

Ontogenetic and Environmentally Induced Changes in Cuticular Hydrocarbons of *Oryzaephilus surinamensis* (Coleoptera: Cucujidae)

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Ann. Entomol. Soc. Am. 88(4): 485-495 (1995)

ABSTRACT The influence of short-term environmental challenges such as desiccation and temperature extremes on the cuticular hydrocarbon composition of insects is not well understood. We report here the effects of chilling (4°C), exposure to moderate relative humidity (≈30%), and exposure to dusts of silica gel, diatomaceous earth, and freeze-dried, ice-nucleating bacteria (*Pseudomonas syringae*) on the cuticular hydrocarbons of larvae and adults of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), a major pest of stored grain. Larvae and adult beetles have some hydrocarbons in common (particularly n-alkanes and small quantities of internal methyl branched alkanes), but they differ substantially in alkene composition. Unstressed larvae have ≈4% of Z-(9)-alkenes (C₂₅ to C₃₁) and 1% of a homologous series of Z,Z-(6,9)-dienes of the same chain lengths. Adult beetles, however, in unstressed situations have none of the larval dienes on their cuticle, and possess only ≈3% of the Z-(9)-monoenes. When the adult beetles were environmentally stressed for 24 h, they released up to 3% of phenotypically larval dienes onto their cuticle. In addition, desiccation stresses resulted in adults substantially increasing the quantity of monoenes on their cuticle (up to 5% of the total), whereas low temperature caused them to reduce the quantity of monoenes. Larvae exposed to similar stresses did not increase the quantity of dienes on their cuticle, but rather decreased their alkenes and increased the abundance of alkanes. The release of the dienes by the adults was shown not to be from new biosynthesis, but rather to result from release of stored dienes. The physiological and ecological ramifications of these changes in cuticular hydrocarbon profile by the various life stages of the sawtoothed grain beetle in response to environmental stresses are discussed.

KEY WORDS *Oryzaephilus surinamensis*, cuticle, stress physiology

CUTICULAR HYDROCARBONS OF insects serve a variety of physiological and ecological functions (Howard and Blomquist 1982, Howard 1993). One important function is water conservation (Edney 1977), and different life stages of a species occupying different habitats frequently have very different hydrocarbon compositions that reflect differing water conservation needs (Arnold et al. 1969, Arnold and Regnier 1975, Baker et al. 1979, Howard et al. 1990). When immature and adult forms occupy the same habitat, they tend not to show large differences in hydrocarbon composition (Howard and Blomquist 1982, Blomquist et al. 1987). Although it is well known that insects undergo marked changes in their cuticular hydrocarbon profiles during the first 48–72 h of adult life (Howard and Blomquist 1982, Blomquist et al. 1987), little is known of the effects of marked short-term (a few hours to a few days) environmental changes (stresses) on their cuticular hydro-

carbon composition. Such stresses for stored-product insects might include relatively large (roughly 15–20°C) temperature drops (or increases) (Fields 1992), exposure to substantial quantities of desiccating agents (such as diatomaceous earth or silica gel aerosols) (Quarles 1992a, b), or exposure to potential pathogens such as bacteria or fungi (Lee et al. 1992). We report here our findings on the effects of short-term stresses such as these on the cuticular hydrocarbons of larvae and adults of a major cosmopolitan stored-grain insect pest, the sawtoothed grain beetle. Distinct ontogenetic differences were observed in this insect in the absence of environmental stresses. In the presence of the stresses, the adult forms release dienes that are typical of the larval stage and are absent from unstressed adult forms. The intensity of this response varies with the particular stress, desiccation causing a greater response than low temperature. These phenotypic responses were shown to be time dependent, and partially reversible for the adults. These phenotypically larval dienes are not rapidly biosynthesized in response to the stress, but are stored internally and released as needed. Under these same environmental stresses the lar-

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vae do not express additional quantities of dienes, but tend to increase the proportion of alkanes on their cuticle. The monoenes also were reduced in those larvae exposed to silica gel, ice-nucleating bacteria, and low temperature.

Materials and Methods

Insects. All experiments used laboratory cultures of *Oryzaephilus surinamensis* (L.) established several years ago from Kansas populations and periodically refreshed with wild stock from Kansas. Insects were cultured in whole wheat flour enriched with 5% brewer's yeast and a small amount of rolled oats ($\approx 5\%$ of total mixture). Insects were isolated from culture media by sieving the media through standard 25- and 50-mesh sieves with gentle agitation, and then held for at least 30 min in glass petri dishes before any subsequent experimental manipulation.

Chemical Analyses. Cuticular hydrocarbons were isolated by extracting insects in three 1-ml portions of hexane for 1 min each, combining the extracts, concentrating the extract to ≈ 0.01 ml with nitrogen, and then chromatographing it over BioSil A (BioRad Laboratories, Richmond, CA) in a minicolumn contained in a Pasteur pipette, eluting with 2-column volumes of hexane (Howard et al. 1978). The hexane eluant was concentrated to dryness with nitrogen and then made up to a known volume for qualitative analysis by gas chromatography-mass spectrometry (GC-MS) or for quantitative analysis by gas chromatography with flame ionization detection (GC-FID). GC-MS analyses were conducted using either a Hewlett-Packard 5790A GC and HP5970A mass-selective detector with an HP 9133 data system or an HP5890 series II gas chromatograph coupled to an HP 5971 A mass-selective detector and an HP 486/33T workstation with HP chemstation software (Hewlett-Packard, Palo Alto, CA). Both mass selective detectors were operated in the electron impact mode at 70 eV. Capillary columns used were either a DB-5 (10 m by 0.19 mm) (J and W Scientific, Rancho Cordova, CA) or a Supelco SPWax-10 column (30 m by 0.32 mm) (Supelco, Bellefonte, PA). Samples were injected in a splitless mode, and the carrier gas was high purity helium at a head pressure of 3.5 kg/cm². The DB-5 column was temperature programmed from 150 to 320°C at 10°/min, with an initial hold of 2 min and a final hold of 30 min. The SP-Wax 10 column was temperature programmed from 150 to 260°C at 2°/min, with an initial hold of 2 min and no final hold period. Equivalent chain-length values for all hydrocarbons were calculated by comparison to retention times of a n-alkane standard mixture.

