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Cite this article: Hatano E, Wada-Katsumata A, Schal C. 2020 Environmental decomposition of olefinic cuticular hydrocarbons of *Periplaneta americana* generates a volatile pheromone that guides social behaviour. *Proc. R. Soc. B* **287**: 20192466.
http://dx.doi.org/10.1098/rspb.2019.2466

Received: 22 October 2019
Accepted: 5 February 2020

Subject Category:
Behaviour

Subject Areas:
behaviour, ecology, environmental science

Keywords:
Periplaneta americana, American cockroach, cuticular hydrocarbons, environmental reaction, habitat selection, pro-semiochemicals

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Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.4853601.

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Environmental decomposition of olefinic cuticular hydrocarbons of *Periplaneta americana* generates a volatile pheromone that guides social behaviour

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Once emitted, semiochemicals are exposed to reactive environmental factors that may alter them, thus disrupting chemical communication. Some species, however, might have adapted to detect environmentally mediated breakdown products of their natural chemicals as semiochemicals. We demonstrate that air, water vapour and ultraviolet (UV) radiation break down unsaturated cuticular hydrocarbons (CHCs) of *Periplaneta americana* (American cockroach), resulting in the emission of volatile organic compounds (VOCs). In behavioural assays, nymphs strongly avoided aggregating in shelters exposed to the breakdown VOCs from cuticular alkenes. The three treatments (air, water vapour, UV) produced the same VOCs, but at different time-courses and ratios. Fourteen VOCs from UV-exposed CHCs elicited electrophysiological responses in nymph antennae; 10 were identified as 2-nonanone, 1-pentanol, 1-octanol, 1-nonanol, tetradecanal, acetic acid, propanoic acid, butanoic acid, pentanoic acid and hexanoic acid. When short-chain fatty acids were tested as a mix and a blend of the alcohols and aldehyde was tested as a second mix, nymphs exhibited no preference for control or treated shelters. However, nymphs avoided shelters that were exposed to VOCs from the complete 10-compound mix. Conditioned shelters (occupied by cockroaches with faeces and CHCs deposited on the shelters), which are normally highly attractive to nymphs, were also avoided after UV exposure, confirming that breakdown products from deposited metabolites, including CHCs, mediate this behaviour. Our results demonstrate that common environmental agents degrade CHCs into behaviourally active volatile compounds that potentially may serve as necromones or epideictic pheromones, mediating group dissolution.

1. Introduction

Mobile animals must assess habitat conditions to secure resources, such as shelter, food or mates, and avoid risks, such as predators, parasites or pathogens. Olfactory cues (semiochemicals) play a prominent role in habitat assessment because they convey information downwind before the receiver contacts the potentially hazardous source [1]. For many species, aggregation pheromones—semiochemicals emanating from conspecifics—signal that a resting or feeding site may be suitable [2]. On the other hand, epideictic (marking) pheromones and necromones convey risk of competition [3], hazards [4] and potentially even cannibalism [5].

Once excreted, volatile pheromones are exposed to a harsh atmosphere that may degrade the pheromone before it reaches a receiver. For instance, the honeybee Nasonov pheromone [6] is readily oxidized in air, and even plant volatile organic compounds (VOCs), like terpenes, may polymerize or oxidize [7]. Thus, exposure to environmental factors that degrade semiochemicals is considered detrimental to communication.

Nonetheless, because chemical communication has evolved under the same harsh atmospheric conditions for millions of years, it is possible that insects

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evolved adaptive responses. Indeed, it has been shown that behaviourally inactive insect-produced chemicals are activated and mediate attraction upon exposure to environmental factors. For instance, air oxidation of the unsaturated cuticular hydrocarbons (CHCs) of female yellowheaded spruce sawfly, *Pikonema alaskensis*, produced (*Z*)-10-nonadecenal which was highly attractive to males [8]. Ozonolysis of CHCs of the wheat stem sawfly, *Cephus cinctus*, also produced the attractant 9-acetyloxynonanal which yielded large trap catches of males and females in the field [9]. The authors speculated that attractive VOCs might be formed by natural oxidation of double bonds of unsaturated CHCs [9]. Other volatile oxidation products with similar pheromonal activity were also reported in other insect species (*Macrocentrus grandii* [10], *Anoplophora glabripennis* [11] and *Drosophila melanogaster* [12]). Therefore, environmentally mediated production of volatile semiochemicals from CHCs demonstrates that some species exploit seemingly harsh environmental factors in their chemical communication.

CHCs are excellent targets for their adaptive degradation by atmospheric factors into semiochemicals. CHCs are species-specific blends of aliphatic chains that cover the entire insect body surface [13,14] and serve multiple functions, including as pheromones [15,16]. Insect CHCs are also ingested during grooming behaviour, and defecated, thus accumulating on resting sites, such as shelters [17]. The process of CHC degradation is similar to rancidification of lipids in food [18] and skin [19], cleaving the double bonds in unsaturated CHCs into short-chain VOCs, such as aldehydes, ketones and fatty acids. The multiple functions of CHCs, and their loss from the cuticle through contact, grooming and environmental degradation, may drive their relatively high turnover to maintain a fresh CHC layer and homeostasis [20].

We hypothesized that various abiotic environmental agents degrade unsaturated CHCs deposited by *Periplaneta americana* in shelters, and the resulting VOCs could then be used by conspecifics as a pheromone to assess shelter suitability for aggregating with conspecifics. *Periplaneta americana* is particularly suitable for this investigation because the major component of its CHCs is a 27-carbon diene (two double bonds) [21], unlike most insect species that possess alkanes as major CHCs.

2. Methods

(a) *Periplaneta americana* rearing

Periplaneta americana colonies were maintained at 27°C under a 12 : 12 light–dark photoperiod in plastic containers with access to water, dry LabDiet rodent chow (Purina No. 5001; PMI Nutrition International, St Louis, MO, USA), and moulded fibre egg cartons as shelters.

(b) Cuticular hydrocarbon extract

Fifth instar *P. americana* nymphs were placed in plastic boxes (25 × 8 × 8 cm). Within 24 h after moulting to adults, 10 females were reared in another box for 7 days and frozen. Females were immersed in 200 ml hexanes (Fisher Scientific, Fair Lawn, NJ, USA) in a clean beaker (500 ml), gently swirled for 2 min and the extract was transferred to another beaker. This procedure was repeated twice more, and extracts were concentrated with a rotary evaporator (Büchi, Flawil, Switzerland).

CHCs were purified using silica column chromatography (ϕ = 2 cm; silica gel: 7 g, 70–230 mesh, EM Science, Gibbstown, NJ, USA). CHCs were eluted with 31.5 ml hexane, and concentrated with a rotary evaporator. Silver nitrate (10%) in silica gel (230 mesh, Sigma-Aldrich, St Louis, MO, USA) was used to separate saturated from unsaturated CHCs by eluting the column with 31 ml hexane followed by 62 ml hexane/acetone (4 : 1). Fractions were confirmed using gas chromatography (see electronic supplementary material), and fractions containing the unsaturated CHCs were concentrated under a N_2 stream and suspended in hexane to yield a concentration of 1 cockroach equivalent per millilitre. Solutions were stored in a −30°C freezer.

(c) Cuticular hydrocarbon degradation and sampling of volatile organic compounds

A 50 µl aliquot from the unsaturated CHCs solution was transferred to a clear borosilicate vial (12 × 32 mm, National, Rockwood, TN, USA) that was placed in a horizontal position and hexane was allowed to evaporate for 20 min at room temperature. Vials were flushed with a stream of N_2 for 5 min followed by dry medical grade air (19.5–23.5% oxygen and 76.5–80.5% nitrogen; Airgas National Welders, Radnor, PA, USA, product code: 001002) for 10 s.

CHCs were exposed to different agents to assess the production of odourants. For air treatment, the vials were immediately capped (polypropylene cap, PTFE/silicone septa, Thermo Scientific, Germany) after flushing with dry medical grade air. For water vapour treatment, 4 µl of HPLC grade water (Fisher Scientific, Fair Lawn, NJ, USA) was added to each vial and the vials were capped. This volume was sufficient to form condensation in the vial within 24 h. Controls were empty clean vials that were treated as the treatment vials. Vials were kept inside a fibreboard box at room temperature for 1, 3 or 7 days.

Vials for ultraviolet (UV) exposure were capped and placed 15 cm under a transilluminator (302 nm, TM-40, Ultra Violet Products, San Gabriel, CA, USA) for 1, 6 or 12 h. Caps were covered with aluminium foil to protect them from degradation under UV. Control vials also contained CHCs and were placed under the transilluminator, but they were fully covered with aluminium foil. A box fan was placed in front of the transilluminator to prevent excessive heating of the vials.

After exposing CHCs to the different agents, odourants were collected with SPME (50/30 µm, DVB/Carboxen/PDMS, 2 cm fibre length, Supelco, Bellefonte, PA, USA) by exposing the fibre for 30 min to the interior of capped vials. Odourants were then analysed by either GC–EAD or GC–mass spectrometry (GC–MS).

(d) Electrophysiology

Antennal responses of second instar *P. americana* nymphs to volatile collections from CHC breakdown products were studied by GC–EAD. An antenna was ablated at the base and inserted into a reference glass electrode filled with Ringer, while the recording electrode was connected to the cut tip of the antenna and connected to a custom-made amplifier [22]. The amplifier was connected to a G3456-60010 AIB board in a 7890 GC (Agilent Technologies, Palo Alto, CA, USA) which synchronized the outputs of the FID and EAD.

The GC was equipped and operated according to details in electronic supplementary material. The effluent capillary for the EAD passed through a water-cooled odour delivery tube (30 cm × 8 mm) set at 19°C, where it was mixed with humidified medical grade air (80 ml min^{-1}). The insect antenna was positioned 0.5 cm from the outlet of the odour delivery tube.

(e) Identification of bioactive compounds

Volatile collections were analysed on a GC–MS (6890 GC and 5975 MS, Agilent Technologies) and operated according to details in

electronic supplementary material. Compounds were identified based on Kovats indices, electron ionization mass spectra and comparison with authentic synthetic standards.

(f) Preparation of synthetic mixtures

Synthetic compounds were systematically mixed to reproduce the amount of odourants emitted from cuticular alkenes when exposed to UV light for 12 h. These mixes were achieved by examining the volatiles released from each mix. A 4 µl aliquot of each mix was applied to a piece of filter paper (2 × 2 cm). After 10 min at room temperature, each filter paper was transferred to a 2 ml borosilicate vial and tested. The emission rate of VOCs from filter papers was assessed by sampling the headspace of capped vials with SPME and analysed by GC using the same procedure described for analysing VOCs from CHC degradation.

