Research Article

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Behavioral responses of field-collected German cockroaches to pyrethroids and pyrethroid-formulated insecticides

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Abstract

BACKGROUND: Pyrethroids are synthetic insecticides with low mammalian toxicity and broad-spectrum activity across insects. One major challenge with pyrethroids is their perceived repellency. This perception can influence decisions made by pest control operators, especially when insecticides are used to reduce insect entry into or movement within structures. One major indoor pest that has been repeatedly shown to be repelled by some pyrethroids is the German cockroach, *Blattella germanica*. However, most experiments evaluating pyrethroid repellency in the German cockroach have used end-point assays, which do not provide information on the movement that led to the final position. Therefore, we evaluated the kinetic behavioral response of field-collected German cockroaches to five pyrethroid-based products and their active ingredients (A.I.) in open behavioral arenas using advanced video tracking software. In addition, in an effort to compare our free-moving experiments with end-point assays, we evaluated sheltering behavior using two-choice harborage arrestment assays where German cockroaches were provided a choice between pyrethroid-treated and untreated shelters.

RESULTS: All pyrethroid-formulated products and their respective A.I.'s failed to affect field-collected German cockroach movement behavior in free-moving assays, while positive controls (DEET, corn mint oil) resulted in reduced time spent by German cockroaches in treated areas. However, despite their willingness to move over pyrethroids-treated surfaces, field-collected German cockroaches displayed a reduced propensity to arrest on pyrethroids treated tents.

CONCLUSION: While most pyrethroids/pyrethroid-formulated products affected German cockroach arrestment, pyrethroids and pyrethroid-formulated products failed to change German cockroach movement behavior in free-moving assays. These results indicate the pyrethroids tested act as contact irritants rather than true-spatial repellents on field-collected German cockroaches. This distinction is critical to refining pest management strategies involving pyrethroids.

Keywords: pyrethroids; repellency; irritancy; live video tracking; arrestment assay

1 INTRODUCTION

Pyrethroids are synthetic insecticides originally derived from natural pyrethrins.[1] They possess low mammalian toxicity and broad-spectrum activity across insects.[2,3] Pyrethroids in various forms (e.g., residual, aerosol) are commonly used by pest management professionals and homeowners indoors for the control of various urban pests such as ants, bed bugs, cockroaches, and occasional invaders. Despite varying contact/residual effectiveness, they have been reported to possess repellent properties against a variety of insects, including mosquitoes, and German cockroaches.[4–11]

Repellency is a complex topic, which incorporates how an insect interacts with a chemical and how it responds after that interaction.[12,13] Due to the complexity of repellency, and the variety of ways it can be classified based on observations of insect behavior, Deletre *et al.*[14] categorized repellency into five categories; (1) true/spatial repellents (orient away from the source without direct contact), (2) contact irritancy/excito-repellency/landing inhibition (orient away from source after direct contact), (3) odor masking (reduction of the attractiveness of host or disruption of the location of the host through masking effect), (4) visual masking (reduction of the attractiveness of host or disruption of the location of the host through masking effect), and (5) antifeedant/deterrence (feeding activity disrupted by contact or ingestion of a chemical). Physiological detection mechanisms, chemical concentrations, experimental conditions, and additional experimental variables all contribute to the insect's response, making it difficult to compare results among different studies on different species.[14,15] Despite a broad characterization of the behavioral responses of arthropods towards pyrethroids, many reports focus on mosquitoes or other disease vectors, typically capable of flight. While urban pests, such as the German cockroach, *Blattella germanica* (L.), have been evaluated for their behavioral responses to pyrethroids, most assays focus exclusively on endpoint data, with no or limited information on real-time movement.[9,16–21] While these assays provide critical

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information on German cockroach behavior, they fail to capture data on the behavior/movement which led to the final position.

German cockroaches are almost exclusively indoor pests, and remain one of the most important urban pest species globally. This is due in large part to their ubiquity in disadvantaged communities and the negative effects they have on human health (e.g., production of allergens, transmission of pathogenic microorganisms).[22–25] While multiple control options are available, residual products containing pyrethroids remain one of the most commonly used options for residual control by pest control operators.[9,26] Despite the common use of pyrethroids, efficacy is often poor due to extensive and pervasive pyrethroid resistance in German cockroaches.[27–32] Despite poor efficacy, pyrethroids are also assumed to be highly repellent based on prior reports.[9,16,18,21,33,34] As such, and based on a general definition of repellency provided by these papers, it would be expected that German cockroaches would (1) avoid treated areas, and (2) move out of treated areas into neighboring areas. Furthermore, there is also a risk of treatment contamination if products such as gel baits (another commonly employed control tool for German cockroaches) are exposed to pyrethroids, which could lead to loss of efficacy if these items are no longer visited or consumed.[35] Therefore, it is critical to characterize the behavioral responses of field-collected German cockroaches to pyrethroid-treated surfaces.

To comprehensively document the behavioral responses of field-collected German cockroaches towards pyrethroid insecticides, we evaluated five professional-grade products containing pyrethroids and their respective active ingredients in open behavioral arenas using live video tracking and in harborage arrestment (endpoint) assays. The results from this work are discussed in relation to cockroach control and the current misconceptions surrounding the use of pyrethroids in and around structures.

