Journal of Economic Entomology, 118(4), 2025, 1842–1849
https://doi.org/10.1093/jee/toaf124
Advance Access Publication Date: 19 June 2025
Research

ENTOMOLOGICAL SOCIETY OF AMERICA
SHARING INSECT SCIENCE GLOBALLY

OXFORD

Household and Structural Insects

Increase in insecticide susceptibility after sublethal exposure to deltamethrin in the German cockroach (Blattodea: Ectobiidae)

Shao-Hung Dennis Lee[1,*,†], Man Zhao[1,2,†], and Chow-Yang Lee[1,*]

[1]Department of Entomology, University of California, Riverside, CA, USA
[2]Present address: Henan International Laboratory for Green Pest Control, College of Plant Protection, Henan Agricultural University, Zhengzhou, China

[*]Corresponding authors. Shao-Hung Dennis Lee, Department of Entomology, University of California, Riverside, CA 92521, USA (Email: slee228@ucr.edu); Chow-Yang Lee, Department of Entomology, University of California, Riverside, CA 92521, USA (Email: chowyang.lee@ucr.edu).

[†]These authors contributed equally to this work.

Subject Editor: Arthur Appel

Received on 2 March 2025; revised on 18 April 2025; accepted on 25 April 2025

The German cockroach, *Blattella germanica* (L.), is a major urban insect pest primarily controlled by insecticides, including pyrethroid sprays, fipronil baits, and indoxacarb baits. However, widespread pyrethroid resistance increases the probability that cockroaches will survive treatments in the field. Many insecticides are applied concurrently or repetitively on a periodic basis, meaning survivors have the chance to be re-treated, but any lasting physiological effects from initial survival may impact the performance of subsequent treatments. We investigated the effects of sublethal deltamethrin exposure on the susceptibility of susceptible (UCR) and resistant (Ryan) strains of German cockroaches. Ineffective pyrethroid treatment was simulated by treating cockroaches with the LD_{20} of deltamethrin. Survivors were treated again with the same dose of deltamethrin, a food source containing the LC_{50} of fipronil, or a food source containing the LC_{50} of indoxacarb. Both strains of cockroaches experienced greater mortality when treated with deltamethrin 3 d (49.8% to 67.6%), after the sublethal exposure. No impact on fipronil or indoxacarb susceptibility was observed after pre-treatment with deltamethrin. Because surviving a deltamethrin treatment did not induce tolerance to deltamethrin or other insecticides, this suggests no within-generation drawback of current conventional management practices of applying multiple treatments in the same site. Furthermore, sequential exposure to deltamethrin caused greater mortality than expected, indicating that this insecticide may remain useful even if initial efficacy is low. Comprehensive examinations of additional resistant strains treated with formulated products are warranted to understand whether these effects may impact field control.

Keywords: pyrethroid resistance, fipronil, indoxacarb, sublethal, hormetic priming

Introduction

German cockroaches, *Blattella germanica* (L.), are cosmopolitan urban pests that infest indoor environments such as residences, food preparation areas, hotels, schools, and hospitals (Lee and Wang 2021). Insecticides are the most effective tools to address active cockroach infestations, but insecticide resistance continues to pose significant problems (Scharf and Gondhalekar 2021). In particular, well-documented pyrethroid resistance in field-collected strains discourages reliance on such formulations (DeVries et al. 2019, Hu et al. 2020, Boné et al. 2022, Lee et al. 2022a, Tisgratog et al. 2023, Gordon et al. 2024, Kruaysawat et al. 2024). However, pyrethroids remain common for German cockroach control because of their ease of application, quick action, and low cost, which are important qualities in low-income areas disproportionately affected by infestations (Zha et al. 2018, Miller and Smith 2020).

Continuing to use ineffective insecticides will only worsen resistance issues, resulting in a growing probability of sublethal exposures

© The Author(s) 2025. Published by Oxford University Press on behalf of Entomological Society of America. All rights reserved. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.