In the final formulation, acetic, propanoic, butanoic, pentanoic and hexanoic acids (Supelco, Bellefonte, PA, USA) were mixed in hexane at concentrations of 760, 38, 38, 38 and 760 ng μl^{-1}, respectively, and henceforth called fatty acids mix. The remaining compounds, 1-pentanol, 1-octanol, 1-nonanol (Supelco) and 2-nonanone (Sigma-Aldrich, St Louis, MO, USA) were mixed at 1225, 5.5 and 7.5 and 22.5 ng μl^{-1}, respectively, and tetradecanal (Supelco) was prepared as a separate solution (100 ng μl^{-1}), and henceforth called other VOCs mix. For behavioural assays, 4 µl of each mix was applied to filter papers, solvent was evaporated at room temperature for 10 min and papers were inserted into vials that were then used as odour sources in behavioural assays.

(g) Behavioural assays

All assays were conducted in a walk-in chamber (40% RH, 27°C), 1 h after the beginning of the scotophase under red light. A two-choice arena was constructed of a polystyrene Petri dish (ϕ = 8.5 cm, H = 1.5 cm, Fisher Brand; electronic supplementary material, figure S1) with two holes (ϕ = 1 cm) equidistant from the centre of the dish (2.75 cm). Under the dishes, the holes were covered with a metal screen (500 µm mesh) and a vial cap without septum (Thermo Scientific) was attached using hot plastic glue. The cap was used to attach a borosilicate vial (12 × 32 mm) that contained the test material (e.g. treated CHCs, synthetic mixtures or conditioned filter papers). Therefore, the screen prevented nymphs from contacting the interior of vials, and their behavioural preference was solely mediated by VOCs from vials. Inside the Petri dish, a piece of filter paper (2.5 × 2.5 cm, no. 1, Whatman) folded in an 'M' shape was placed on the top of each screen-covered hole and served as the shelter.

Aluminium foil that wrapped control vials was removed before it was inserted into the arena. A shelter made of an 'M'-shaped piece of filter paper (2.5 × 2.5 cm) was placed on top of each opening. Four second instar nymphs were released and the dish was covered. After 20 min, the number of nymphs resting on each shelter was counted. The positions of test materials were randomized to avoid side bias.

(h) Conditioning of shelters

Sheets of Whatman no. 1 filter papers (ϕ = 25 cm) were cut into rectangles (15 × 22 cm) and cleaned by immersing the bottom part in acetone (Sigma-Aldrich) in a chromatography glass chamber. The solvent was added to the bottom of the chamber and was drawn through the filter papers by capillary action. Once the solvent reached the top, filter papers were removed from the chamber and dried. This procedure was repeated twice and the top 1 cm of the filter papers was discarded.

The filter papers were folded accordion-like and placed inside plastic boxes ($L:W:H$ = 22 × 13 × 10 cm) with 15 adult female *P. americana*. Water and chow pellets were offered ad libitum but food was placed inside the base of a Petri dish (ϕ = 10 cm) to avoid contaminating the filter paper shelters. Control filter papers were similarly set but without cockroaches.

After 48 h of conditioning, filter papers were cut into pieces (1.5 × 2 cm) and individually placed inside borosilicate vials (12 × 32 mm). Capped vials were either exposed to UV for 12 h or protected by covering them with aluminium foil. Control filter papers were treated in the same manner. Vials were either tested in behavioural assays, used for VOCs analysis by GC using SPME, or deposited metabolites were extracted from these shelters.

(i) Extraction and analysis of cuticular hydrocarbons from conditioned shelters

After treatment in vials, conditioned filter papers were washed with 1 ml of hexanes containing 5 ng μl^{-1} of octadecane as internal standard, vortexed for 1 min and the extract was transferred to a borosilicate vial (15 × 45 mm, Thermo Fisher, Rockwood, TN, USA). This procedure was repeated twice more with clean hexane, and the extracts were combined. To obtain hydrocarbons, each extract was fractionated on a silica gel (800 mg) column made of Pasteur pipette (14.6 cm, Fisher Scientific) with glass wool retaining the silica gel; the column was conditioned with hexane. Hydrocarbons were eluted with 4 ml hexane and the solvent was evaporated under nitrogen flow. Hydrocarbons were analysed by a 7890 GC (see details in electronic supplementary material).

(j) Statistical analysis

All statistical analyses were performed in R (v. 3.5.1) [23]. Behavioural responses of nymphs under each shelter were analysed as proportions by linear mixed-effects models (lme) using the package 'lme4' [24] with positions of arenas and experiment blocks (experimental days) as random effects. Correlation coefficients between profiles of volatile products from air, water vapour and UV as degrading agents of unsaturated CHCs in a time series, and the respective p-values were calculated based on Spearman's rank correlation using the package 'Hmisc' [25] and plotted using the package 'corrplot' [26]. Amounts of the three main *P. americana* CHCs recovered from filter papers after UV exposure were analysed by ANOVA. All test statistics are presented in electronic supplementary material, table S1.

3. Results

(a) Generation of cuticular hydrocarbon breakdown products

We first optimized the breakdown conditions of CHCs and sampling of VOCs using UV radiation as a standard method. All methods and results (electronic supplementary material, figures S2–S4) are described in the electronic supplementary material. Based on these results, all subsequent experiments used borosilicate vials, positioned 15 cm from a UV-transilluminator, and exposed for up to 12 h.

(b) Behavioural response to cuticular hydrocarbon breakdown products

We validated that the two-choice aggregation assay with *P. americana* lacked any side bias with two blank vials (figure 1*a*; p = 0.396), as well as UV-exposed versus UV-protected blank vials (figure 1*h*; p = 0.321). Nymphs avoided the shelters associated with VOCs from CHCs exposed to air for 7 days and preferred to aggregate under blank shelters (figure 1*d*; p < 0.001), but shorter exposure to air did not

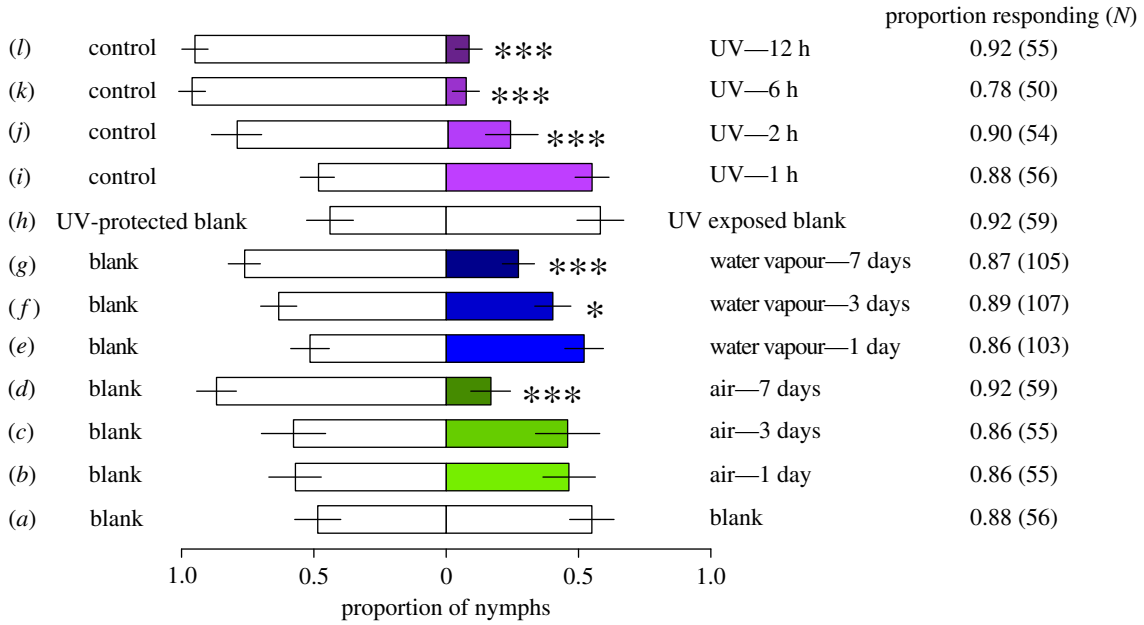

Figure 1. Behavioural responses of *P. americana* nymphs to breakdown VOCs of unsaturated CHCs. Groups of four second instar nymphs were tested in two-choice shelter assays in Petri dishes. The source of VOCs was a vial positioned under each shelter. Experiments were to test (*a*) for side bias using only vials treated with hexane (blank); (*b–d*) breakdown VOCs produced when CHCs were exposed to air for 1, 3 and 7 days (air); (*e–g*) breakdown VOCs produced when CHCs were exposed to water vapour for 1, 3 and 7 days (water vapour); (*h*) blank vials exposed to UV (UV-exposed blank) and protected from UV (UV-protected blank) for 12 h; and (*i–l*) breakdown VOCs produced when CHCs were exposed to UV radiation for 1, 2, 6 and 12 h (UV). Bars represent mean (±s.e.) proportions of nymphs (lme, $*p < 0.05$, $***p < 0.001$, $N = 15–30$). The proportion of tested nymphs that responded to both stimuli and respective number of nymphs (*N*) are shown next to each bar. (Online version in colour.)

generate avoidance responses (1 day exposure: figure 1*b*; $p = 0.352$; 3 days exposure: figure 1*c*; $p = 0.597$). Nymphs also avoided VOCs from CHCs exposed to water vapour for 3 days (figure 1*f*; $p < 0.05$) and 7 days (figure 1*g*; $p < 0.001$) but not for 1 day (figure 1*e*; $p = 0.636$).

Exposure to UV radiation elicited avoidance of the resulting VOCs at much shorter periods of exposure, with 1 h exposure to UV eliciting no avoidance (figure 1*i*; $p = 0.476$), but 2, 6 and 12 h of exposure resulting in strong avoidance (figure 1*j–l*; $p < 0.001$).

(c) Analysis of volatile organic compounds

When unsaturated CHCs were exposed to air, no VOCs were detected on day 1 compared to the respective control vial (electronic supplementary material, figure S5I). Small amounts of VOCs appeared after 3 days (electronic supplementary material, figure S5II) and more compounds at higher amounts appeared after 7 days (electronic supplementary material, figure S5III). Unsaturated CHCs exposed to water vapour followed a similar pattern, but the ratios of compounds were different between the two treatments (electronic supplementary material, figure S5IV–VI). By contrast, exposure of alkenes to UV radiation produced VOCs within 1–6 h and large amounts of VOCs were generated after 12 h of UV exposure (electronic supplementary material, figure S5VII–IX); notably, unsaturated CHCs that were protected from UV did not produce VOCs (electronic supplementary material, figure S5VII–IX) nor did clean borosilicate vials exposed to UV radiation (electronic supplementary material, figure S6). We calculated correlation coefficients for each treatment pairing based on the relative abundance of GC peaks, and significant correlations are displayed in a correlation matrix (electronic supplementary material, figure S7). The VOCs from exposure of unsaturated CHCs to air or water vapour for 7 days, or to UV for 6 or 12 h were significantly positively correlated (electronic supplementary material, figure S7). At these time points, the VOC compositions were similar, but their concentrations and ratios differed (electronic supplementary material, figure S5III, VI, VIII and IX); yet, all three methods elicited the same behavioural responses in *P. americana* nymphs. Of the three methods, however, UV produced the highest amounts of VOCs in the shortest time, including all the VOCs produced under the effects of water vapour and air. Therefore, UV exposure was used for all subsequent analyses, including GC–EAD and GC–MS (figure 2).