2 MATERIALS AND METHODS

2.1 Cockroach population and rearing

An apartment-collected population of German cockroaches (CC29; also referred to as 'field-collected German cockroaches' or 'German cockroaches' as per relevancy through the manuscript) was used in all experiments. Population CC29 was originally collected in 2019 from an apartment in Raleigh, North Carolina, and has been maintained in the laboratory since this time. Population CC29 is also known to be resistant to pyrethroids (between 10- and 100-fold resistant).[36] For consistency among behavioral assays, only adult males were used for all experiments. Cockroaches were reared under standard laboratory conditions (25 ± 2 °C, 40–70% RH, 12:12 L:D cycle) inside plastic bins with cardboard harborages. Water and food (Mazuri Rat & Mouse Diet; Mazuri Exotic Animal Nutrition, St Louis, MO) were provided *ad libitum*. Cockroaches were acclimated for a minimum of 1 week to the behavioral assay room before experiments were initiated. It should be noted, we were unable to test a pyrethroid-susceptible (laboratory) population due to high mortality/moribundity (40%) observed in this population during preliminary trials after only 30 min of exposure.

2.2 Insecticides

Five pyrethroid-based insecticide products and their active ingredients (AIs) were used (Table 1). Insecticides and corresponding AIs were used at both the label rate and in some situations, 10-times the label rate. Multiple concentrations of DEET (N, N-Diethyl-meta-toluamide; Fisher Scientific, Hampton, NH) and corn mint oil (*Mentha arvensis*; Rocky Mountain Oils; Orem, UT) were used as positive controls. Pyrethroid-based products were diluted in water according to the label rate, whereas active ingredients and positive controls were diluted in acetone.

2.3 Free moving assays using video tracking

All experiments took place in a dedicated behavioral room (~45.3 m^3). During experiments, there was no air flow, with natural air exchange allowed in between assays by leaving the door open and/or running the air conditioning in the room. Behavioral assays were conducted in open-top plastic arenas (size: Length: 13.7 cm, breadth: 8.3 cm, height: 5.9 cm; PrepNatural, Philadelphia, PA), divided into two equal halves (Fig. 1(a)). Two equal pieces of white paper (type: seamless background paper; Savage Paper Specialties, Chandler, AZ) lined the bottom of the arena. Each piece of paper was first treated with either an insecticide solution, individual AI, or solvent (acetone, water) only. Active ingredients (including DEET and corn mint oil) were diluted in acetone and applied to the paper substrate directly (1 mL) using a pipette. The amount of active ingredient applied was either a range of doses (DEET, corn mint oil) or an amount based on the label rate (see Table 1). Formulated pyrethroid products were diluted in water and sprayed using a glass spray bottle (50 mL) (Wedama Spray bottle, Amazon) at a rate of 1 gal per 1000 ft^2 (4.07 μL/cm^2) applied to a 58 cm^2 area (236 μL), per label instructions. Shortly after evaporation of the solvent (acetone or water), the treated paper was attached to the bottom of the arena using double-sided tape, and the side walls were greased lightly with mineral oil to keep cockroaches in the arena. In control tests, both halves were treated with solvent only (Fig. 1). In the control group, one side was designated the untreated side and the other side was designated the treated side at random. This allowed us to compare between the treated side of the control group and the treated side of all experimental treatment groups. In experimental treatment tests, one half was treated with insecticide, and the other half was treated with solvent only (Fig. 1). The behavioral data generated from treated zones of treatment groups was used for behavioral analysis.

Behavioral recordings were conducted using high-resolution GigE cameras (model: acA1300-60gc; Basler AG; Ahrensburg, Germany) with manual iris and focus lens (4.5–12.5 mm with 0.5 in. manual iris) and fitted with IR pass filters (model: Infrarot RG 850; Heliopan US, White Plains, NY). One IR illuminator light (model: CM-IR110; CMVision, Houston, TX) was used to illuminate each arena. EthoVision XT version 15 software (Noldus Information Technology Inc., Lessburg, VA) was used to capture and live track German cockroach behavior during a 30 min recording period (Fig. 2). The static subtraction method was used for detecting insects/insect movement, with a sample rate of 25 frames/s. To initiate behavioral assays, German cockroaches were introduced and acclimated in the center of the solvent-only side of the arena for 5 min by placing a single cockroach under an inverted 60 mL plastic jars (Consolidated Plastics, Stow, OH) upside down. After 5 min, cockroaches were released from the jars, and tracking was started. Arenas were set up with alternating spatial arrangements (switching the solvent-only and treatment sides) to control for any side bias. In total, 14–16 replications were conducted for each testing parameter. Video recordings where more than 60 s of tracking were lost (not able to be processed by EthoVision) were discarded. It should also be noted that no mortality in field-collected population (inability to right themselves and make coordinated movement away when being

Table 1. List of pyrethroids insecticides used in the assays

Insecticides	Active ingredients (AI)	Final AI Concentration (%)*	Amount of AI Applied (μg/cm²)†	Manufacturer
Suspend Polyzone®	Deltamethrin	0.06	2.44	Envu‡, Cary, NC
Demand CS®	Lambda-cyhalothrin	0.06	2.44	Syngenta Crop Protection, LLC, Greensboro, NC
Cy-Kick CS®	Cyfluthrin	0.10	4.06	BASF Corporation, Research Triangle Park, NC
Permethrin SFR®	Permethrin	0.5	20.34	Control Solutions, Inc., Pasadena, TX
Demon Max®	Cypermethrin	0.2	8.13	Syngenta Crop Protection, LLC, Greensboro, NC

*A 1% weight by volume solution equates to 1 g/100 mL.
† Amount applied is based on an application rate of 1 gal/1000 ft² (4.07 μL/cm²) applied over a 58 cm² area (236 μL).
‡ Formerly Bayer Environmental Sciences, Cary, NC.