Analyses by GC-FID were conducted on a Shimadzu GC-14A chromatograph (Shimadzu Scientific Instruments, Columbia, MD) connected to a Shimadzu C-R4A workstation. Split injections were made on a Supelco SPWax-10 column (30 m

by 0.32 mm), temperature programmed in the same manner as the qualitative runs on GC-MS, and used ultrapure helium as the carrier gas. All GC-FID analyses used a quantitative internal standard of n-eicosane. Statistical analyses in which the data were expressed as percentage values were conducted using arcsin square-root transformed values, but reported means are of the untransformed data. Statistical analyses in which the data are expressed in absolute units (nanogram per insect) did not involve any prior transformations.

Location of Double Bonds in Alkenes and Alkadienes. Double-bond locations in alkenes and alkadienes were obtained by preparing dithiome-thyl ethers (Francis and Veland 1981) and examining their mass spectra. Stereochemistry of underivatized alkenes and alkadienes was established from Fourier transform infrared analyses. Fourier transform infrared vapor-phase spectra were obtained on a Hewlett-Packard 5890 GC with a 5965B FTIR detector and a 7958A data system. A SPWax-10 capillary column (30 m by 0.32 mm) using chromatographic conditions identical to those described above was used. Confirmation that the dienes contained no other functional group was established by conducting a microhydrogenation using the method of Schwartz et al. (1972) followed by mass spectrometry of the reaction products.

Experimental Treatments. Experiment 1: Effects of Environmental Stress on Hydrocarbon Profiles. Insects were sieved from the culture media, held for 30 min in glass petri dishes, and either immediately placed in hexane (control treatment 1), held 24 h in the petri dish at 30°C and $\approx 30\%$ RH and placed in hexane (control treatment 2), or placed in a glass petri dish with either 1 g of amorphous silica gel (treatment 1), diatomaceous earth (treatment 2), or a freeze-dried, ice-nucleating *Pseudomonas syringae* bacteria preparation known as sno-max (Snomax, Division Genencor, Rochester NY) (treatment 3). These 3 experimental treatments were also held at 30°C and $\approx 30\%$ RH for 24 h. An additional treatment (No. 4) involved placing the insects in a glass petri dish and holding them for 24 h in an incubator held at 4°C for 24 h. The insects from the 4 treatments containing particulate matter were removed from their petri dishes, gently shaken in a 50-mesh sieve to dislodge as many adhering particles as possible, and then extracted in hexane as described above. All extracts were then analyzed by GC-MS and GC-FID. In addition, 0.1-g portions of the silica gel, diatomaceous earth, and freeze-dried bacteria were extracted with hexane, chromatographed over BioSil A and analyzed by GC-MS to confirm that they did not contain any contaminating hydrocarbons.

Experiment 2: Ability to Recover from Environmental Stress. In this experiment, 100 adult *O. surinamensis* were held at 30°C and $\approx 30\%$ RH without food for 48 h (the stress period) and were

Table 1. Cuticular hydrocarbons of larval and adult *O. surinamensis* with diagnostic data used to identify hydrocarbons

Compound	Equivalent chain length		Diagnostic EI-MS ions ^a
	DB-5	SPWax-10	
C21	21.00	21.00	296
C22	22.00	22.00	310
C23	23.00	23.00	324
C24	24.00	24.00	338
C25	25.00	25.00	352
Z-9-C25:1	24.74	25.09	350; [173, 271; 444]
C26	26.00	26.00	366
C27	27.00	27.00	380
11-,13-MeC27	27.32	27.16	169, 253, 379; 197, 225, 379
7-MeC27	27.40	27.25	113, 309, 379
5-MeC27	27.50	27.35	85, 337, 379
3-MeC27		27.65	365, 337, 379
Z,Z-6,9-C27:2	26.68	27.54	376; [131, 371, 323; 299, 203, 155; 502]
Z-9-C27:1	26.72	27.10	378; [173, 299; 472]
C28	28.00	28.00	394
C29	29.00	29.00	408
11-,13-MeC29	29.31	29.16	169, 281, 407; 197, 253, 407
Z,Z-6,9-C29:2	28.68	29.59	404; [131, 399, 351; 327, 203, 155; 530]
Z-9-C29:1	28.72	29.12	406; [173, 327; 500]
C30	30.00	30.00	422
C31	31.00	31.00	436
11-,13-M3C31	31.31	31.26	169, 309, 435; 197, 281, 435
Z,Z-6,9-C31:2	30.68	31.63	432; [131, 427, 379; 355, 203, 155; (558) ^b]
Z-9-C31:1	30.72	31.14	434; [173, 355; 528]
C32	32.00	32.00	450
C33	33.00	33.00	464
11-,13-MeC33	33.31	33.19	169, 337, 463; 197, 309, 463
C35	35.00	35.00	492
11-,13-MeC35	35.33	35.19	169, 365, 491; 197, 337, 491

^a Ions in brackets are for dimethylsulfide derivatives of alkenes.

^b Ion not observed.

placed in petri dishes containing 5 g of dietary media, held for 8 h, 1, 3, and 10 d (the recovery period) at 30°C and 70% RH, and were extracted as described above for analysis by GC-MS. Three replicate dishes were set up for each treatment interval. An internal standard of 1 heptadecene was used to convert total ion current areas of alkenes and dienes into nanogram values for statistical analysis.