(d) Identification of bioactive volatile organic compounds

Active VOCs from UV-exposed unsaturated CHCs were recognized using gas chromatography coupled to electroantennographic detection (GC–EAD) with nymph antennae (figure 2). Fourteen compounds elicited antennal responses and 10 of these were identified by mass spectrometry (figure 2). Consistent with the behavioural results, bioactive compounds gradually accumulated over time of UV exposure of unsaturated CHCs. Among these, five compounds were short-chain fatty acids (acetic, propanoic, butanoic, pentanoic and hexanoic acids) which comprised 68% of the total mass of volatiles. The other compounds (26%) included tetradecanal, 1-pentanol, 1-octanol, 1-nonanol and 2-nonanone. Unsaturated CHCs that were not exposed to UV did not produce any EAD-active compounds (electronic supplementary material, figure S8).

(e) Behavioural response to identified compounds

We prepared two synthetic mixes from the identified EAD-active compounds: one mix contained the five fatty acids, and the other mix contained the remaining five compounds

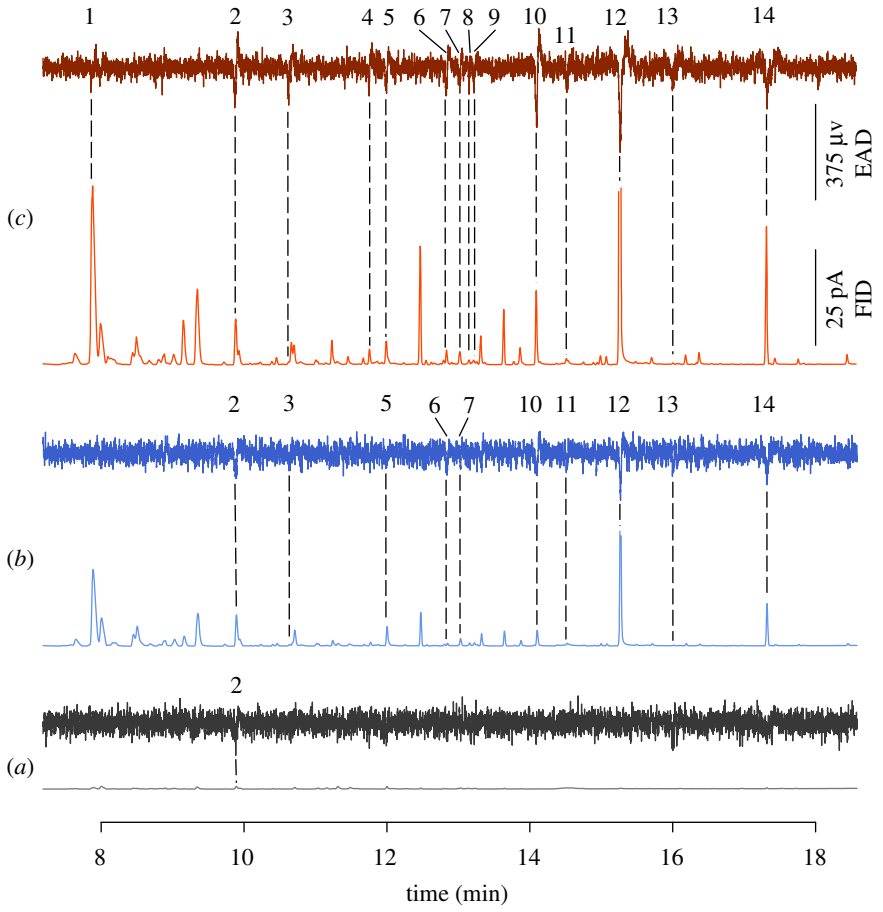

Figure 2. Antennal electrophysiological responses of second instar *P. americana* nymphs to volatile compounds from the UV-mediated decomposition of unsaturated CHCs for (*a*) 1, (*b*) 6 and (*c*) 12 h. In each panel, the bottom (upwards going peaks) shows the FID output chromatogram, and the top (downward-going EAD responses) shows the antennal responses; larger downward-going responses indicate greater response to the corresponding FID peak (dashed lines connect FID peaks and EAD responses). Antennal response is represented by the median of EAD recordings ($N = 6$–9). Compounds: 1-pentanol (1), 2-nonanone (2), acetic acid (3), propanoic acid (4), 1-octanol (5), butanoic acid (6), unknown (7), unknown (8), 1-nonanol (9), pentanoic acid (10), unknown (11), hexanoic acid (12), tetradecanal (13) and unknown (14). Scale bars for all FID chromatograms and EAD traces are 25 pA and 375 µV, respectively. (Online version in colour.)

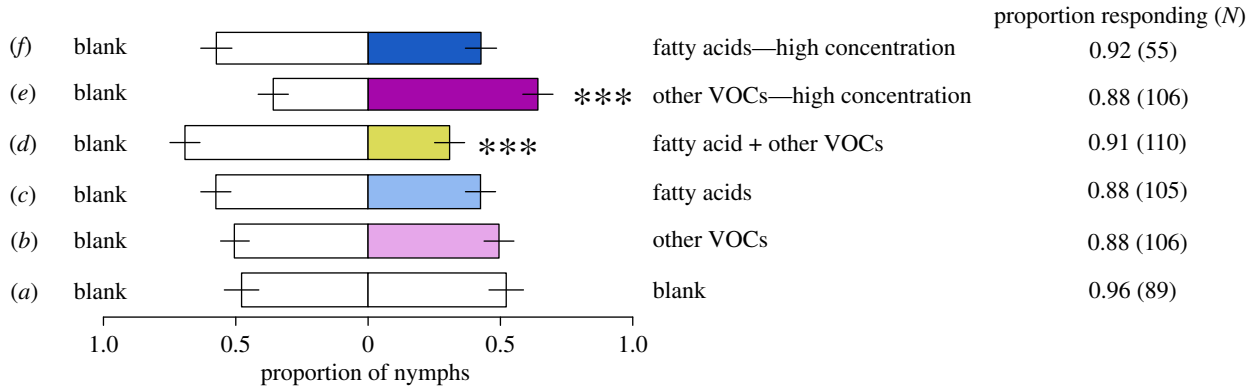

Figure 3. Behavioural responses of *P. americana* nymphs to synthetic mixes of EAD-active CHC breakdown VOCs. Groups of four second instar nymphs were tested in two-choice shelter assays and nymphs on each shelter were counted after 20 min. Experiments were to test (*a*) for side bias using vials treated with hexane control (blank); (*b*) the effect of a mix of 1-pentanol, 2-nonanone, 1-octanol, 1-nonanol and tetradecanal (other VOCs); (*c*) a mix of acetic, propanoic, butanoic, pentanoic and hexanoic acids (fatty acids); and (*d*) a combination of both mixes (acids+other VOCs). In order to compensate for the higher concentration of fatty acids in the total VOCs, the (*e*) other VOCs mix and (*f*) fatty acids mix were tested at concentrations matching those of the total VOCs. Bars represent mean (±s.e.) proportions of nymphs (lme, ***$p < 0.001$, $N = 15$–30). (Online version in colour.)

(alcohols, ketone and aldehyde). These compounds were carefully mixed and validated by sampling the headspace to reproduce the concentrations observed in the VOCs from CHC degradation (electronic supplementary material, figure S9). Bioassays with hexane demonstrated that cockroaches did not prefer either shelter position in the two-choice arena, that is, there was no side bias (figure 3*a*; $p = 0.403$). Neither mix alone affected the choice of nymphs for shelters (figure 3*b*,*c*; fatty acids mix: $p = 0.138$; other VOCs mix: $p = 0.359$). However, when both mixes were combined, cockroaches avoided the shelters positioned over the combined mixes and aggregated under the control shelters

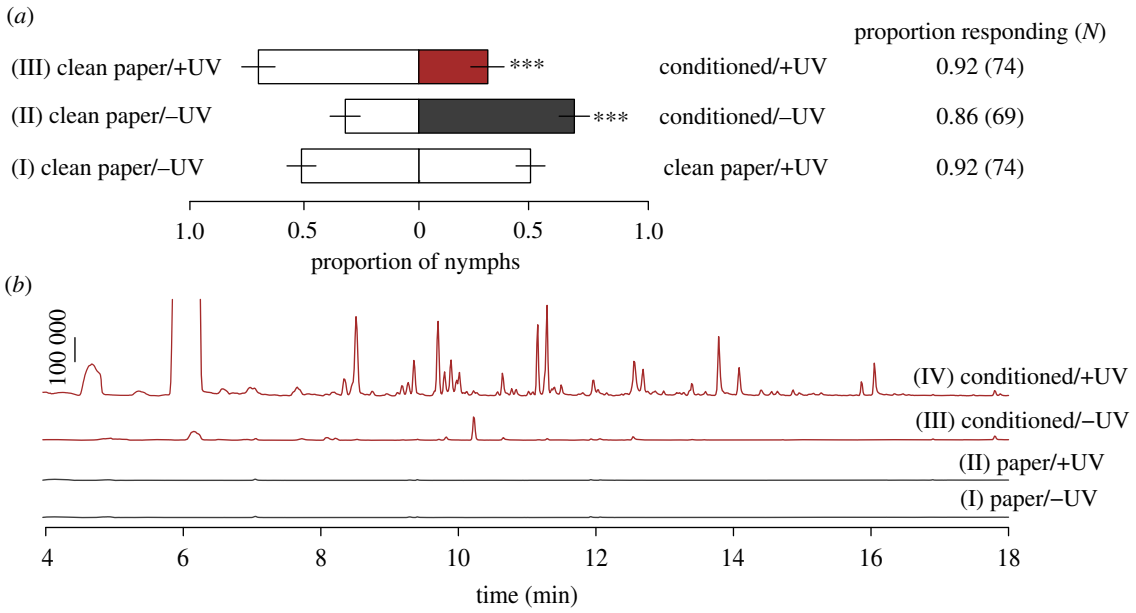

Figure 4. Effect of UV radiation on CHCs deposited on conditioned filter papers. Pieces of conditioned or clean filter papers were placed inside vials and exposed to UV radiation for 12 h. (*a*) Aggregation of *P. americana* nymphs on cockroach-conditioned shelters. Groups of four second instar nymphs were tested in two-choice shelter assays and nymphs on each shelter were counted after 20 min. The behavioural assays tested the effect of (I) clean containers using clean filter paper protected from UV (clean paper/—UV) and exposed to UV (clean paper/+UV); (II) filter papers conditioned by adults and protected from UV (conditioned paper/—UV); and (III) filter papers conditioned by adults and exposed to UV (conditioned paper/+UV). Bars represent mean (±s.e.) proportion of nymphs (lme, $^{**}p < 0.01$, $N = 15$–30). (*b*) Total ion chromatograms of odourants collected from (I) clean filter paper protected from UV and (II) exposed to UV radiation, (III) shelters conditioned with cockroaches protected from UV and (IV) exposed to UV radiation. Scale bar, 100 000 abundance. (Online version in colour.)