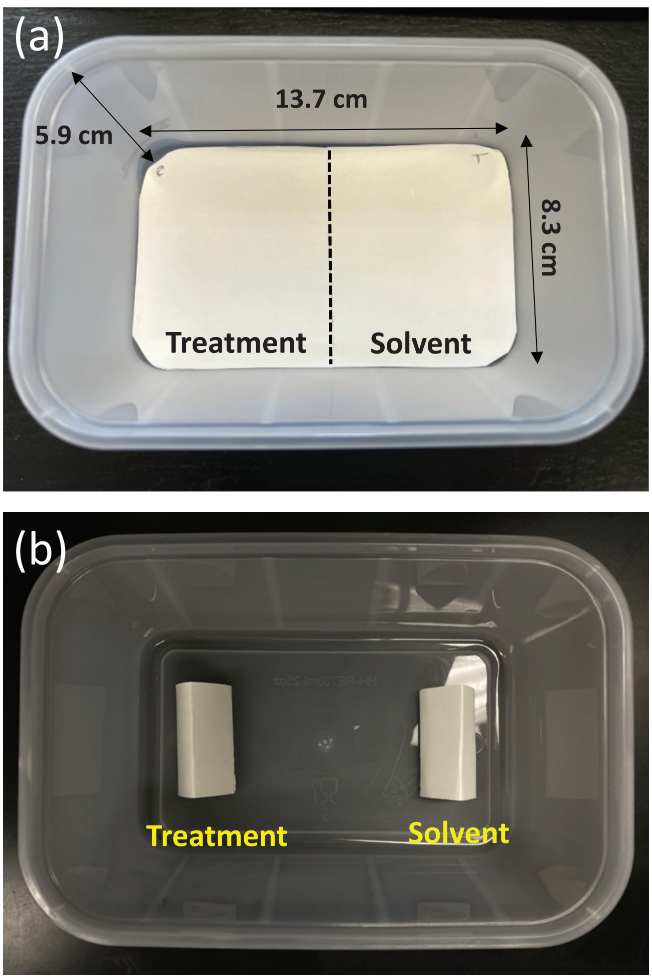

Figure 1. Behavioral tracking and harborage arrestment arena designs. (a) free-moving behavioral tracking assays were performed in rectangular arenas, with one half treated with an insecticide (AI or formulated product) and the other half treated with solvent only (both sides treated with solvent in control assays). (b) Harborage arrestment two choice assays were conducted in similar arenas where two triangular tents were provided on two sides of the arena, one tent treated with insecticide (AI or formulated product) and the other tent treated with solvent only (both sides treated with solvent in control assays).

probed) was observed in any of the free moving experiments after the 30 min experimental period.

2.4 Harborage arrestment assays

To make these results comparable to previous arrestment (end-point) assays, we also conducted harborage arrestment assays, looking for harborage preference between insecticide treated and untreated shelters. Harborage arrestment assays were conducted in open-top plastic arenas (size: Length: 13.7 cm, breadth: 8.3 cm, height: 5.9 cm; PrepNatural) (Fig. 1(b)). Each arena contained two harborage tents constructed from white paper (type: seamless background paper; Savage Paper Specialties). The paper substrates were cut into strips (3.6 × 8 cm), then treated with either an insecticide solution, individual AI, or solvent-only control as described above. Both sides of the paper were treated (58 cm²) then allowed to dry. After drying, each paper strip was folded four times lengthwise to produce a 3.6 cm long triangular tube with two overlapping sides (subsequently referred to as a tent or harborage). Each arena contained a solvent-only treated tent and either a second solvent-only treated tent (control) or an insecticide treated tent. The placement of these harborages was alternated between trials to control for any side bias. The side walls of containers were greased with mineral oil to prevent cockroach escape.

After setup, a single male cockroach was released into the middle of the arena using soft forceps 4 h before the photophase began. Cockroaches were then given 24 h to make a choice, with position checked 4 h into the photophase on day 2 (after 32 h of time spent in the arena). When determining final position, cockroaches were scored as selecting a specific harborage tent if they made any contact with that tent. Those cockroaches not touching any of the harborages were counted as non-responders. Control groups were conducted similarly and run concurrently with the experimental treatments. In the control group, both harborages were treated with solvent only (water or acetone). Very limited mortality in field-collected population (inability to right themselves and make coordinated movement away when being probed) was observed among all assays performed (6 out of 475 total assays performed) at the conclusion of each assay.

2.5 Statistical analysis

Time spent, distance traveled, and velocity of cockroaches in treated zones (positive controls, pyrethroid active ingredients,