Experiment 3: Source of Dienes in Adults. Unstressed adult *O. surinamensis* were placed in a 100-ml glass beaker and killed by pouring liquid nitrogen over them. When the liquid nitrogen had evaporated (≈5 min), the beetles were extracted 3 times with hexane, chromatographed over BioSil A, and analyzed by GC-MS.

Statistical Methods. All experiments were replicated 5 times unless otherwise indicated. Data were analyzed by 1-way analysis of variance using Statgraphics Plus for personal computers (Manugistics 1992). Proportional data were arcsine square-root transformed before analysis, but untransformed data were used to generate percentage means reported in the tables.

Voucher Specimens. Voucher specimens have been deposited in the research collection of the Department of Entomology, Kansas State University, Manhattan.

Results

Identification of Cuticular Hydrocarbons.

The cuticular hydrocarbons of the sawtoothed grain beetle are dominated by n-alkanes with chain lengths from C₂₁ to C₃₅ (major components C₂₅ to C₃₃), lesser amounts of monoenes and dienes (stage specific) with chain lengths of C₂₅ to C₃₁ and even lesser amounts of 3-, 5-, 7-, 11-, and 13-monomethylalkanes of chain lengths C₂₇ to C₃₅ (Table 1). The monoenes and dienes were inadequately resolved on the 10 m nonpolar DB-5 column, and, therefore, quantitative analyses were conducted using a Supelcowax-10 polar column, which readily separated not only the alkenes but also all the saturated hydrocarbons. The most striking differences between unstressed larval and adult cuticular hydrocarbons were found in the alkene fraction. Larvae contain both Z-(9)-monoenes and Z,Z-(6,9)-alkadienes whereas unstressed adults contain only the Z-(9)-monoenes on their cuticle. Because the dienes eluted unexpectedly close in retention time to the corresponding monoenes on the nonpolar DB-5 column, experiments were conducted to verify that they were normal dienes and not branched ones, or that they did not contain additional functionality. Hydrogenation yielded only n-alkanes, ruling out either of these alternative possibilities. Confirmation of the location of the double bonds

was achieved by preparing the thiomethyl derivatives. Monoenes gave the expected addition products whose mass spectra are straightforwardly interpreted (Francis and Veland 1981) and the dienes yielded heterocyclic products whose mass spectra were diagnostic for 6,9-dienes (Vicenti et al. 1987). Verification of stereochemistry was achieved by examination of the FT-IR spectra of the dienes, and only evidence for Z-stereochemistry was found (absence of a strong band at 970 cm^{-1} and presence of a weak-to-moderate band at 1650 cm^{-1}) (Nakanishi 1962).

Hydrocarbon Compositions as a Function of Environmental Stresses. Tables 2 and 3 list the mean percentage of compositions of adult and larval sawtoothed grain beetle, respectively. For both stages there are significant compositional differences between beetles taken directly from stock cultures (control group) and those held in petri dishes and exposed to either desiccating conditions or low temperatures. For the adults, 15 of the components were significantly different from the control ($P \leq 0.05$), whereas for the larvae, 17 of the components were significantly different from the control ($P \leq 0.05$). Because these differences occurred across all 4 hydrocarbon types, hydrocarbon components were pooled by type (alkanes, alkenes, monoenes, and dienes) and statistically compared.

Figures 1 and 2 show how the various treatments affected the cuticular hydrocarbon classes of the adults and larvae, respectively. For adults, the alkanes were unaffected by the low temperature, whereas all the desiccating treatments (including that of simple exposure to low relative humidity [treatment C2]) resulted in a significant reduction in the proportion of alkanes, diatomaceous earth having the greatest effect (Fig. 1A). Correspondingly, as the alkanes went down in relative abundance, the alkenes increased in importance, except in the low temperature treatment (Fig. 1B). The major contributors to these changes in the total alkenes were the dienes, where all treatments were significantly different from the control group (Fig. 1D). Only the diatomaceous earth treatment also had a significant increase in monoenes (Fig. 1C). Somewhat different trends were found for the larvae (Fig. 2). Here, the diatomaceous earth treatment and the treatment of holding the larvae without food at 30% RH did not differ significantly from the control group in terms of either alkanes or alkenes. In contrast, the ice-nucleating bacteria, silica gel, and low temperature treatments did all differ significantly from the controls. In particular, for these 3 treatments, the proportion of alkanes increased and the proportion of alkenes decreased (Fig. 2 A and B). Unlike with the adult stage, however, the alkene component that significantly decreased was the monoenes, and not the dienes (Fig. 2 C and D).

Recovery of Normal Hydrocarbon Profiles. When adults were replaced into their normal cul-

ture media conditions after having been stressed for 48 h at 30% RH without food, and then followed with time to see if they returned to an unstressed hydrocarbon profile (Fig. 3), clearly they only partially did so after 10 days of recovery. Indeed, they seemed to continue to release the larval dienes for 24 h after being removed from the stress and only then to begin to replace the dienes with the typical adult hydrocarbon components (Fig. 3).

Source of Adult Dienes. Because adults taken directly out of culture jars have no detectable dienes on their cuticle, the question of the source of these stress-related dienes was of some interest. Two hypotheses were considered. Dienes could be the result of de novo biosynthesis in response to the environmental stress, or they could be stored in the adult and released to the cuticle only when needed. To distinguish between these 2 hypotheses, adult beetles taken directly out of the culture jar were immersed in liquid nitrogen, extracted, and their hydrocarbons examined by gas chromatography. More dienes were present in these extracts than in any of our previous experiments ($\approx 35\%$ of total hydrocarbons), clearly showing that the dienes must be stored internally, for the liquid nitrogen instantly kills the tissues, precluding any biosynthesis. The ratio of the monoenes to dienes in these extracts were approximately the same as for the cuticular extracts from stressed beetles. The internal storage sites of these lipids is unknown.