(figure 3*d*; $p < 0.001$), as they did in response to the natural VOCs of CHC degradation. In order to assess the effect of quantitative differences between the two blends, the concentration of the fatty acids mix and the other VOCs mix was increased 10% and 90%, respectively, to match the total mass of all the identified bioactive volatile products. We found no difference in the aggregation of cockroaches under shelters in response to the high concentration of the fatty acids mix (figure 3*f*; $p = 0.076$). By contrast, the high concentration of the other VOCs mix (alcohols, ketone, aldehyde) elicited significant aggregation in the shelter above this mix (figure 3*e*; $p < 0.001$).

(f) Behavioural responses to cockroach-conditioned shelters

Filter papers were conditioned by cockroaches, a procedure that results in the deposition of faecal and oral secretions and cuticular lipids on the papers. Exposure of clean shelters to UV did not affect the aggregation responses, as evidenced by the lack of preference for UV-exposed versus UV-protected clean filter papers (figure 4*a*(I); $p = 0.78$). As expected, the odour from conditioned shelters that were protected from UV were significantly more attractive to cockroaches than clean shelters treated in the same manner (figure 4*a*(II); $p < 0.001$). However, this preference was reversed when both types of shelters were exposed to UV (figure 4*a*(III); $p < 0.001$).

(g) Accumulation and degradation of cuticular hydrocarbons on shelters and formation of volatile products

The chemical profiles from UV-exposed and UV-protected clean shelters were similar, with little VOCs detected in either treatment (figure 4*b*(I,II)). Headspace analysis with solid-phase microextraction (SPME) confirmed that conditioned shelters exposed to UV produced more and different VOCs than UV-protected shelters (figure 4*b*(III,IV)). Furthermore, the odour profile from UV-exposed conditioned papers was strikingly similar to the profile of UV-exposed unsaturated CHCs, which, as shown before, also differed from the VOCs emanating from UV-protected CHCs (electronic supplementary material, figure S10A).

GC analysis of shelter extracts showed that UV-exposed shelters contained less of the main CHC, 6,9-heptacosadiene, than UV-protected shelters (electronic supplementary material, figure S10B; $p < 0.05$), while amounts of the main saturated CHCs, *n*-pentacosane and 3-methylpentacosane, did not differ (electronic supplementary material, figure S10B; $p = 0.761$ and $p = 0.937$, respectively).

4. Discussion

The majority of insect semiochemicals have been collected and tested under controlled environmental conditions to avoid contamination and decomposition, which may result in the loss of bioactivity. Natural field conditions abound with oxidizing agents (e.g. oxygen, ozone, water vapour, light and UV radiation), which are often unavoidable, and can modify the emitted semiochemicals. These agents may significantly reduce the lifespan (half-life) of semiochemicals, disrupting chemical communication between organisms [6]. However, insects have evolved and perfected chemical communication mechanisms for millions of years under these harsh environmental conditions. We hypothesized that insects exploit these environmental agents to decompose pro-semiochemicals resulting in decomposition products that serve as semiochemicals.

The most prominent potential pro-pheromones that are readily available for interaction with atmospheric agents are CHCs. These long-chain compounds are ubiquitous in insects, occur in relatively large amounts and in species-specific blends, serve multiple unrelated physiological and behavioural functions, and are maintained at homeostatic levels through regulated biosynthesis, deposition on the cuticle and grooming [27,28]. Some insect species contain a mix of saturated, unsaturated and methyl-branched CHCs, while others display only saturated CHCs [13]. Notwithstanding, the CHC profile of *P. americana* is quite unique among insects in general because it includes several alkenes that constitute more than 70% of total CHCs, and more than 68% is represented by a single alkadiene, (*Z*,*Z*)-6,9-heptacosadiene [21,29]. A review by Lockey [13] concluded that less than 50% of 119 insect species contained unsaturated CHCs, and 36 species had alkadienes and/or alkatrienes, but most in small amounts. It is important to note, however, that alkenes may occur in small amounts (e.g. less than 1% of total CHCs) and thus excluded from functional analysis, and they may be obscured by methylalkanes in GC analysis [30], so the representation of alkenes in insects may be underestimated.

The CHCs of *P. americana* are thought to mediate species-specific aggregation [29], but it is unknown why *P. americana* evolved such a high proportion of a single alkadiene. We suggest that the prominence of 6,9-heptacosadiene in all stages of *P. americana* make it a potential pro-pheromone for producing volatiles via environmentally mediated decomposition. Its presence in nymphs, males and females [29] would suggest that volatile reaction products might serve as pheromones perceived by all developmental stages. We focused on early instar nymphs because they exhibit strong gregarious behaviour and response to semiochemicals [31], as in other cockroaches [32]. In this study, we demonstrated that atmospheric agents degrade *P. americana* CHCs, releasing volatile semiochemicals that repel nymphs from settling in a shelter exposed to these VOCs. These pheromones might serve to identify old-colonized or over-crowded shelters (epideictic) or as necromones.

Several metabolites from *P. americana* that were thought to be intrinsically biosynthesized have been identified as aggregation pheromones [33], sex pheromone [34] and necromones [35]. We found that VOCs for this species were produced from the interaction of CHCs with air, water and UV radiation, and all elicited behavioural responses. It is unlikely that *P. americana* would be exposed to UV, because it is nocturnal and it inhabits UV-protected but chemically complex environments such as sewers, bat guano in caves and dump sites [36]. These habitats contain high concentrations of numerous chemical agents (e.g. ammonia, hydrogen sulfide, acids) [37] that could interact with CHCs to form various VOCs, including the ones found in our study. Therefore, UV radiation was used here as a model environmental agent to effect the timely decomposition of CHCs and facilitate exploration of the function of the reaction products. Because the reaction of CHCs with air and water vapour produced the same behavioural responses as to UV-produced volatiles, the results of this study should compel future studies of the effects of various ecologically relevant environmental factors, alone and in combinations, on semiochemical production.

The production of insect attractants via oxidation of long-chain CHCs was demonstrated for *P. alaskensis* [8], *M. grandii* [10], *A. glabripennis* [11], *C. cinctus* [9] and *D. melanogaster* [12]. Other metabolites may also serve as precursors to volatile cues, such as tetracosyl acetate from *Diaphorina citri* [38]. Most of these studies focused on the effect of a single component within the complex VOCs (but see Wickham *et al.* [11]) and on the effect of oxidation in air or with synthetic reagents. However, a wide range of VOCs can be formed from lipid degradation simultaneously, as multiple factors affect the process [39], such as the type of precursors (e.g. chain length, number and position of double and triple bonds), environmental factors (e.g. oxidant, temperature, radiation, metal ions) and period of exposure. While the formation of some oxidation products from unsaturated hydrocarbons can be relatively straightforward [39,40], it may still yield multiple unpredicted components. For instance, ozonolysis of the hydrocarbon (*R*)-(+)-limonene produces approximately 1200 different compounds, among which at least 75 were of low molecular weight [41]. In our studies, the composition of *P. americana* CHC decomposition products varied with degradation agent and exposure period, suggesting that the quality and quantity of decomposition products could be plastic and shaped by abiotic habitat conditions.

Fatty acids trigger different behavioural responses in different species. Some medium-chain fatty acids, like linoleic and oleic acids, act as necromones for *Apis mellifera* [42], *P. americana* [35], termites [43] and isopods [4], repelling them from sites with dead conspecifics to avoid the potential risk of predation or pathogenesis. However, short- and long-chain fatty acids from faeces of *Blattella germanica*, some of which were microbial-mediated [44], elicited aggregation response [45]. Here, *P. americana* CHC reaction products included compounds of different chemical classes, but short-chain fatty acids were highly predominant in quantity. Given their prominence in the emitted VOCs, and the importance of fatty acids in cockroach behaviour, we examined the role of these fatty acids and the remaining VOCs separately. Our results suggest that the fatty acids alone are not responsible for repellence, and either a subset or all components in both mixes are required to elicit repellence. This observation is consistent with studies showing that combinations of components may elicit stronger behavioural responses than individual components from insects, including cockroaches [45]. Furthermore, we were unable to elicit the shelter avoidance response by increasing the concentration of fatty acids, confirming that the pheromone is defined primarily by the odourant composition of the mix and secondarily by its concentration. Conversely, increasing the concentration of the Other VOCs mix (i.e. alcohols, ketone and aldehyde) elicited attraction of nymphs to the shelter that received this treatment. If other environmental conditions could favour the formation of these non-fatty acid compounds from CHCs, attraction and aggregation might be mediated in *P. americana*, rather than repellence. However, we are unaware of any environmental agent that facilitates the production of these attractive compounds and, therefore, whether *P. americana* could be attracted to CHC breakdown products under certain conditions.

The majority of the investigated cockroach species are gregarious, living in groups for long periods with few individuals dispersing to distant areas [46,47]. Recognition of their own group is mediated through chemical cues emitted directly from the cockroaches and from organic materials deposited on or near the refugium, like volatiles from faeces and associated microbes [44], and contact metabolites, like CHCs (but see Hamilton *et al.* [17]). We hypothesize that as faeces

and CHCs accumulate on shelters and react with environmental factors, VOCs signal colony conditions, such as size, demographic composition and health. We observed that cockroach-conditioned shelters that were initially attractive to nymphs were later avoided after exposure to UV radiation, which triggered changes in the VOCs, largely through degradation of cuticular alkenes. We attribute the change in VOCs to an 'ageing' effect on the accumulated CHCs imposed by environmental agents. Although early developmental stages of *P. americana* tend to readily form aggregations compared to later nymphal stages and adults, they are also more susceptible to injury and cannibalism in dense aggregations [31]. Thus, it is plausible that early-stage nymphs benefit most from assessing the chemical cues that emanate from refugia and increase their survival rate by avoiding or dispersing from aggregations that impose a high risk of mortality.

In this report, we used *P. americana* and UV radiation as a proof-of-concept model for UV-mediated production of semiochemicals from unsaturated CHCs. We recognize that *P. americana* would rarely be exposed to UV, and instead, other environmental factors would cause the CHC decomposition. Other insect species, however, might display behavioural adaptations to prevent or facilitate UV exposure and VOC production. Basking in many diurnal insects, for example, might serve not only the recognized functions of thermoregulation and intraspecific display, but it might facilitate the generation of semiochemicals from CHCs. Environmentally mediated generation of semiochemicals has been documented in several species from different taxonomic groups (i.e. Lepidoptera, Coleoptera, Hymenoptera, Diptera, Hemiptera and now Blattodea), mostly under laboratory conditions. Although field trapping assays from other studies have demonstrated that artificial breakdown products are behaviourally active, the detection and characterization of these products under various natural conditions have yet to be demonstrated and coupled to behavioural observations to validate this phenomenon as an evolutionary innovation of insect chemical communication.

Data accessibility. All data presented in this manuscript are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.n8pk0p2r3 [48].