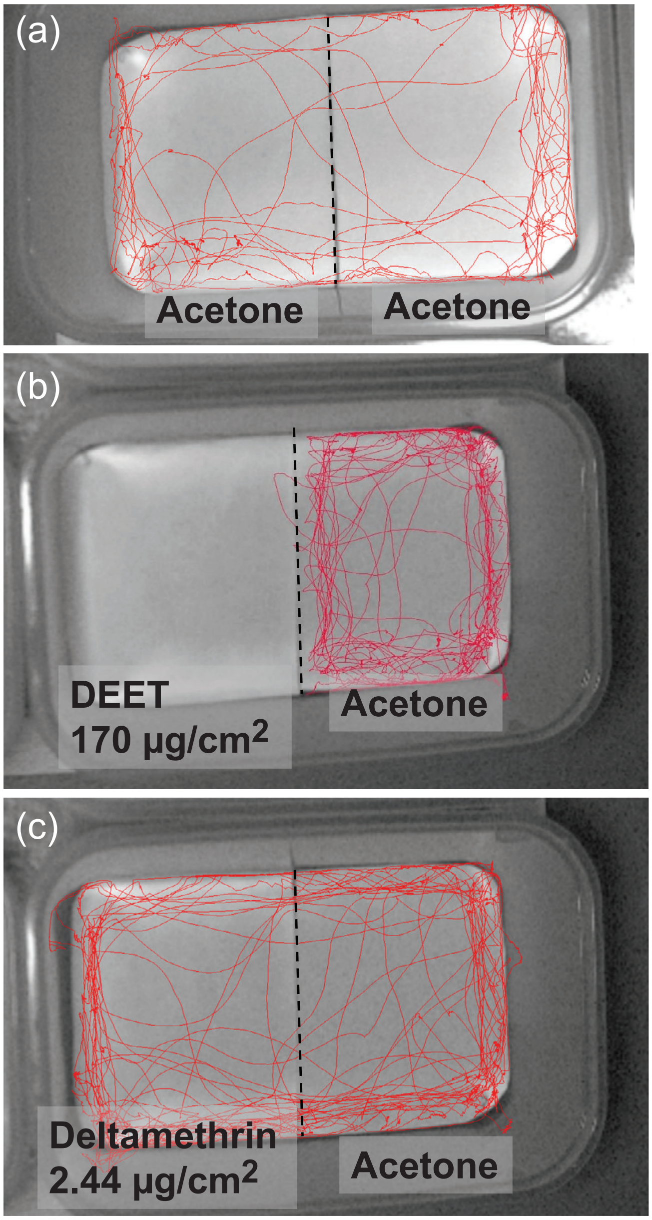

Figure 2. Examples of tracks from the video analysis (30 min). (a) Example recording when a German cockroach was tested with acetone only, showing free movement between both sides. (b) Example recording when a German cockroach was faced with a true repellent (DEET), showing clear side preference for the solvent (acetone) treated side. (c) Example recording when a German cockroach was tested with the pyrethroid Deltamethrin. Similar results were obtained for all pyrethroids tested.

and formulated products) were compared to corresponding values from the treated zones in control groups (e.g., acetone or water only) using one-way ANOVA followed by Dunnett's test at the significance level of $\alpha = 0.05$. A Chi-square goodness of fit test was used to compare the responses of cockroaches to insecticide treated *versus* solvent-only treated tents in all harborage arrestment bioassays, with the null hypothesis that cockroaches do not respond differentially to treated tents, that is they display equal preference for both sides of the assay. All tests were conducted in SPSS Version 26 (IBM Corp., Armonk, NY).

3 RESULTS

3.1 Reponses of cockroach to insecticides in live video tracking assays

German cockroaches displayed strong spatial repellency towards DEET and corn mint oil, both in a dose-dependent manner (Fig. 3). Cockroaches spent significantly less time in the DEET-treated zones at concentrations greater than or equal to 90 µg/cm^2 compared to the control (Fig. 3(a); $F_{5,90} = 27.78$; $P < 0.001$), although there was no difference when tested at a concentration of 30 µg/cm^2. Similar to DEET, German cockroaches spent significantly less time in corn mint oil treated zones at concentrations greater than or equal to 160 µg/cm^2 compared to the control (Fig. 3(b); $F_{3,64} = 19.36$; $P < 0.001$).

In contrast to DEET and corn mint oil, German cockroaches displayed no spatial repellency towards pyrethroids (AIs) applied at the label rate, spending similar amounts of time on treated and untreated surfaces (Fig. 4(a); $F_{5,\ 90} = 0.283$, $P > 0.05$). When pyrethroids were tested at 10-times the label rate, we found treatment (pyrethroid) to have a significant effect on the time spent in the treated zone (Fig. 4(b); $F_{5,85} = 2.51$, $P = 0.036$). However, the only pyrethroid found to cause significant reductions in time spent on the treated zones was deltamethrin, with all other pyrethroids resulting in no significant changes in the time spent in the treated zone when compared with the control (Fig. 4(b)). German cockroaches also showed no changes in the time they spent in zones treated with pyrethroid-formulated products, spending similar amounts of time on treated and untreated surfaces (Fig. 5; $F_{5,86} = 1.37$, $P > 0.05$).

In addition to spending less time in sides treated with DEET and corn mint oil, German cockroaches also traveled less distance in areas treated with higher concentrations of DEET (90, 170, 860, 1720 µg/cm^2; $F_{5,90} = 46.64$; $P < 0.001$) and corn mint oil (780, 1550 µg/cm^2; $F_{3,64} = 10.49$, $P < 0.01$) treated areas compared to controls (Table 2). In contrast, there were no differences in the distance traveled by cockroaches in pyrethroid treated (AI at label rate) areas when compared to the controls (Table 2; $F_{5,91} = 2.18$, $P = 0.062$). When we increased the concentration of pyrethroids A.I.s to 10-times the label rate, we found insecticide treatment to have a significant effect on distance traveled (Table 2; $F_{5,85} = 4.17$, $P = 0.002$). However, Dunnett's test revealed only distance traveled on cyfluthrin treated areas was significantly greater than the control (Table 2), with the significant differences due to differences among the individual compared with each other rather than the control. Treatment had a significant effect on distance traveled in pyrethroid-formulated product treated areas ($F_{5,86} = 2.52$, $P = 0.035$). However, when values were compared with the control using Dunnett's test, there were no significant differences.