Discussion

The cuticular hydrocarbons of well over 100 insect species have now been described (Lockey 1988, Howard 1993), including many stored product insects. The closest ecological analogue of *O. surinamensis* for which cuticular hydrocarbons have been described (Howard 1992) is another cucujid beetle, *Cryptolestes ferrugineus* (Stephens); the 2 species are frequently sympatric. Both species are granivorous, and the larvae of both are internal seed feeders. The adults are external seed feeders and are most frequently found in the upper 20% of the bulk of stored grain (Hagstrum 1989). Despite these similarities in their niches, the cuticular hydrocarbons of *C. ferrugineus* and *O. surinamensis* differ substantially, both in chemical identity and in ontogenetic relationships. Whereas *O. surinamensis* is characterized by a predominance of n-alkanes, a very low abundance of methyl-branched alkanes, and only moderate abundances of alkenes, *C. ferrugineus* is characterized by nearly equal quantities of n-alkanes and alkenes and moderate abundances of methyl-branched alkanes. Furthermore, although both species have Z-(9)-monoenes, they differ markedly in the nature of their dienes, *O. surinamensis* having Z,Z-(6,9)-dienes and *C. ferrugineus* having Z,Z-(8,22)-, Z,Z-(9,23)-, and Z,Z-(9,25)-dienes (Howard 1992). Furthermore, the ontogenetic re-

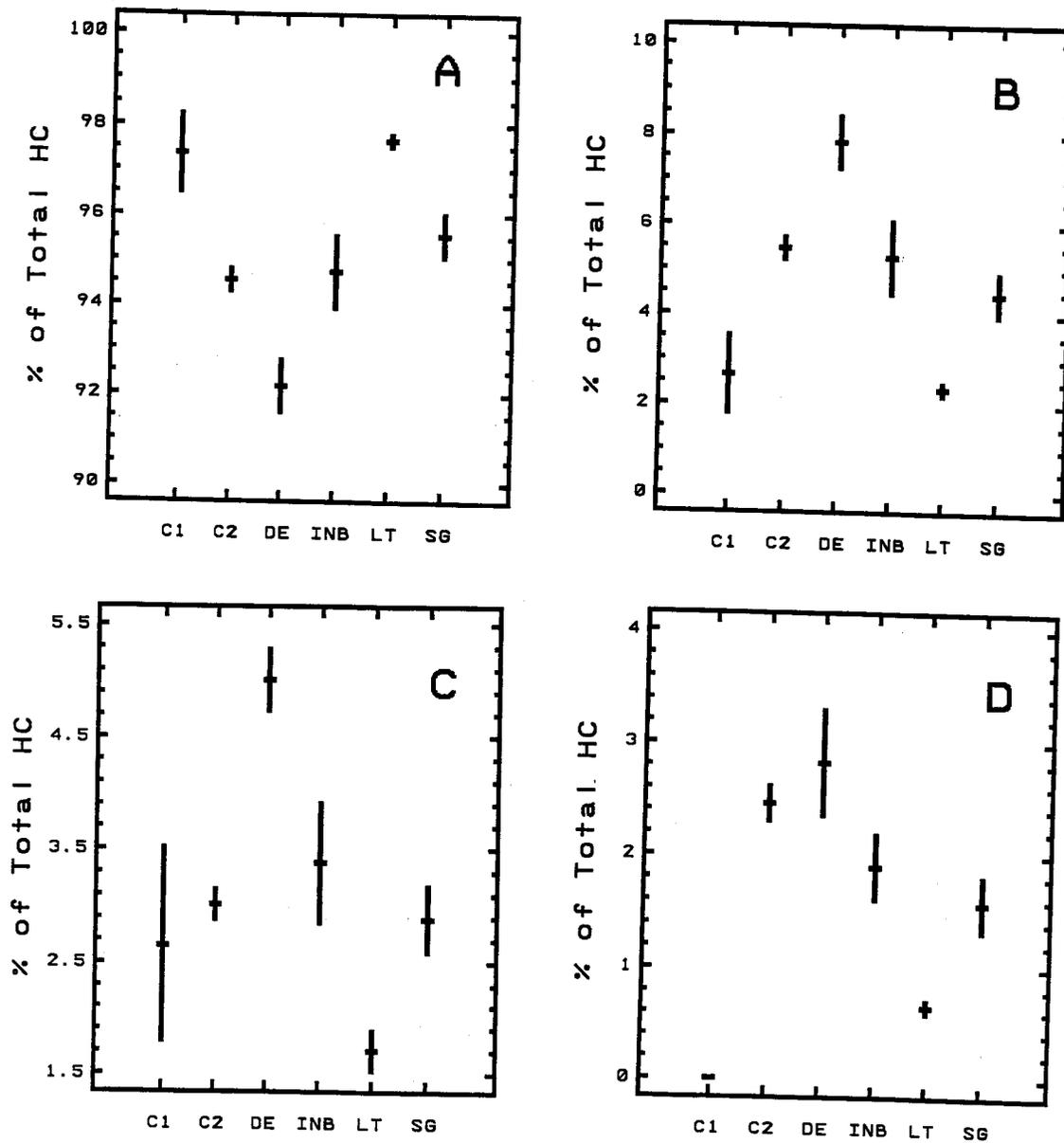


Fig. 1. Mean (SE) values of cuticular hydrocarbon classes of adult *O. surinamensis* exposed to various short-term environmental stresses. A, alkanes; B, alkenes; C, monoenes; D, dienes. Treatments groups: C1, beetles taken directly from culture jar; C2, beetles held at room temperature and $\approx 30\%$ RH for 24 h, DE, diatomaceous earth; INB, ice-nucleating bacteria; LT, low temperature; SG, silica gel.

relationships of cuticular hydrocarbon profiles among the stages of each species are fundamentally different. *O. surinamensis* larvae are characterized by the presence of the Z,Z-(6,9)-dienes, whereas the adults, unless stressed, do not have these dienes as a normal part of their cuticular composition. In contrast, larvae and adults of *C. ferrugineus* possess the same cuticular hydrocarbons and in approximately the same relative proportions

(Howard 1992; R.W.H., unpublished data).