Authors' contributions. E.H., A.W.-K. and C.S. designed the study; E.H. collected data and performed statistical analysis; E.H. wrote the first draft of manuscript; all authors contributed to revisions.

Competing interests. We declare we have no competing interests.

Funding. This work was supported in part by the United States National Science Foundation (grant no. IOS-1557864) and the Blanton J. Whitmire endowment at North Carolina State University.

Acknowledgements. We thank Rick Santangelo and Brandy Simmons for maintaining the insect colonies, Judy Elson for tests in the Xenon-weather-ometer and three anonymous reviewers for helpful comments.

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Supplementary Material

Environmental decomposition of olefinic cuticular hydrocarbons of *Periplaneta americana* generates a volatile pheromone that guides social behaviour

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Materials and methods

Optimization of CHC degradation

To investigate the effect of radiation on CHCs, we first tested three types of clear containers on the breakdown of CHCs (fig. S2A). Containers were 2 ml borosilicate vials (12 x 32 mm, National, Rockwood, TN, USA), 3 ml UV-quartz cuvettes (Z276669, Sigma, St. Louis, MO, USA) and 2 ml fused quartz tubes (5 mm ID x 7 mm OD, 50-872-632, Fisher Scientific, Fair Lawn, NJ, USA). A CHC aliquot corresponding to 5% male-cockroach-equivalent, containing 200 µg of tetracosane as internal standard, was transferred to each container (N = 5 per treatment) and hexane was allowed to evaporate for 20 min during which vials were kept horizontally. Uncapped vials were then positioned inside a solar simulator (Atlas Ci3000+ Xenon-weather-ometer, borosilicate S/borosilicate S filters, 24°C, Chicago, USA). After 6 and 24 hr of light exposure CHCs were recovered by rinsing the containers with 100 µl hexane and transferred to 300 µl conical glass inserts. Controls were set using the same procedure but not exposing the containers to the solar simulator. A second set of CHCs in quartz tubes were exposed to UV-radiation using a transilluminator (302 nm, TM-40, Ultra Violet Products, San Gabriel, CA, USA) for 24 hr.

To select the optimal distance that maximizes the breakdown of CHCs, we set 2 ml borosilicate vials containing 10% male-cockroach-equivalent containing 200 µg of tetracosane as internal standard at 15 and 30 cm under the transilluminator. After 6 hr, CHCs were recovered from vials using the same procedure previously described. Control vials were set by extracting CHCs soon after evaporating the hexane.

All extracts were then analysed by GC and amounts of the three main CHCs, *n*-pentacosane, 3-methylpentacosane and 6,9-heptacosadiene were quantified relative to the IS. CHC extracts were analysed on a 7890 GC equipped with a DB-5 column (20 m × 0.18 mm, df = 0.18 µm, Agilent Technologies, Palo Alto, CA, USA) and operated in splitless mode (11 psi for 1 min, then 10 psi). Injector temperature was set to 300 °C. Hydrogen was used as the carrier gas at an average linear velocity of 35 cm/s. The oven program was set to 50 °C for 1 min, increased at 20 °C/min to 150 °C and then at 5 °C/min to 300 °C and held for 5 min. The flame ionization detector (FID) was set at 300 °C.

Gas Chromatography-ElectroAntennographic Detection (GC-EAD)

The GC was equipped with a DB-WAXetr column (30 m × 0.25 mm, df = 0.25 µm, Agilent Technologies, Palo Alto, CA, USA) and operated in pulsed splitless mode (15 psi for 0.5 min, then 10 psi). Hydrogen was used as the carrier gas at an average linear velocity of 45 cm/s. The oven program was set to 40 °C for 2 min, increased at 10 °C/min to 250 °C. The FID was set at 280 °C. At the end of the capillary column, the effluent was split 1:1 between the FID and the EAD. The effluent capillary for the EAD passed through a modified MS transfer line set at 270 °C, and into a custom-made water-cooled glass odour delivery.

All FID and EAD traces were exported as CSV files. Drift of the EAD baseline was corrected by modelling the signal using a Local Polynomial Regression Fitting (α=0.05) in R (version 3.5.1, R Core

Team) and subtracting for the respective trace. Medians of FID and corrected EAD signals were then each calculated at each time-point using all replicates.

Gas Chromatography-Mass Spectrometry (GC-MS)

Volatile collections were analysed on a GC-MS (6890 GC and 5975 MS, Agilent Technologies, Palo Alto, CA, USA). The GC was operated in pulsed splitless mode (15 psi for 0.5 min, then 6 psi) and equipped with a DB-WAXetr column (30 m × 0.25 mm, df = 0.25 µm, Agilent Technologies, Palo Alto, CA, USA), and helium was used as the carrier gas at an average velocity of 34 cm/s. The oven temperature program was the same as in GC-EAD. Injector temperature was set to 250 °C, transfer line temperature was 260 °C, and MS quadrupole was 150 °C. The mass-to-charge ratio range was from 33 to 650.

GC analysis of CHCs from cockroaches and conditioned shelters

CHCs extracted from female *P. americana* and conditioned shelters were analysed on a 7890 GC (Agilent Technologies, Palo Alto, CA, USA) equipped with a DB-5 column (20 m × 0.18 mm, df = 0.18 µm, Agilent Technologies, Palo Alto, CA, USA). The GC was operated in splitless mode (11 psi for 0.5 min). Hydrogen was used as the carrier gas at an average linear velocity of 35 cm/s. The oven program was set to 50 °C for 1 min, increased at 20 °C/min until to 150 °C, increased at 5 °C/min until to 300 °C for 5 min. The FID was set at 300 °C.

Statistical analysis

Analysis of amounts of the three main *P. americana* CHCs (*n*-pentacosane, 3-methylpentacosane and 6,9-heptacosadiene) recovered from vials, tubes and cuvettes during our

optimization experiments were analysed by ANOVA. The effects of distance to the UV source (control, 15 and 30 cm) and time (0, 6 and 24 hr) on the CHC breakdown process were analysed by ANCOVA.

Results

Optimization of CHC degradation

Experimental conditions for CHC breakdown were analysed using several containers, radiation sources, distance to sources and time of exposure. To select a container that would allow UV-radiation to interact with CHCs, we loaded CHCs into borosilicate vials, quartz cuvettes and quartz tubes and exposed them to UV in a solar simulator. Quartz cuvettes and tubes served as positive controls that allowed for maximum UV transmission and borosilicate vials were used because they are convenient to SPME sampling. Amounts of the saturated CHCs, *n*-pentacosane and 3-methylpentacosane, did not change with vial type ($F_{2,39}$=2.319, p=0.112 and $F_{2,39}$=0.948, p=0.396, respectively; fig. S2B) and time of exposure ($F_{1,39}$=1.185, p=0.283 and $F_{1,39}$=0.195, p=0.661, respectively; fig. S2B). The amounts of the unsaturated CHC, 6,9-heptacosadiene, however, were significantly reduced with time of exposure ($F_{1,39}$=137.03, p<0.001; fig. S2B) but were independent of vial type ($F_{2,39}$=0.081, p=0.922; fig. S2B). Therefore, we continued using borosilicate vials because they were convenient to contain the breakdown volatiles for analysis and bioassays. As expected, 6,9-heptacosadiene was the only CHC that was significantly reduced upon exposure to solar radiation, due to the presence of double bonds which facilitated the breakdown process. To maximize the production of VOCs, we used only purified unsaturated CHC in our experiments.

When comparing the solar simulator and the UV-transilluminator to the control, there were small variations in the amounts of the saturated CHCs, pentacosane (ca. 4%; $F_{2,12}$=105.7, p<0.001; fig. S3) and 3-methylpentacosane (ca. 2%; $F_{2,12}$=5.49, p<0.05; fig. S3). However, the amounts of 6,9-heptacosadiene declined much more when exposed to the solar simulator (97.2%; t_{12}=50.11, p<0.001; fig. S3) and UV-radiation (98.2%; t_{12}=50.62, p<0.001; fig. S3), and there was no significant

difference between the effects of the two radiation sources (t_{12}=0.512, p=0.618; fig. S3). We selected the UV-transilluminator as radiation source in our study because it was readily available in our laboratory.

Finally, we tested two distances between the vials and UV-transilluminator (15 and 30 cm) for 6 hr to optimize the breakdown process. As we previously observed while testing the effect of radiation source, we observed minor changes in the amounts of pentacosane and 3-methylpentacosane when exposed to UV-radiation at 15 (ca. 0.1% and 2%, respectively, fig. S4) and 30 cm (ca. 0.5% and 0.4%, respectively, fig. S4) compared to the control ($F_{2,12}$=90.61, p<0.001; $F_{2,12}$=116.6, p<0.001; respectively, fig. S4). The amount of the unsaturated CHC, 6,9-heptacosadiene, declined dramatically when exposed to UV ($F_{2,12}$=799.4, p<0.001; respectively, fig. S4) with higher degradation at 15 cm distance (28%; t_{12}=27.32, p<0.001; fig. S4) than 30 cm (20%; t_{12}=38.95, p<0.001; fig. S4). We, therefore, exposed CHCs to UV-radiation at 15 cm.

References

1. Harrell Jr FE, Many with contributions from CD and. 2019 Hmisc: Harrell Miscellaneous. R package version 4.2-0. https://CRAN.R-project.org/package=Hmisc.
2. Wei T, Simko V. 2017 R package 'corrplot': Visualization of a correlation matrix (Version 0.84). Available from https://github.com/taiyun/corrplot.

Figures

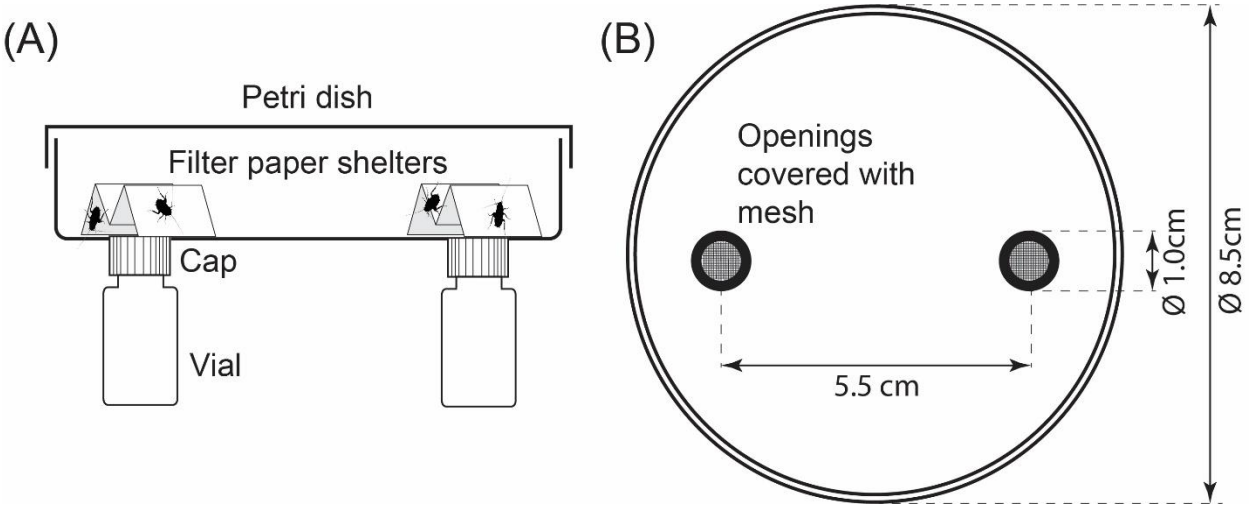

Figure S1. Schematic representation of the arena used for behavioural assays. (A) Side view and (B) top view of the arena. Arenas were built of polystyrene petri dishes (ø=10 cm) with two holes (ø=7 mm) equidistant from the centre of the dish (3 cm). The holes were covered with metal screen (500 µm mesh) and a vial cap without septum was attached under the petri dish using hot plastic glue. The cap was used to attach a borosilicate vial that contained the test material to the arena. Inside the dish, a piece of filter paper (2.5 x 2.5 cm) folded in an "M" shape was placed on top of the hole. Four cockroach nymphs were released inside the arena and their position was recorded after 20 min.