German cockroaches were also found to move significantly faster in areas treated with DEET (170, 860, 1720 µg/cm^2; $F_{5,89} = 8.78$; $P < 0.001$) and corn mint oil (780, 1550 µg/cm^2; $F_{3,64} = 20.13$, $P < 0.001$) (Table 2) in comparison to control. Treatment had a significant effect on movement speed of German cockroaches for AIs at the label rate ($F_{5,91} = 2.94$, $P = 0.016$) and the pyrethroid formulated products ($F_{5,86} = 2.58$, $P = 0.032$), but not for AIs tested at 10-times the label rate ($F_{5,85} = 1.97$, $P = 0.091$; Table 2). However, when values were compared with the control using Dunnett's test, there were no significant differences detected.

3.2 Reponses of cockroach to insecticides in harborage arrestment assays

German cockroaches showed no harborage preference when both sides were treated with solvent only (Fig. 6; acetone *versus* acetone:

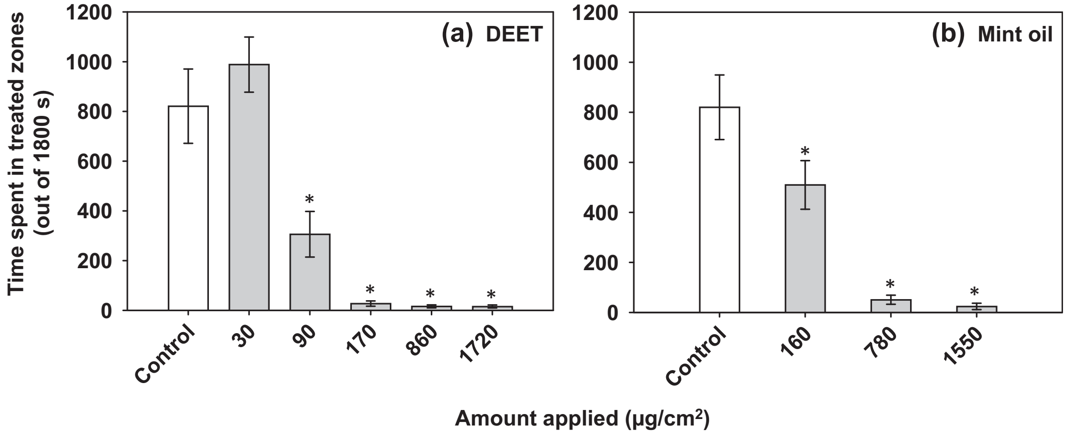

Figure 3. Time spent by German cockroaches in treated areas (DEET, corn mint oil). Asterisks (*) Indicate significant differences from control within each group (Dunnett's test, $P < 0.05$). Error bars represent the standard error of the mean.

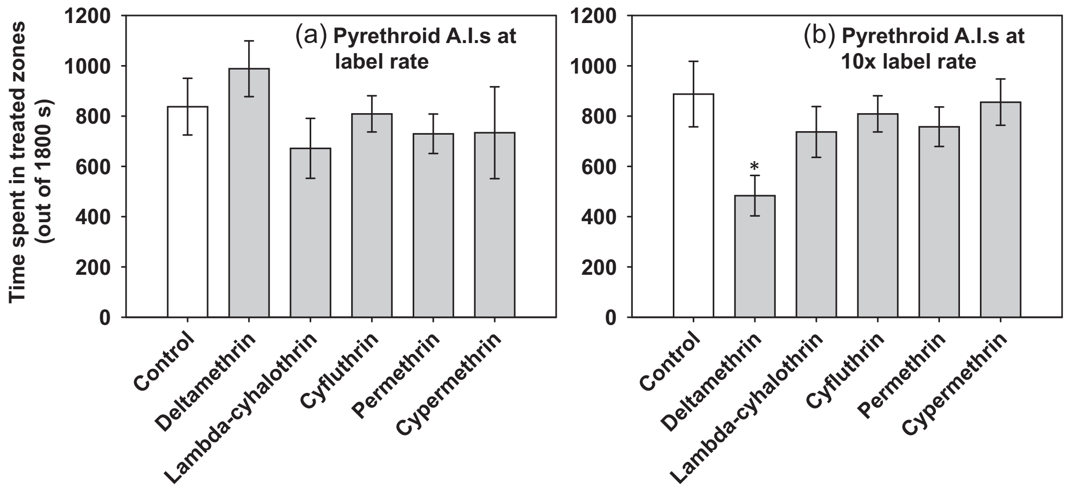

Figure 4. Time spent by German cockroaches in treated areas (pyrethroid Ais diluted in acetone). Asterisks (*) Indicate significant differences from control (or 0% concertation) within each group (ANOVA followed by Dunnett's test, $P < 0.05$). Error bars represent the standard error of the mean.