Beyond these basic chemical compositional differences, preliminary unpublished experiments show that *C. ferrugineus* adults (larvae were not examined) show little obvious change in their cuticular hydrocarbon profiles when exposed to desiccants or low temperatures. This striking difference between *C. ferrugineus* and *O. surinamensis*

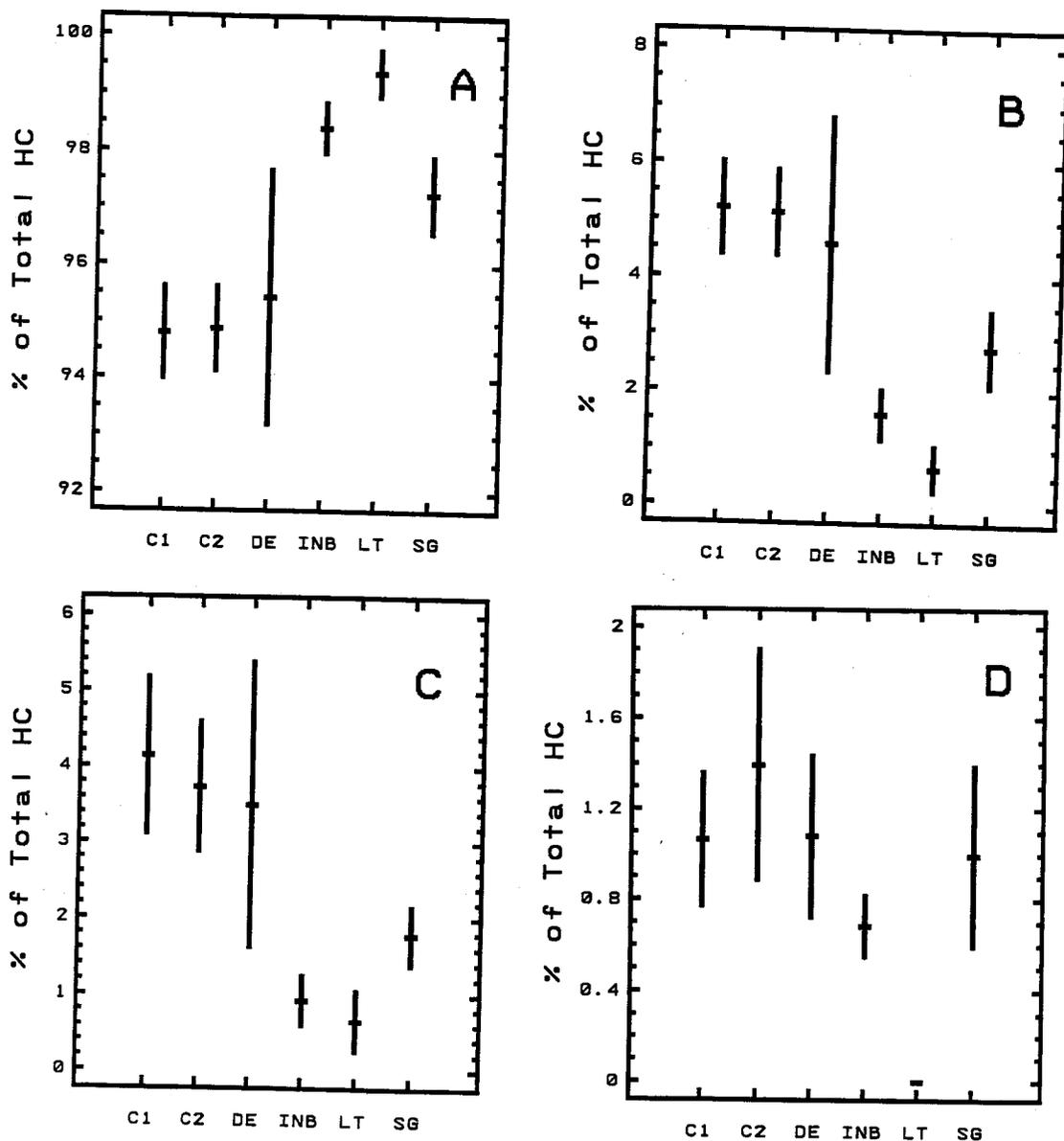


Fig. 2. Mean (SE) values of cuticular hydrocarbon classes of larval *O. surinamensis* exposed to various short-term environmental stresses. A, alkanes; B, alkenes; C, monoenes; D, dienes. Treatments groups: C1, beetles taken directly from culture jar; C2, beetles held at room temperature and $\approx 30\%$ RH for 24 h, DE, diatomaceous earth; INB, ice-nucleating bacteria; LT, low temperature; SG, silica gel.

is intriguing and will require further study to ascertain whether it reflects basic phylogenetic differences or whether unknown alternative phenotypic responses of *C. ferrugineus* to environmental stresses remain to be discovered.

The effects of environmental stresses on cuticular permeability and cuticle biochemistry of insects and other arthropods have been extensively investigated (Neville 1975, Edney 1977, Hadley 1984), but most studies have dealt with single life-stages and with long-term seasonal effects. In ad-

dition, many older studies were conducted before adequate instrumentation was available to examine critically changes in cuticular chemistry. Despite this, many important findings were made that warrant discussion here. These findings may be grouped under the general headings of the effects of temperature and seasonality, relative humidity, dessicants, and fungal infections.

