$	76.3 ± 5.9	59, 72, 240 (M-43), 283 (M^+)
$C_{18}H_{37}CONH_2$	0.5 ± 0.2	59, 72
$C_{19}H_{39}CONH_2$	15.5 ± 6.2	59, 72, 268 (M-43), 311 (M^+)
$C_{20}H_{41}CONH_2$	1.4 ± 0.9	59, 72
$C_{21}H_{43}CONH_2$	Trace[b]	59, 72

[a] Calculated from *m/z* 59 extracted ion analysis, $N = 2$.
[b] Less than 0.1 percent, but detectable.

DISCUSSION

Despite the burgeoning literature on cuticular hydrocarbons of insects, as well as a lesser literature on other cuticular lipid constituents of insects, large groups of taxa remain unexplored. The Psocoptera represent one such example. As this report shows, novel compounds remain to be discovered in these unstudied taxa. The cuticular hydrocarbons of *L. bostrychophila* contain a diversity of saturated

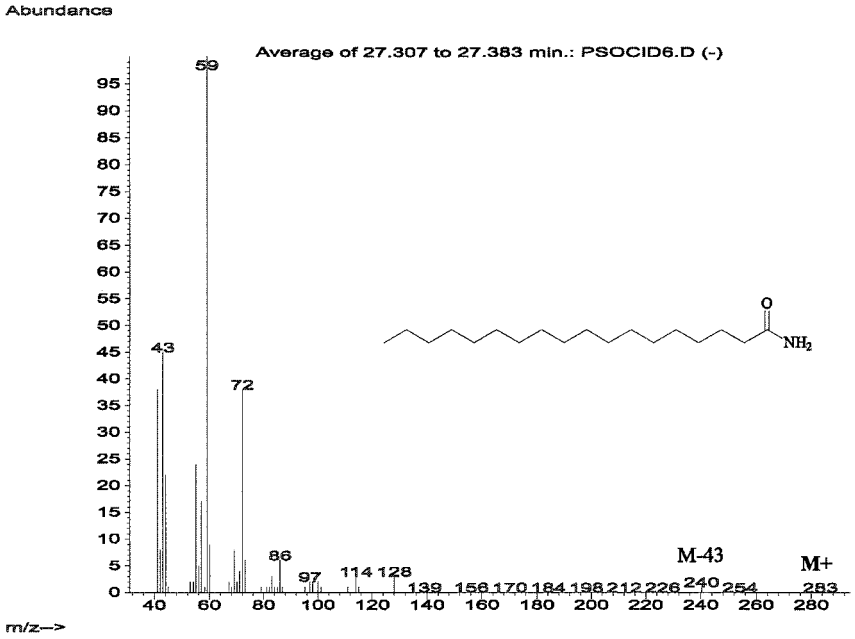

FIG. 1. EI mass spectrum of stearoyl amide isolated from the cuticle of *Liposcelis bostrychophila*.