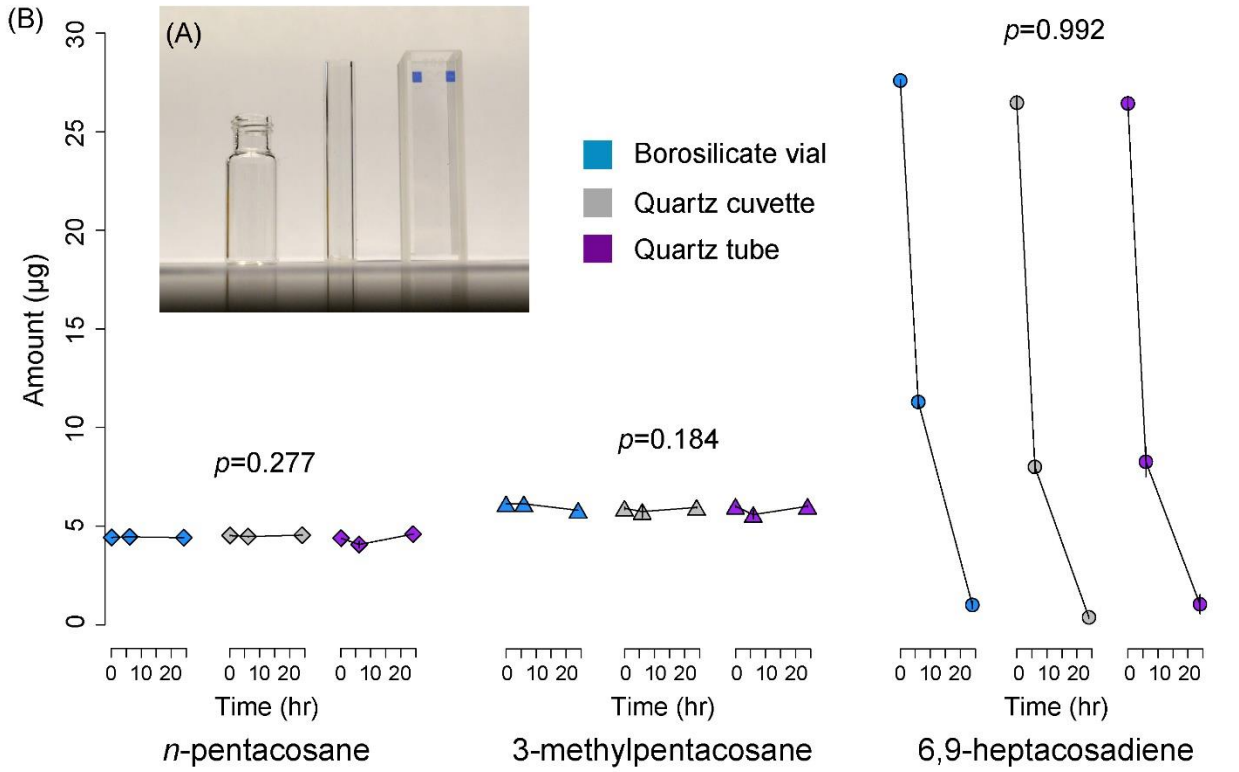

Figure S2. Effect of three types of containers on the CHC breakdown process by solar radiation. (A) The three types of containers: borosilicate vial (left), quartz tube (centre) and quartz cuvette (right). (B) After exposure to solar radiation for 6 or 24 hr, the remaining CHCs were recovered and the main CHCs, *n*-pentacosane, 3-methylpentacosane and 6,9-heptacosadiene, were quantified by GC. As controls, CHCs were placed in tubes but not exposed to solar radiation. Points represent mean amounts (µg±SE) of CHCs. Different point types (diamond, triangle and circle) represent different CHCs (*n*-pentacosane, 3-methylpentacosane and 6,9-heptacosadiene, respectively). Different colours (blue, grey and purple) refer to different containers (borosilicate vial, quartz cuvette and quartz tube, respectively). *P*-values refer to comparisons among vial types along time of exposure within the same CHC type (N = 5). Only amounts of the unsaturated hydrocarbon, 6,9-heptacosadiene, significantly decreased over time (ANCOVA, $p<0.001$) and no differences were found among container types (ANCOVA, $p=0.922$).

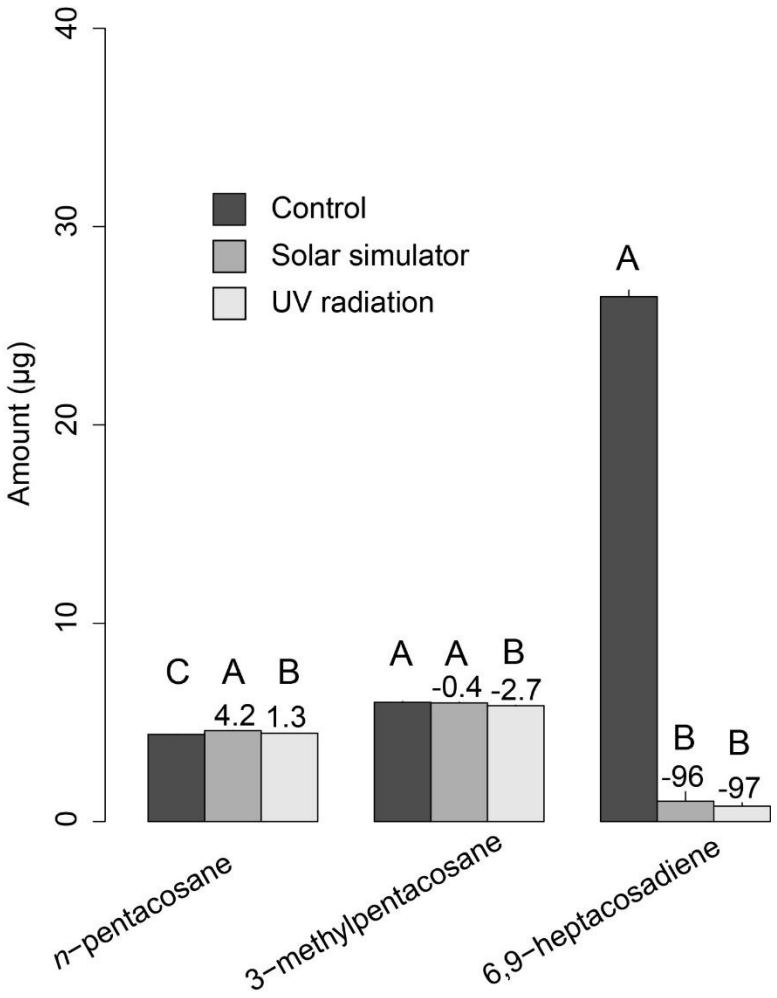

Figure S3. Comparison of solar and UV-radiation on the breakdown of CHCs. After exposure to radiation for 24 hr in quartz tubes, CHCs (5% male equivalent) were recovered and the main CHCs, *n*-pentacosane, 3-methylpentacosane and 6,9-heptacosadiene, were quantified by GC. Control tubes contained CHCs but were not exposed to radiation. Bars represent mean amounts (µg±SE). Different letters denote significantly different responses compared to control within each CHC (ANOVA, $p<0.01$, $N = 5$). Numbers on top of each bar are proportions of change in amounts of each CHC relative to the respective control based on means of each treatment.

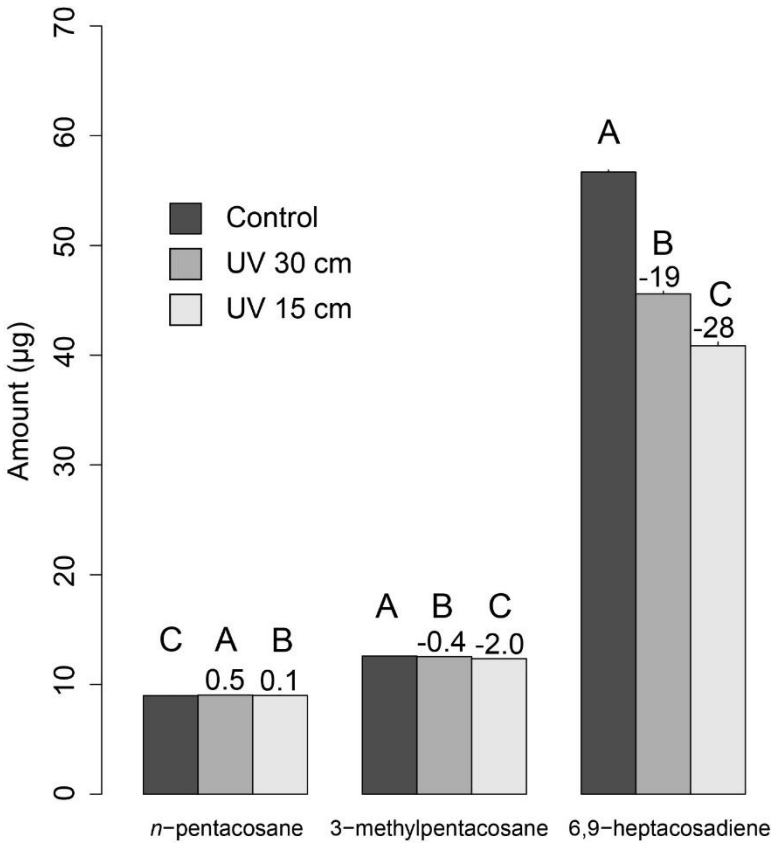

Figure S4. Comparison of distance (15 or 30 cm) from the UV-transilluminator on the breakdown of CHCs. After exposure to UV-radiation for 6 hr in quartz tubes, CHCs (10% male equivalent) were recovered and the main CHCs, *n*-pentacosane, 3-methylpentacosane and 6,9-heptacosadiene, were quantified by GC. Control tubes that contained CHCs were not exposed to UV-radiation. Bars represent mean amounts (µg±SE). Different letters denote significantly different responses within each CHC (ANOVA, $p < 0.001$, $N = 5$). The unsaturated CHC, 6,9-heptacosadiene, declined more when exposed at 15 cm than 30 cm (28% and 20%, respectively, relative to the control). Numbers on top of each bar are proportions of change in the amount of each CHC relative to the respective control based on means of each treatment.