In an early study on the effects of temperature on insect hydrocarbon composition, Hadley (1977, 1978) examined cuticular permeability and hydro-

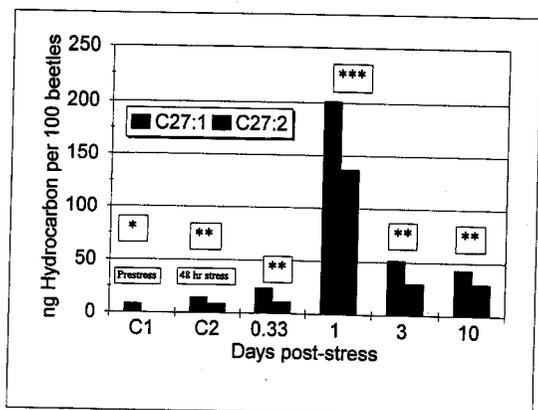


Fig. 3. Mean values of cuticular $C_{27:1}$ and $C_{27:2}$ of adult *O. surinamensis* exposed to desiccation for 48 h and then returned to normal culture conditions. Bars with the same symbol above them are not statistically different at $P = 0.05$.

carbon compositions of 5 species of desert tenebrionid beetles taken in either the summer or winter, and then acclimated for several weeks to the alternative seasonal regime. Warm-acclimated and summer-active beetles contained greater quantities of hydrocarbon and had distributions with a greater overall average chain length than those from cold-acclimated or winter-active beetles. All hydrocarbon components in these desert beetles were saturated and varied by species with chain lengths of 26–44 carbons. The percentage of n-alkanes varied from 7.7 to 78.5% among the various species. Despite this tremendous variability in the ratio of normal to branched alkanes among species, all 5 desert species showed far lower rates of water loss with time than comparable arthropods from moist habitats (Hadley 1978). Toolson and Hadley (1979) extended these studies to examine year round seasonal changes in lipid composition and cuticular permeability in the desert scorpion *Centruroides sculpturatus* Ewing. The hydrocarbons of scorpions collected in fall, winter, and early spring were characterized by higher proportions of shorter n-alkanes, whereas the scorpions collected in spring and summer had predominantly long-chain branched alkanes. Correspondingly, water loss rates for winter scorpions were substantially greater than for those of summer scorpions, and Toolson and Hadley (1979) postulate that the longer hydrocarbons found on the summer scorpions have greater Van der Waal interactions with each other than do the hydrocarbons on the cooler weather scorpions, resulting in a greater barrier to water loss. These authors note, however, that they and other workers could show only a weak correlation between cuticular permeability and hydrocarbon epicuticular composition in many species (Hadley and Jackson 1977; Toolson and Hadley 1977, 1979; Hadley 1978) suggesting that other layers of the

cuticle are also important in the total water conservation mechanisms of the animals.

Although many desert arthropods possess only saturated alkanes on their cuticle, some desert dipterans have been shown to possess appreciable quantities of long-chain unsaturated cuticular hydrocarbons (Toolson 1982, Toolson et al. 1990) and these compounds also vary with temperature and seasonality. *Drosophila pseudoobscura* Frolova adults have a complex mixture of normal and methyl-branched alkanes, alkenes, and alkadienes with chain lengths of 21–33 carbons (Toolson 1982), but the exact mixture varied according to the temperature of the rearing regime the pupae (but not larvae) were exposed to. Adults arising from pupae held at 17°C had more short-chain methyl-branched alkanes and short-chain alkadienes than adults arising from pupae held at 24°C whose hydrocarbon profiles were dominated by longer-chain methyl-branched alkanes and longer-chain alkadienes. Water loss measurements agreed with these differences, the flies raised at lower temperatures showing greater water loss rates than the flies raised at higher temperatures. Toolson (1982) argues that the exposure of the fly pupae to the higher temperatures leads to an adaptive response in which the adults have a cuticular hydrocarbon composition suitable for hot desert temperatures and low relative humidity. Although these flies also had slightly greater absolute quantities of hydrocarbons on their cuticle, Toolson felt that this was of only minor significance (Toolson 1988).

Temperature effects were also observed on the cuticular hydrocarbon composition of another desert fly, *Drosophila mojavensis* Patterson & Crow. The cuticular hydrocarbons of this fly contain 23–39 carbon atoms; >96% of the mixture were of chain lengths of C_{29} – C_{37} , and a substantial portion of the compounds were highly unusual symmetrical dienes (Toolson et al. 1990). When newly emerged adults (all held at 24°C as pupae) were held for 8 d at 17, 24, or 34°C, the proportion of the long chain hydrocarbon components also increased (Markow and Toolson 1990). A complicating factor in this scenario is that freshly collected wild populations of *D. mojavensis* consistently had lower absolute abundances of cuticular hydrocarbons than did laboratory cultured material (Toolson et al. 1990) (see below).

Although most studies have focused on temperature effects, relative humidity is also an environmental parameter of some significance to water relationships in an arthropod. Perhaps the most dramatic example of this occurs in the studies of Toolson and Kuper-Simbrón (1989) with *D. pseudoobscura*. They found that newly captured wild flies had cuticular hydrocarbon compositions dominated by branched alkanes and <1% alkadiene. Within 1 generation in laboratory culture the alkadienes had become the dominant cuticular hydrocarbon components, although it took >2 yr be-

fore the laboratory cultures settled down to a consistently alkadiene-dominated hydrocarbon composition. Wild *D. pseudoobscura* live in a microhabitat that ranges between 25 and 40% RH, whereas the standard *Drosophila* laboratory culture media has a 95–98% RH. This shift in hydrocarbon phenotype was shown to be a result of gene frequency change and not simple acclimation. Although not so dramatic, Toolson (1988) also found that different wild populations of *D. pseudoobscura* living in similar temperature regimes but differing relative humidity regimes also had different cuticular hydrocarbon profiles that fit the water deficit environment that they were in. Hadley (1979) has also reported on a desert tenebrionid whose body color depends on the relative humidity at which it is held. This insect, *Cryptoglossa verrucosa* (LeConte), gets its color from the physical structure of the waxy hydrocarbon coating on its cuticle, the low relative humidity leading to the production of a highly complex mesh of wax filaments that appear blue, whereas high relative humidity leads to discrete droplets of wax that appear black. Although little actual chemistry was presented, it appears that the percentage of composition of the secretion in the 2 color phases is very similar.