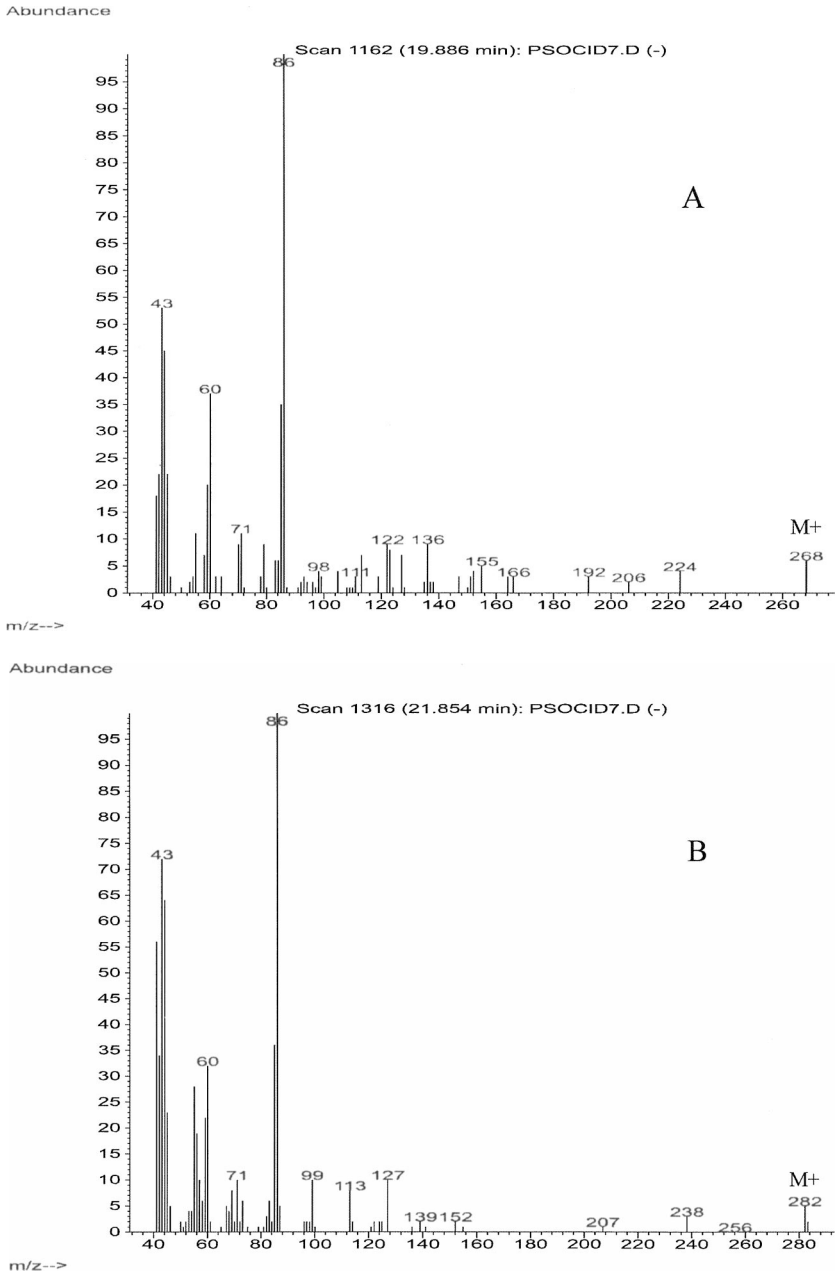

FIG. 2. EI mass spectrum of *N*,*N*-dimethylhydrazones of pentadecanal (A), hexadecanal (B) and heptadecenal (C) isolated from the cuticle of *Liposcelis bostrychophila*.

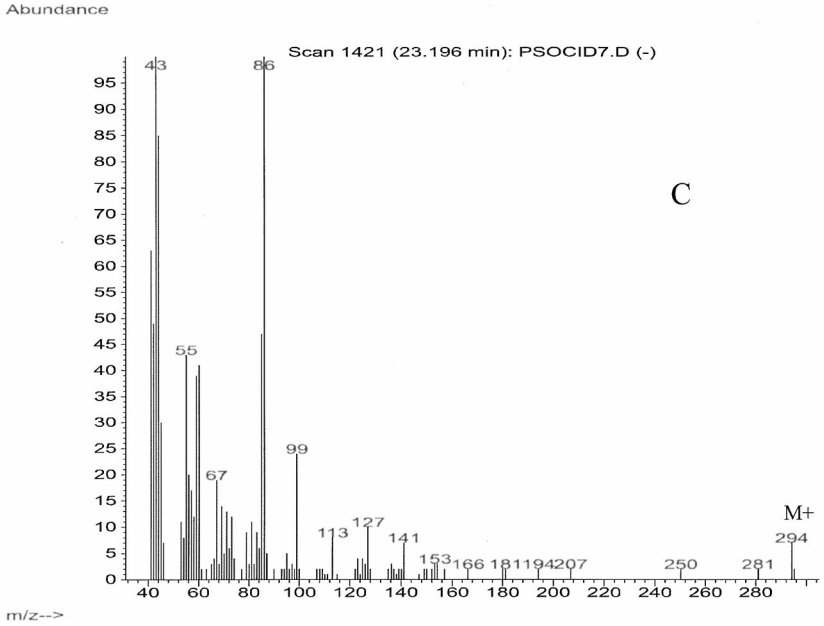

FIG. 2. Continued.

alkanes of medium to long chain length. Although the particular complex of hydrocarbons found is unique to this species, the individual components are all known from previous insect studies (Howard, 1993; Nelson, 1993). No unsaturated hydrocarbons were detected, but there are many other insect taxa where unsaturated hydrocarbons are also missing. Of particular note, the hydrocarbons recovered from *L. bostrychophila* were not the same as those from the rusty grain beetle, which has numerous unsaturated hydrocarbons (Howard, 1992), thus ruling out any possibility that the psocid hydrocarbons were contaminants from the rearing media.

The small amount of hydrocarbons (ca. 0.01% of total biomass) on this psocid is similar to the quantities found on subterranean termites (0.0001–0.01%) (Howard et al., 1978). These abundances may be compared to the few other studies where lipid has been expressed as a function of biomass: values of 0.13–1.8% for a flesh fly (Jackson et al. 1974) and five species of desert inhabiting tenebrionid beetles with values of 0.02–0.18% (Hadley, 1978). It is difficult to make exact comparisons among these taxa, however, as they differ greatly in size (and consequently in surface-to-volume ratio) and ecological habitat. Cuticular hydrocarbons are used by many insects as a barrier to the loss of internal water (Edney, 1977; Gibbs, 2002). In habitats with high humidity, however, an abundance of hydrocarbons may be less critical to a species and the metabolic energy needed to produce them can be used for other basic physiological needs.