$\chi^2_{1,23} = 0.043$, $P = 0.835$; water *versus* water: $\chi^2_{1,27} = 0.333$, $P = 0.564$). However, German cockroaches showed a significant preference for the solvent (acetone) treated tent when the other tent was treated with DEET (170 μg/cm²) or corn mint oil (780 μg/cm²) (Fig. 6; DEET 170 μg/cm²: $\chi^2_{1,33} = 33$, $P < 0.001$; corn mint oil 780 μg/cm²: $\chi^2_{1,23} = 19.17$, $P < 0.001$). When the tents were treated with pyrethroids, we found similar results to the positive controls (except for permethrin), where German cockroaches showed a significant preference to arrest on the solvent treated tents *versus* those treated with deltamethrin (Fig. 6; $\chi^2_{1,25} = 4.84$, $P = 0.28$), lambda-cyhalothrin (Fig. 6; $\chi^2_{1,28} = 9.14$, $P = 0.002$), cyfluthrin (Fig. 6; $\chi^2_{1,40} = 12.10$, $P < 0.001$) and cypermethrin (Fig. 6; $\chi^2_{1,26} = 7.53$, $P = 0.006$). However, German cockroaches showed no significant preference when tents were treated with permethrin (Fig. 6; $\chi^2_{1,34} = 1.05$, $P = 0.303$). German cockroaches also showed significant preference for solvent (water) treated tents *versus* those treated with formulated pyrethroids products including Suspend Polyzone (Fig. 7; $\chi^2_{1,18} = 5.55$, $P = 0.018$), Demand CS (Fig. 7; $\chi^2_{1,22} = 6.54$, $P = 0.11$), Cy-Kick CS (Fig. 7; $\chi^2_{1,20} = 7.20$, $P = 0.007$), and Demon Max (Fig. 7; $\chi^2_{1,35} = 10.31$, $P = 0.001$). However, German cockroaches showed no significant preference when tents were treated with Permethrin SFR (Fig. 7; $\chi^2_{1,47} = 1.04$, $P = 0.307$).

4 DISCUSSION

Repellency is a complex process that has traditionally been oversimplified, with many studies focusing only on the final position of insects and not movement patterns or behaviors. Our work investigating the behavioral responses of field-collected German cockroaches towards pyrethroids was designed based on the German cockroach's morphological characteristics (non-flying) and known foraging behavior (indoors and nocturnal).[37] Therefore, we assessed the response of German cockroaches to pyrethroids using advanced video tracking in free-moving arenas, where one side was treated with chemicals and the other is treated with solvent control. This experimental design has been used previously to measure the repellency of chemicals against other species of cockroaches.[38,39] Prior to testing pyrethroids, we validated the experimental design using both DEET and corn mint oil, which were previously reported to repel German cockroaches.[39–41] In our study, both DEET and corn mint oil significantly reduced German cockroach movement on treated surfaces, in a dose-dependent manner. In addition, cockroaches were shown to travel a shorter distance at a higher velocity in the area treated with DEET and corn mint oil, further supporting a direct/immediate behavioral response that would be expected

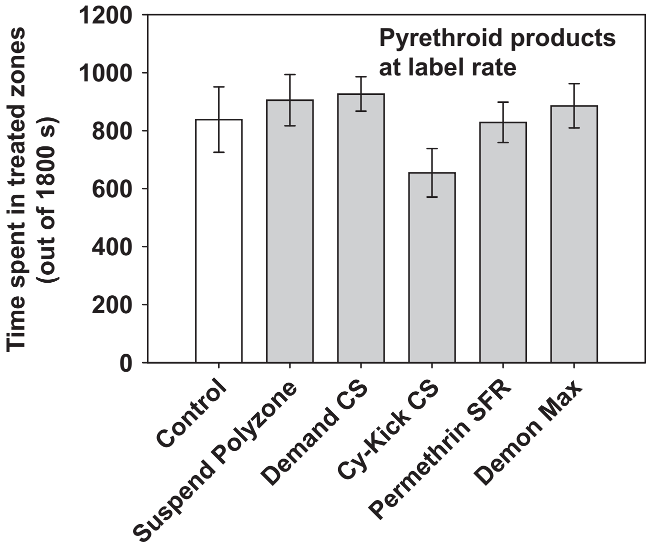

Figure 5. Time spent by German cockroaches in treated areas (pyrethroid-formulated products diluted in water). There was no significant difference in control (0% concentration) and any of the pyrethroid-formulated product treatments (ANOVA, $P > 0.05$). Error bars represent the standard error of the mean.

of a true repellent. However, it should be noted that the assays used in this study did not evaluate behavior without direct contact. It should also be noted that contrary to a recent report that showed adult male German cockroaches spend approximately 30% of their time on DEET treated surfaces at a concentration of 195 μg/cm^2,[39] we found adult males spent less than 2% of their time on DEET treated surfaces at a similar concentration (170 μg/cm^2), as would be expected with a repellent such as DEET.

In contrast, most pyrethroid AIs tested (1× and 10×) and products formulated with these AIs failed to significantly alter German cockroach movement behavior in free-moving assays when applied at the label rate, with the exception of deltamethrin (10× label rate, decrease in time spent in treated zone) and cyfluthrin (10×, increase in distance traveled). Further, no differences in the distance traveled and velocity of cockroaches on AI and product treated surfaces compared to the untreated surface show that pyrethroid AIs and formulated products fail to alter movement behavior. If these products acted as true repellents, we would have expected to see behavior similar to what was observed with DEET and mint oil, namely a decrease in the distance traveled and an increase in velocity. These data show a consistent pattern of only limited changes in German cockroach movement behavior when exposed to some of the most commonly used indoor

Table 2. Distance traveled and average velocity of German cockroaches in treated areas