Although desiccants have been used for insect control for some time (Quarles 1992a, b), very few studies actually examine possible changes in cuticular hydrocarbon composition as a function of the desiccants. Baker et al. (1978) examined the effects of tricalcium phosphate on adult *Tribolium castaneum* (Herbst) either directly applied as a dust or incorporated into a dietary media. Although dusting the beetles with the chemical led to a 55% loss of hydrocarbon mass, most of this was accounted for by absorption by the alkenes and alkadienes associated with the paired prothoracic and abdominal glands (Howard 1987 and references therein). The cuticular hydrocarbons of this species are all saturated, and to the extent that the tricalcium phosphate dust affected them, it was by slightly increasing the proportion of n-alkanes and reducing the proportion of branched alkanes (Baker et al. 1978). When the desiccant was mixed in the insect diet, an even larger effect on hydrocarbons was seen, with a 74% reduction in the glandular alkenes and alkadienes, and a marked increase in cuticular hydrocarbons, especially heptacosane. Significantly, when the beetles were held at low relative humidity without any physical desiccants, no changes in their hydrocarbon composition were observed. Baker (1978) also examined the effects of tricalcium phosphate on the larvae of the black carpet beetle, *Attagenus megatoma* (F.). This insect is less susceptible to desiccation with dusts than many other stored product insects, and indeed, hydrocarbons make up only ≈12% of its cuticular lipids, which consist of 98% n-alkanes, 2% branched alkanes, and no alkenes. As might be expected, the tricalcium phosphate dusts did not af-

fect the hydrocarbon profiles of this insect (Baker 1978).

Besides serving as a major line of defense against the loss of body water, arthropod cuticle also serves as a first line of defense against invasion by fungal and other microbial pathogens (Neville 1975). As with desiccants, little attention has been paid to possible changes in hydrocarbon composition in the presence of such biotic agents. One intriguing study is that of Lecouana et al. (1991); the cuticular hydrocarbons of 2 lepidopteran larvae, *Ostrinia nubilalis* (Hübner) and *Melolontha melolontha* L., were challenged with several virulent and avirulent strains of the fungal pathogen *Beauveria bassiana* (Bals.) Vuill. They found that within 6 h of applying the fungal spores several monomethyl alkanes on the larval cuticle disappeared completely; within 72 h up to 86% of the hydrocarbons of *O. nubilalis* and 51% of the hydrocarbons of *M. melolontha* were gone. The compositional changes were complex, and the authors indicate that much remains to be learned about how these transformations are achieved.

As noted by Toolson and Kuper-Simbrón (1989), arthropod cuticular hydrocarbon compositions can be highly labile and sensitive to a variety of environmental influences. The exact extent of this lability is in our judgment not sufficiently appreciated. Our results, however, support the contention that the insects are capable of relatively rapid responses to environmental challenges. We have not found reports of other insects in which a larval-specific hydrocarbon is stored in the adult stage and transported to the cuticle for combating environmental stresses. We suspect this is simply a reflection of insufficient examination rather than anything special about the sawtoothed grain beetle.

As indicated above, larvae and adults of *O. surinamensis* respond differently to low relative humidity, desiccants, and low temperatures in terms of alterations in their cuticular hydrocarbon profiles. These differences may be related to the fact that larvae are internal seed feeders and restricted in their choices of habitat, whereas the adults are external seed feeders and can readily disperse to more favorable habitats. A striking feature of the larval sawtoothed grain beetle response to low relative humidity and physical desiccants is that they substantially increase the n-alkanes, particularly n-C₂₉ and n-C₃₁ and reduce the proportions of the unsaturated components (Table 3). This should have the effect of reducing cuticular permeability and, therefore, internal body water loss (Toolson 1982). The larval responses to low temperatures are similar except that besides reducing the alkenes they also reduce most of the n-alkanes, except for n-C₂₉ and n-C₃₁, which they markedly increase. These changes should increase their resistance to water loss even more. Adult *O. surinamensis* respond to these stresses by markedly increasing their unsaturated components (particularly the dienes) while moderately decreasing the n-

Table 2. Percentage of composition of cuticular hydrocarbons of adult *O. surinamensis* exposed to various environmental stresses (*n* = 5)

Compound	Control 1	24-h control	DE	INB	LT	SG
C21	1.45	0.34	0.93	0.25	0.69	0.41
C22	0.81	0.33	0.60	0.23	0.29	0.27
C23	1.05	0.42	1.14	0.35	0.49	0.39
C24	0.69	0.36	0.84	0.39	0.31	0.36
C25	2.38	1.36	2.49	1.34	2.04	1.47
Z-9-C25:1	<0.01	<0.01	<0.01	0.07	<0.01	<0.01
C26	0.88	0.57	1.12	0.67	0.63	0.64
C27	11.83	10.66	14.00	12.16	11.39	11.08
11-,13-MeC27	<0.01	0.08	0.29	<0.01	<0.01	<0.01
7-MeC27	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
5-MeC27	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3-MeC27	0.23	0.29	0.20	0.64	0.32	0.34
Z,Z-6,9-C27:2	<0.01	0.98	1.00	0.50	0.33	0.52
Z-9-C27:1	0.98	1.62	2.22	1.27	0.73	1.18
C28	1.15	0.99	1.56	0.83	1.13	1.06
C29	17.07	19.84	18.37	19.91	17.85	19.04
11-,13-MeC29	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Z,Z-6,9-C29:2	<0.01	1.12	1.49	0.90	0.32	0.92
Z-9-C29:1	0.72	0.88	1.83	1.23	0.51	1.06
C30	2.71	2.80	3.19	2.89	3.01	2.80
C31	51.17	52.80	43.17	51.59	54.76	53.56
11-,13-MeC31	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Z,Z-6,9-C31:2	<0.01	0.36	0.34	0.50	<0.01	0.13
Z-9-C31:1	0.95	0.51	0.96	0.81	0.46	0.64
C32	2.14	1.04	1.48	0.97	1.57	1.11
C33	3.79	2.65	2.79	2.50	3.15	3.00
11-,13-MeC33	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
C35	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
11-,13-MeC35	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Control 1 (C1) taken immediately from culture jar; 24-h control (C2) beetles held at room temperature and ≈30% RH for 24 h. DE, diatomaceous earth; INB, ice nucleating bacteria; LT, low temperature; SG, silica gel.