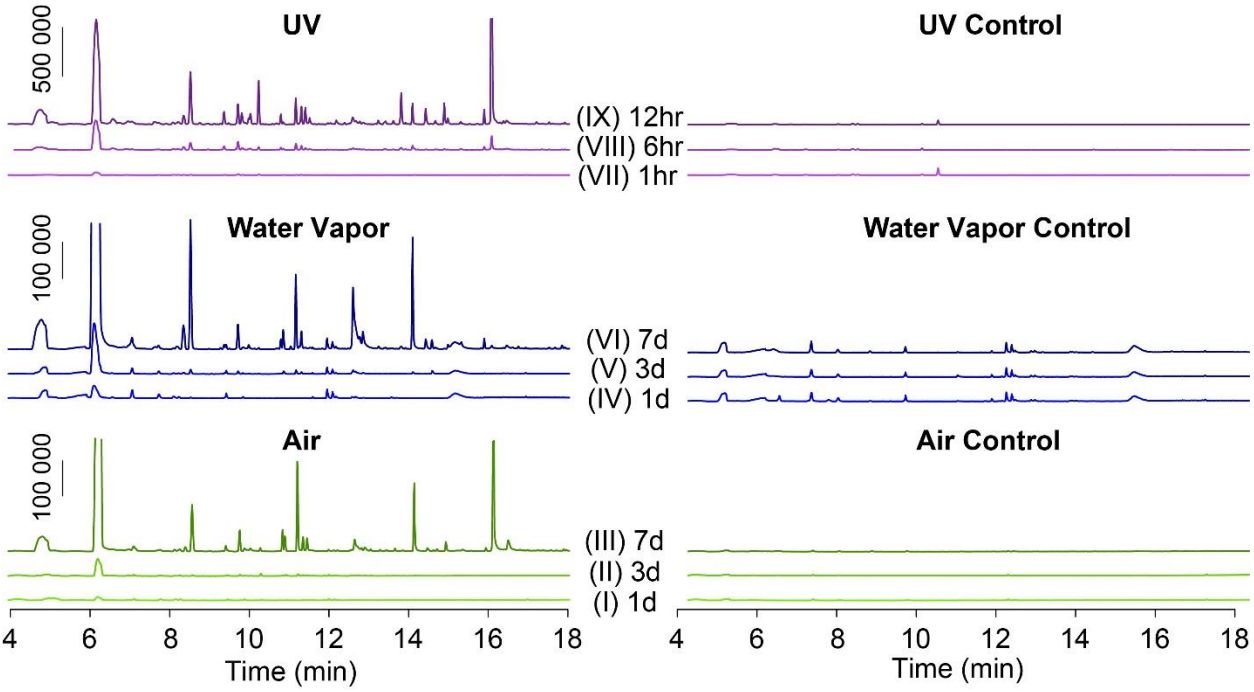

Figure S5. Time-courses of production of VOCs from degradation of *P. americana* unsaturated CHCs. CHCs were exposed to air, water vapour or UV-radiation. Total ion chromatograms of breakdown VOCs of unsaturated CHCs. Left: VOCs emitted from CHCs exposed to air (I-III) or water vapour (humidified air) (IV-VI) for 1, 3 and 7 days, or UV-radiation (VII-IX) for 1, 6 and 12hr. Right: chromatograms of the respective controls for air (blank vials, I-III), water vapour (blank vials, IV-VI) and UV-radiation (CHCs protected from UV-radiation: VII-IX). VOCs were sampled by SPME.

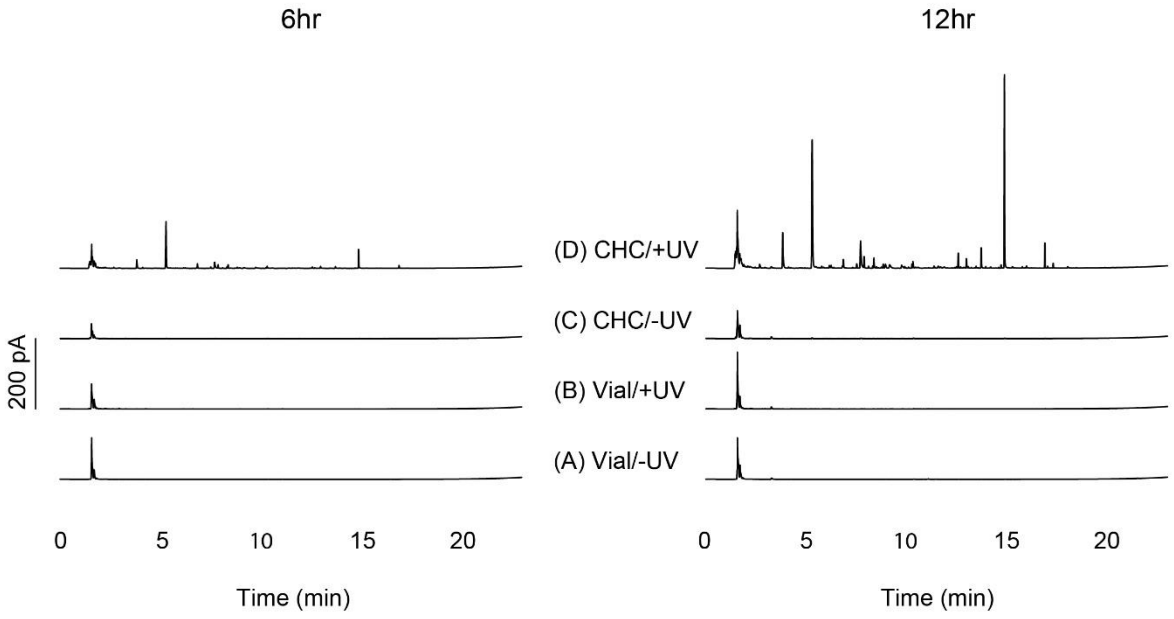

Figure S6. Volatile compounds originate solely from breakdown of *P. americana* unsaturated CHCs when exposed to UV-radiation for 6 hr (left) or 12 hr (right). Borosilicate vials were loaded with either unsaturated CHCs or hexane (carrier solvent). Gas chromatograms of headspace collections from (A) UV-protected vials (Vial / -UV), (B) UV-exposed vials (Vial / +UV), (C) UV-protected CHCs (CHC / -UV), and (D) UV-exposed CHCs (CHC / +UV). Chromatogram traces are medians of recordings (*N*=3). Volatile compounds were sampled by SPME.

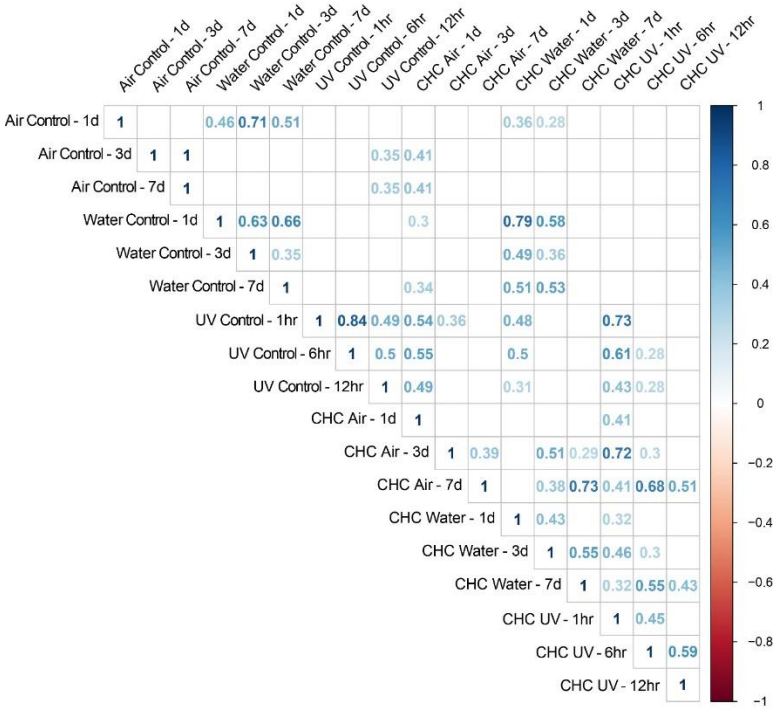

Figure S7. Correlation matrix showing similarity between the VOC patterns of CHCs degraded by air (CHC Air), water vapor (CHC Water) and UV-radiation (CHC UV), and the respective controls (Air Control, Water Control and UV-Control) over time (1, 3 and 7 days for Air and Water Vapor treatments and 1, 6 and 12 hr for UV-radiation). Data consisted of medians of proportions of the integrated area of each GC-MS peak relative to the respective total abundance of all peaks (N=3). Each number represents the pattern correlation coefficients between treatments. Correlations that were not significant (p>0.01) were left blank. Similarities between patterns were tested using Spearman's correlation.