Treatment type	Insecticides	Concentration (μg/cm^2)	Distance traveled (cm) ± SEM†	Velocity (cm/s) ± SEM†
Positive controls	DEET	0.00 (control)	219.2 ± 22.1	0.42 ± 0.07
		30	260.2 ± 18.3	0.32 ± 0.04
		90	140.1 ± 17.8*	0.85 ± 0.11
		170	28.6 ± 10.2*	1.73 ± 0.32*
		860	27.4 ± 6.2*	1.62 ± 0.29*
		1720	28.8 ± 8.2*	2.00 ± 0.34*
	Corn mint oil	0.00 (control)	219.1 ± 17.8	0.37 ± 0.06
		160	200.1 ± 31.8	0.50 ± 0.04
		780	95.8 ± 27.4*	2.11 ± 0.2*
		1550	37.8 ± 15.6*	1.45 ± 0.2*
AIs at label rate	Control	-	336.4 ± 50.1	0.48 ± 0.07
	Deltamethrin	2.44	191.9 ± 20.2	0.34 ± 0.04
	Lambda-cyhalothrin	2.44	338.5 ± 77.9	0.50 ± 0.05
	Cyfluthrin	4.06	334.1 ± 49.2	0.48 ± 0.07
	Permethrin	20.34	339.7 ± 38.2	0.50 ± 0.05
	Cypermethrin	8.13	360.3 ± 29.5	0.66 ± 0.06
AIs at 10× label rate	Control	-	290.9 ± 25.4	0.42 ± 0.05
	Deltamethrin	24.4	247.0 ± 36.0	0.60 ± 0.06
	Lambda-cyhalothrin	24.4	438.1 ± 76.2	0.58 ± 0.06
	Cyfluthrin	40.6	487.8 ± 54.4*	0.60 ± 0.04
	Permethrin	203.4	332.9 ± 34.7	0.47 ± 0.05
	Cypermethrin	81.3	417.7 ± 34.7	0.52 ± 0.03
Formulated products at label rate	Control	-	321.88 ± 56.7	0.41 ± 0.06
	Suspend polyzone	2.44	319.2 ± 37.3	0.39 ± 0.05
	Demand CS	2.44	337.7 ± 26.5	0.37 ± 0.02
	Cy-Kick CS	4.06	299.6 ± 36.8	0.48 ± 0.03
	Permethrin SFR	20.34	447.8 ± 53.0	0.55 ± 0.04
	Demon Max	8.13	446.5 ± 33.4	0.54 ± 0.04

*Indicates significant difference from control (or 0% concertation) within each group (ANOVA followed by Dunnett test, $P < 0.05$).
† SEM represents the standard error of the mean.

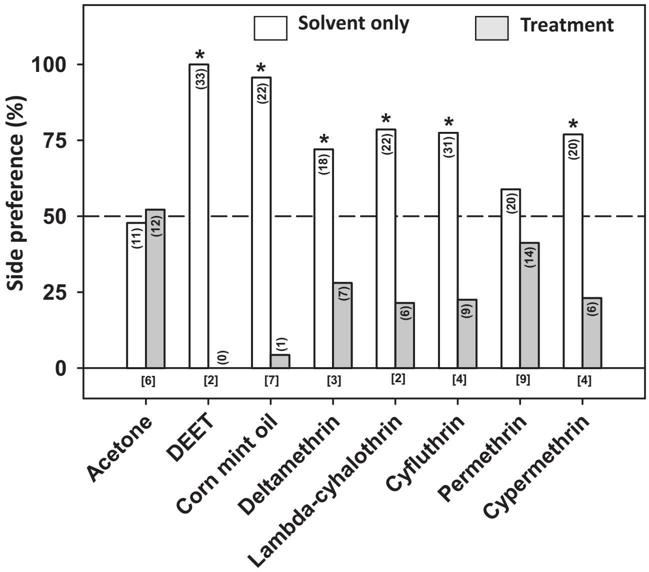

Figure 6. Responses of German cockroaches to shelters treated with DEET, corn mint oil, and pyrethroid active ingredients (diluted in acetone). An asterisk (*) indicates a significant preference for one side of the two-choice assay (Chi-square test, $P < 0.05$). The number of cockroaches that responded is indicated in parentheses for each choice, and the number of cockroaches that failed to respond (i.e., rested on neither tent) is indicated in brackets below each set of choices for each assay.

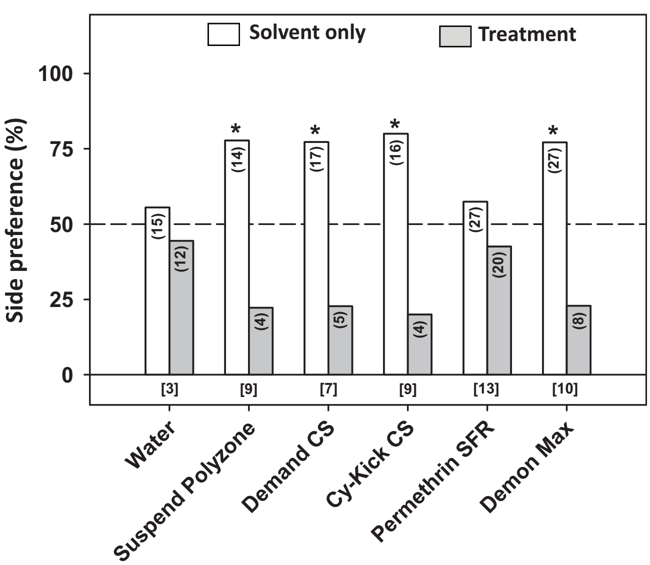

Figure 7. Responses of German cockroaches to shelters treated with pyrethroid formulated products (diluted in water). An asterisk (*) indicates a significant preference for one side of the two-choice assay (Chi-square test, $P < 0.05$). The number of cockroaches that responded is indicated in parentheses for each choice, and the number of cockroaches that failed to respond (i.e., rested on neither tent) is indicated in brackets below each set of choices for each assay.