Table 3. Percentage of composition of cuticular hydrocarbons of larval *O. surinamensis* exposed to various environmental stresses (*n* = 5)

Compound	Control 1	24-h control	DE	INB	LT	SG
C21	0.30	0.72	1.02	0.62	0.11	0.26
C22	0.83	0.40	0.56	0.44	0.22	0.34
C23	1.84	1.30	0.96	1.63	0.51	0.81
C24	1.14	0.44	0.97	0.54	0.29	0.44
C25	7.55	5.21	3.13	5.47	2.51	3.76
Z-9-C25:1	0.87	0.23	0.16	0.23	<0.01	0.08
C26	1.58	0.75	1.54	0.72	0.55	0.68
C27	17.48	11.02	11.83	15.04	10.85	13.00
11-,13-MeC27	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
7-MeC27	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
5-MeC27	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3-MeC27	0.05	<0.01	<0.01	0.20	0.04	0.03
Z,Z-6,9-C27:2	0.14	0.27	<0.01	0.41	0.13	0.39
Z-9-C27:1	2.15	2.38	0.49	1.99	0.40	0.94
C28	2.58	1.02	1.92	1.24	1.09	1.31
C29	18.24	19.38	19.69	23.50	24.63	24.10
11-,13-MeC29	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Z,Z-6,9-C29:2	0.56	0.46	<0.01	0.31	0.17	0.47
Z-9-C29:1	0.56	0.59	<0.01	0.65	0.12	0.31
C30	3.95	3.39	4.17	3.05	3.71	3.49
C31	33.82	47.92	47.95	40.50	50.49	45.64
11-,13-MeC31	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Z,Z-6,9-C31:2	0.36	0.66	<0.01	0.37	0.38	0.14
Z-9-C31:1	0.56	0.52	<0.01	0.63	0.32	0.45
C32	2.31	0.91	1.82	0.68	0.88	0.98
C33	3.12	2.42	3.79	1.77	2.54	2.37
11-,13-MeC33	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
C35	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
11-,13-MeC35	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

See Table 2 for abbreviations.

alkanes. Unlike the larvae, the longer chain n-alkanes do not shift disproportionately to the other n-alkanes, and in the presence of low temperature the n-alkanes do not seem to alter significantly at all (Table 2).

Relating these changes to a prevention of internal body water loss is more difficult because it is usually thought that both methyl branching and unsaturation decrease the strength of Van der Waal bonding among the hydrocarbons, increasing their fluidity and increasing permeability (Toolson 1982). Other instances of this shift to unsaturated hydrocarbons while still maintaining protection against water loss have been reported (Toolson and Kuper-Simbrón 1989, Markow and Toolson 1990), although the alkenes in question in those 2 studies were of much greater total chain length. Their insects were also desert flies, and the degree of aridity and extremes of temperature to which they were exposed were greater than those experienced by stored grain insects. Indeed, most stored grain insects would not normally experience maximal temperatures in excess of 35–40°C or relative humidity <70–80%. Two alternative hypotheses for the increase in unsaturated hydrocarbons on adult cuticles when stressed by desiccants is that the insect is attempting to wash the particulate matter off its cuticle with the more fluid unsaturated hydrocarbons, or that the more fluid cuticular surface would show greater resistant to abrasion.

An unexpected feature in our results for both larvae and adults was the differential responses to the various particulate desiccants. We do not have a completely satisfactory explanation for our observations, but the data perhaps reflect differing particle sizes and relative absorptivity, as well as different surface area relationships between larvae and adult beetles. The freeze-dried (dead) bacteria are a fine powder and we do not think that the insects are responding to them as bacteria, but rather as particulate matter. Additional experiments examining particle size, dose, and insect response time will be needed before we can understand insect responses to these desiccants and relate them to natural events of ecological and evolutionary significance.

Although we considered it unlikely that our laboratory cultures would differ in hydrocarbon composition from freshly collected wild populations to the extent that the flies of Toolson and Kuper-Simbrón (1989) did, we recognized that this was a possibility. We have now examined freshly captured wild *O. surinemensis* adults and their hydrocarbon profiles match closely those of our laboratory cultured material. The biochemical regulatory pathways that govern larval versus adult phenotypes are intriguing and require further examination. Are the alkadienes carried over from earlier life stages, or are they made as adults and just not expressed except under stress? No matter when they are made, where are they stored in the adult insect? And what are the ranges of environmental cues that

trigger their release? Perhaps the greatest need in future studies is to recognize that insect cuticular hydrocarbons in the same macro-environment do show extreme variation on an individual basis, and that the underlying factors that cause this variation need careful examination. Toolson has reported (1982) coefficients of variation of 25–50% for interindividual water loss rates in nearly all arthropods examined to date. We suspect that similar coefficients of variation would also be found for many cuticular hydrocarbon compositions unless some driving force such as semiochemical function transcends other basic physiological parameters to minimize phenotypic variation (Howard and Blomquist 1982, Howard 1993).

Acknowledgments

We thank G. Blomquist (University of Nevada), D. Nelson (USDA-ARS), and L. Seitz (USDA-ARS) for critical comments on an earlier version of the manuscript. We also thank P. Fields (Agriculture Canada) for his generous gift of the ice-nucleating bacteria.

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Received for publication 18 November 1994; accepted 15 February 1995.