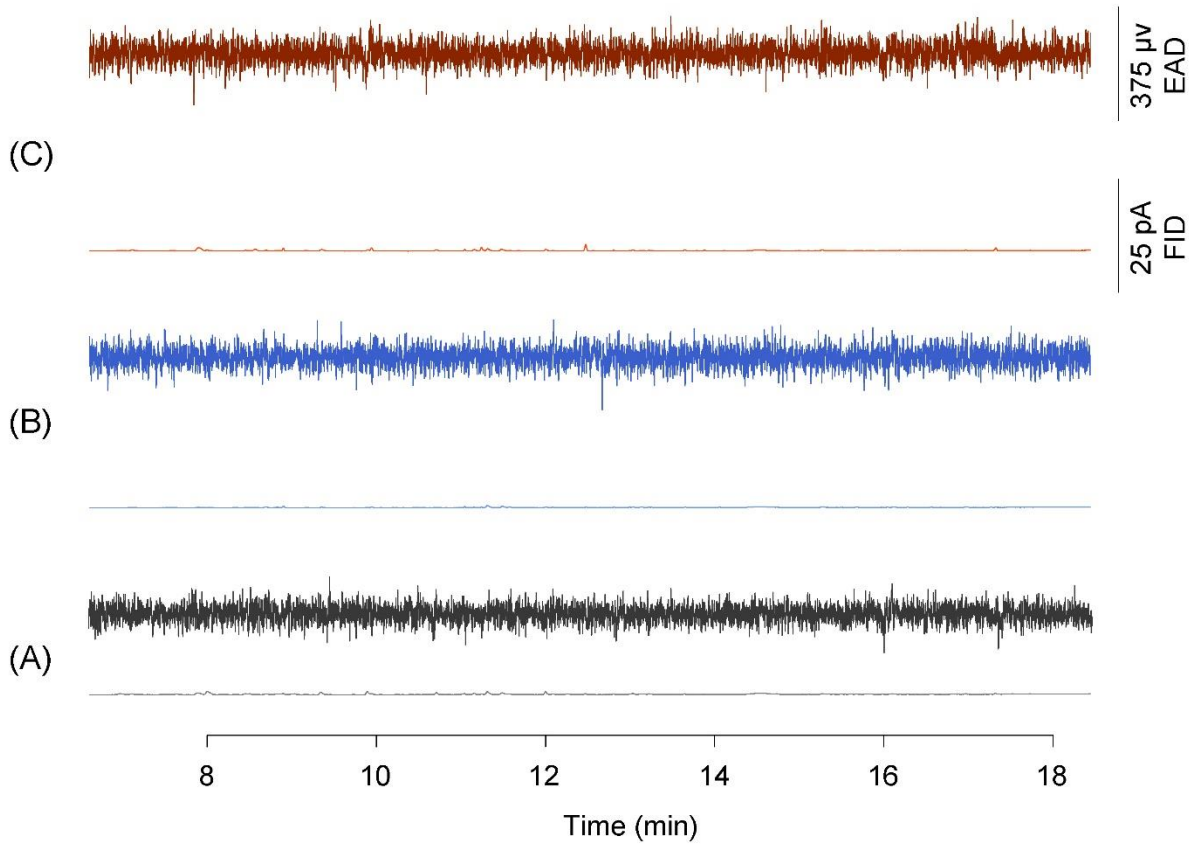

Figure S8. Antennal electrophysiological responses of second instar *P. americana* nymphs to headspace collections from unsaturated CHCs that were protected from UV-radiation for (A) 1 hr, (B) 6 hr and (C) 12 hr. In each panel, the bottom (upwards going peaks) shows the FID output chromatogram, and the top (downward going EAD responses) shows the antennal responses. No antennal response was detected to any headspace collection of protected CHCs. EAD traces are represented by the median of recordings (*N*=6-9). Scale bars for all FID chromatograms and EAD traces are 25 pA and 375 µV, respectively.

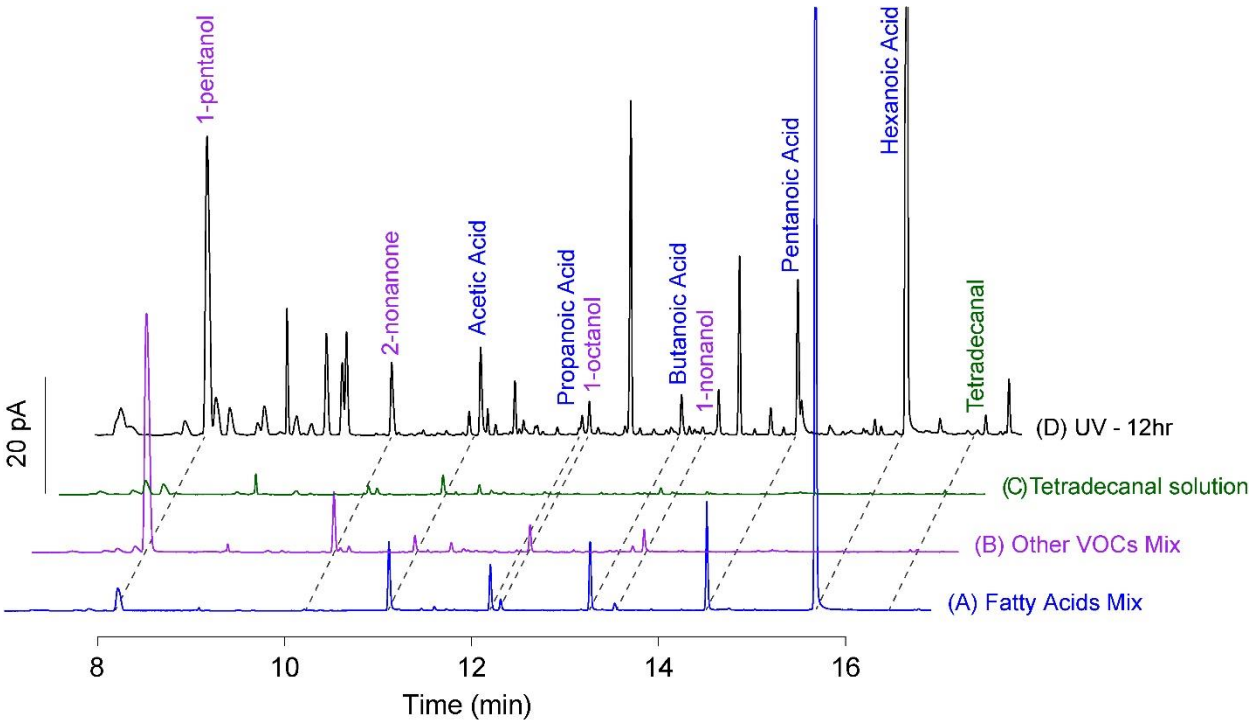

Figure S9. Analysis of volatiles from synthetic mixes. Gas chromatograms of headspace collections from (A) the Fatty Acids Mix, (B) the Other VOCs Mix, (C) tetradecanal and (D) unsaturated CHCs exposed to UV for 12 hr. Synthetic mixes were loaded to filter papers in borosilicate vials and volatiles were sampled by SPME. Dashed lines indicate the same compounds among the chromatograms. Identified EAD-active compounds are shown on top of (D). Fatty acids are coloured in blue, alcohols and the ketone in purple, and tetradecanal in green.

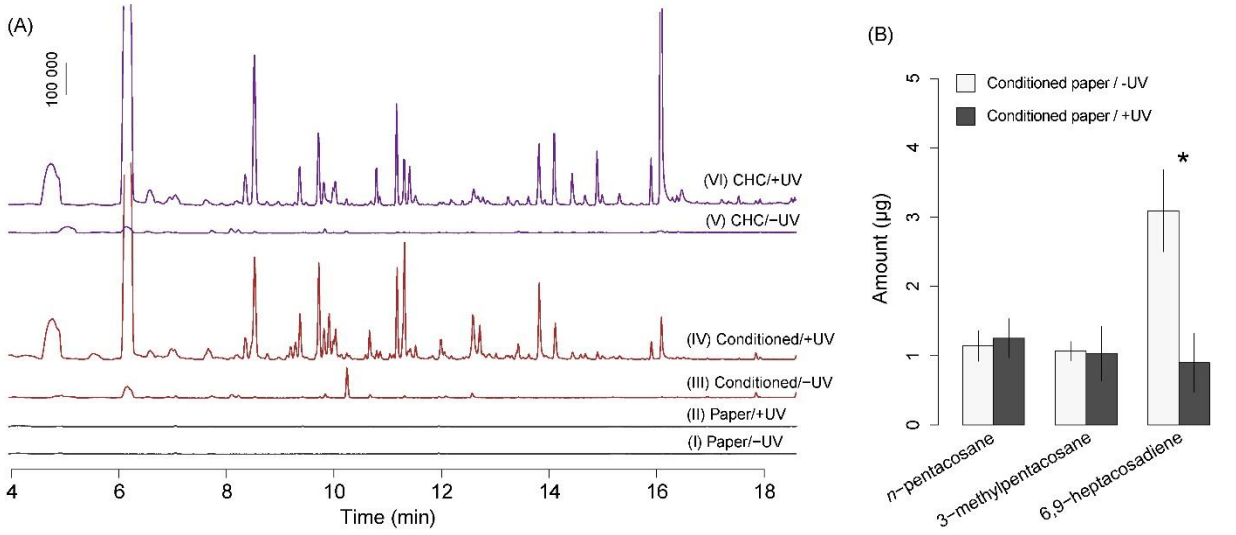

Figure S10. Effect of UV-radiation on CHCs deposited on conditioned filter papers. After conditioning and exposure to UV-radiation, CHCs remaining on pieces of cockroach-conditioned and control filter papers (1.5 x 2.0 cm) were extracted. (A) Total ion chromatograms of odorants collected from clean filter paper protected from (I) and exposed (II) to UV-radiation, shelters conditioned with cockroaches protected from (III) and exposed (IV) to UV-radiation, and extracted *P. americana* CHC alkenes protected from (V) and exposed (VI) to UV-radiation. Scale bar: 100 000 abundance. (B) Degradation of main cuticular hydrocarbons (*n*-pentacosane, 3-methylpentacosane and 6,9-heptacosadiene) of *P. americana* deposited on paper shelters after exposure to UV-radiation. Bars represent mean amounts of CHCs (µg ±SE). Only amounts of the unsaturated hydrocarbon, 6,9-heptacosadiene, significantly decreased after UV exposure (ANOVA, $*p<0.05$, $N=6$).

Table S1. Test statistics of comparisons of behavioural responses to CHC breakdown products (Fig. 1A-L, lme), behavioural responses to synthetic mixes (Fig. 3A-F, lme), behavioural responses to conditioned shelters (Fig. 4AI-III, lme) and quantification of CHCs on conditioned shelters (Fig. S10, ANOVA).

Experiment	**Analysis**	**d.f.**	***t***	***p***	**Figure**
Behavioural response to CHC breakdown products	Blank – Blank	31	0.849	0.396	1A
	Air 1 day – Blank	31	0.932	0.352	1B
	Air 3 days – Blank	31	0.529	0.597	1C
	Air 7 days – Blank	31	6.84	<0.001	1D
	Water vapor 1 day – Blank	59	0.474	0.636	1E
	Water vapor 3 days – Blank	59	2.413	<0.05	1F
	Water vapor 7 days – Blank	59	4.97	<0.01	1G
	UV-exposed blank - UV-protected blank	31	0.992	0.321	1H
	UV 1 hr – Control	31	0.713	0.476	1I
	UV 2 hr – Control	29	3.727	<0.001	1J
	UV 6 hr – Control	31	9.215	<0.001	1K
	UV 12 hr – Control	29	11.17	<0.001	1L
Behavioural response to identified compounds	Blank – Blank	53	0.836	0.403	3A
	Other VOCs Mix – Blank	59	0.916	0.359	3B
	Fatty Acids Mix – Blank	59	1.481	0.138	3C
	Fatty Acids + Other VOCs Mix - Blank	59	4.379	<0.001	3D
	Other VOCs Mix High Concentration- Blank	59	3.759	<0.001	3E
	Fatty Acids Mix High Concentration - Blank	59	1.776	0.076	3F
Behavioural responses to cockroach-conditioned shelters	Clean paper / +UV - Clean paper / -UV	39	0.28	0.78	4AI
	Conditioned / -UV - Clean paper / -UV	39	3.893	<0.001	4AII
	Conditioned / +UV - Clean paper / +UV	39	3.822	<0.001	4AIII
Accumulation and degradation of CHCs on shelters	*n*-pentacosane: -UV - +UV	9	0.312	0.761	S10B
	3-methylpentacosane: -UV - +UV	9	0.08	0.937	S10B
	6,9-heptacosadiene: -UV - +UV	9	3.014	<0.05	S10B