For many insect species, cuticular hydrocarbons are important in a variety of semiochemical roles, including recognition and courtship functions (Howard, 1993). Although psocids, being parthenogenetic, do not need gender recognition cues, they occur in moderate to large aggregations, and cuticular compounds may play a role in maintenance of these aggregations.

Polar lipids are also cuticular constituents of many insects (Buckner, 1993). Of these, free fatty acids are common, with even carbon number components ranging from 14 to 20 being most prevalent. Although these acids are rarely the major cuticular component, they can be present in fairly high abundance [49% of the cuticular lipids of the adult stonefly *Pternonarcys californica* (Armold et al., 1969) and up to 47% of the cuticular lipids of the adult flesh flies, *Sarcophaga bullata* (Jackson et al., 1974)]. However, little is known of the functional significance of these free fatty acids.

Unlike the free fatty acids, long-chain aldehydes are not commonly known components of insect cuticular lipids (Buckner, 1993). The majority of such compounds reported have ranged in carbon number from C_{22} to C_{34}, they usually occur with long-chain alcohols, and they occur in low abundance. Such aldehydes are known, however, as major components in wax particles secreted by whitefly adults (Homoptera) (Nelson and Blomquist, 1995; Nelson et al., 1997). Shorter chain aldehydes (C_{14}–C_{16}, unsaturated C_{18}) have been reported as cuticular components of the cockroach, *Periplaneta americana* (Gilby and Cox, 1963). The psocid aldehydes are structurally similar to those of *P. americana*, with the exception that no evidence of methyl-branched aldehydes was found, and they occur in much lower concentrations than in the roaches. The functions of these various cuticular aldehydes are unknown.

Our finding of free primary fatty acid amides as insect cuticular components is the first such instance that we are aware of. Indeed, amide structures of any kind in insects (other than in peptides) are rare in the literature. Several recent papers have dealt with the amide volicitin and its homologs found in the oral secretions of the beet armyworm (Alborn et al., 2000 and references therein), but these are secondary amides and have numerous other functional groups that undoubtedly contribute to their biological activity.

As noted earlier, the physiological and chemical ecology of these psocids are virtually unknown. The stored grain ecosystem, either in silos or in processed grains, is a somewhat dusty environment. Perhaps the unusual cuticular lipid composition of these insects protects these insects from surface abrasion in the dusty stored grain habitats in which they are frequently found. The insects'cuticular acids, aldehydes, and amides are all relatively low-molecular-weight compounds with some degree of fluidity that may aid in sloughing off dust. This, coupled with the insects' ability to efficiently extract atmospheric moisture (Knülle and Spadafora, 1969; Rudolph, 1982) are likely to be important evolutionary adaptations to the stored grain environment.

We have found *L. bostrychophila*, unlike the majority of insect species, to be unaffected by three entomopathogenic fungi of broad host range: *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces fumosoroseus* (J.C.L., unpublished data). Earlier workers had reported that liposcelidids were highly resistant to other entomopathogenic fungi or bacteria (Turner, 1988). Could the unusual array of polar lipids found on the cuticle of *L. bostrychophila* be responsible for this resistance to fungal infection? Insect resistance to fungal pathogens has been linked to fungistatic aldehydes on the stink bug *Nezara viridula* (L.) (Sosa-Gomez et al., 1997) and short-chain fatty acids (Koidsumi, 1957; Smith and Grula, 1982) in cuticular lipids of Lepidoptera. The aldehydes and fatty acids found on this psocid are longer in chain length than the aldehydes and acids referenced above, and the amides might synergize the putative fungistatic properties of these aldehydes and acids. Indeed, fatty acid amides were among the first surface-active agents to be used by the detergent industry to enhance the detergent properties of sulfate and sulfonate detergents (Schwartz and Perry, 1949). In addition, surfactants have been shown to inhibit enzymes and to enhance the absorption of toxicants through cell membranes (Schwartz et al., 1958). Surfactants also produce "slippery" surfaces, which may disrupt the hydrophobic interactions that are critical for successful fungal attachment to host insects (Boucias et al., 1988). It is, thus, conceivable that the amides, in combination with the fatty acids and aldehydes, present an inhospitable niche to these opportunistic fungi. At this point, the roles of the various cuticular constituents of *L. bostrychophilia* remain speculative. Experiments in progress may elucidate some of the functional roles of these compounds.

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