pyrethroids. Interestingly, our results using live video tracking match those reported for kissing bugs, which showed no behavioral changes when exposed to eight different pyrethroids (includes all AIs tested in the present study) when tested in a similar video tracking assay.[42] These results superficially would appear to contradict the findings reported in previous studies that employed Ebeling choice boxes,[17] where various formulated products containing pyrethroids (the same Ais used in our study) were reported to be repellent against the German cockroach.[9,16,20] The Ebeling Choice box is an enclosed box composed of two compartments (dark side and light side), where the treatment (pesticide) is applied to the dark compartment, with food and water being provided in the light side. The insects are released into the light side, allowed to acclimate, then allowed to freely move among both compartments through a small hole that connects the sides.[17] If insecticides are repellents/irritants, German cockroaches are expected to be found in the light compartment during the photophase, despite their preference to be in dark areas. While this tool has provided useful information in regards to treatments in voids, it fails to provide information on movement patterns of cockroaches over treated surfaces, as would be encountered by German cockroaches following standard crack/crevice type treatments while the cockroaches foraged. As such, both assays appear to provide accurate information on German cockroach behavior, but show that different results can be obtained when the behavioral measurements being collected are different among assays. This also highlights the need to conduct multiple assay types to ensure the behavior of interest is completely characterized.

To better understand the behavioral response of field-collected German cockroaches to pyrethroids, we also conducted two choice harborage arrestment assays. All pyrethroid formulated products (except Permethrin SFR) and all pyrethroid Ais (except permethrin) resulted in a significant reduction in the number of German cockroaches arresting on treated surfaces, which suggests that pyrethroid treated surfaces act as contact irritants, thus forcing German cockroaches to move off of/away from these surfaces. Overall, the harborage arrestment assays provided supporting evidence for previous work done using Ebeling Choice box assays.[9,16] However, when the harborage assays are viewed alongside the free moving assays, it is clear that the pyrethroids tested do not act as true repellents, but rather contact irritants. While not well-described in cockroaches, pyrethroid irritancy has been observed in mosquitoes that rest on several pyrethroid-treated surfaces such as nets and window screens.[6,43,44] In another study, when resting sites are treated with pyrethroids, *Aedes aegypti* did not simply shift towards the untreated sites but rather they became agitated first (increased flights) before shifting to untreated sites.[45]

It is also important to note that our study used a field-collected pyrethroid-resistant population of German cockroaches only. Because we were unable to test a pyrethroid-susceptible population, we are unable to determine what role (if any) pyrethroid resistance plays in the behaviors we observed. That said, the use of a pyrethroid-resistant population is critical to understanding German cockroach behavior, given the ubiquity of pyrethroid resistance in field-collected German cockroaches.[27–32] Future work should explore what role pyrethroid resistance plays in the behavioral response of German cockroaches to insecticides.

Although we tested several pyrethroids and pyrethroid-based products that are commonly used indoors, there are some pyrethroids that have low vapor pressure and high volatility which have been shown to possess true repellent (spatial) properties (e.g., transfluthrin, vapothrin, d-allethrin).[46–50] While much of this work has been done with mosquitoes, a recent study by Boné *et al.*[46] found that d-allethrin and vapothrin vapors spatially repelled German cockroaches while also increasing spontaneous neuronal activity. However, in the same study, permethrin neither spatially repelled nor increased spontaneous electrical activity in the German cockroach.[46] This study confirmed that the vapor pressure (and thus volatility) of pyrethroids plays a major role in determining repellency in German cockroaches. Therefore, it is not surprising that we found no spatial repellency given the low-volatility of the pyrethroids we tested.

When our results are interpreted alongside current pest management practices, there are major implications for the pest management industry. There is a widely held belief that cockroaches and other pests will not walk on/across pyrethroid-treated surfaces, despite this treatment being one of the major tools of pest management industries. Our results demonstrate that German cockroaches will walk freely across pyrethroid treated surfaces. Pyrethroids are also commonly used as perimeter/barrier treatments for a variety of pests. While not tested in this study, these results suggest that while pyrethroids may cause mortality upon contact, they may not keep arthropods from entering structures, although this should be further tested with additional arthropods known to occasionally invade homes. The results from the current study also suggest that cockroach gel baits (another commonly used German cockroach tool) may not be affected by placement on pyrethroid treated surfaces since German cockroaches will freely forage over these areas, but this remains to be tested.

In summary, the pyrethroids tested in the current study prevented arrestment of field-collected German cockroaches on most of the treated surfaces, but failed to alter movement behavior in most free moving assays. Therefore, non-volatile pyrethroids should be characterized as contact irritants rather than true repellents to field-collected German cockroaches. It should also be noted that behaviors are often a function of the assays used to assess them; therefore, future work should look to incorporate a variety of assays when possible, such that behaviors are fully characterized. In the future, multiple life stages, populations, and other cockroach species should be included in the studies to confirm this behavioral pattern further.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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