Downloaded from https://academic.oup.com/jee/article/118/4/1842/8169665 by University of California, Riverside user on 18 September 2025

occurring in the field. At label rates, pyrethroid products typically achieve low kill on field-collected strains through brief exposure. High mortality rates are only achievable through continuous exposure assays (Lee et al. 2022b, Gordon et al. 2024). Extended exposure is unlikely in scenarios that allow natural cockroach behavior such as avoidance of pyrethroid-treated zones, which can further compound the chance of treatment survivors when combined with other resistance mechanisms (Boné et al. 2020, Lee et al. 2022b, Gaire et al. 2024).

Sublethal exposure to insecticides can have unintended impacts that may affect aspects of management beyond low efficacy. The activity or expression of detoxification enzymes such as cytochrome P450 (Khan and Matsumura 1972, Poupardin et al. 2008, Baek et al. 2010, Rix et al. 2016, Cui et al. 2018, Yang et al. 2018, Ullah et al. 2020), esterase (Riaz et al. 2009, Rix et al. 2016), and glutathione S-transferase (Cui et al. 2018) has been shown to change in many pest species after treatment with neurotoxic insecticides. Although many authors suggest that these changes translate to increased tolerance toward additional pesticide stress, within-generation in vivo studies of insecticide-to-insecticide exposure are rare and often inconsistent (Guedes et al. 2017). Susceptible *Aedes aegypti* L. larvae experienced increased temephos tolerance after permethrin exposure but did not experience altered tolerance to permethrin itself (Poupardin et al. 2008). There was no difference between single and double sequential exposure of spinetoram or permethrin on *Drosophila suzukii* (Matsumura) survival (Deans and Hutchison 2022). Dieldrin exposure increased the susceptibility of a field-collected German cockroach strain to carbaryl but not to diazinon (Khan and Matsumura 1972).

There is a lack of understanding of how sublethal exposure to pyrethroids affects an individual German cockroach's toxicological response to subsequent treatments, despite the high likelihood of pyrethroid resistance and asynchronous exposure occurring in the field. For example, cockroaches can receive multiple nonlethal doses of pyrethroids within their lifetimes, such as through repeated visitation to the same application or exposure to different treatments. Similarly, cockroaches may survive an initial pyrethroid treatment and be exposed to bait insecticide later. A single infested site often contains different insecticide applications because of professional management programs incorporating a multiple-attack strategy, unregulated applications by non-professionals, or leftover applications from previous treatments. Baits and sprays are two common formulations that may be used simultaneously in overlapping areas (Fardisi et al. 2019, Appel and Rust 2021, Rust et al. 2022). As a result, cockroaches must traverse pyrethroid-treated zones to reach bait applications, creating a guaranteed situation of sublethal exposure followed by additional treatment if the population is sufficiently resistant. Given the ubiquity of pyrethroids in conventional German cockroach control, knowledge on sublethal pyrethroid exposure will have significant implications on management decisions.

This study aimed to determine whether deltamethrin sublethal exposure alters the susceptibility of German cockroaches toward subsequent insecticide treatment. First, the toxicity of deltamethrin, fipronil, and indoxacarb (the latter two are common bait toxicants) was determined for a susceptible and resistant strain of German cockroach through topical or crude bait (treated dog food) bioassays. Next, the survivors of pyrethroid treatment were obtained by subjecting German cockroaches to a low dose of deltamethrin (LD_{20}). These survivors later received another dose of deltamethrin or were provided food sources containing fipronil or indoxacarb (LC_{50}) to simulate bait exposure. The resulting mortalities are compared, and the implications are discussed.

Materials and Methods

Cockroach Strains

The Ryan strain was collected from an apartment in San Jose, CA in 2020 with previously documented resistance toward insecticides, including deltamethrin, fipronil, and indoxacarb (Lee et al. 2022a, 2022b). It has not been subjected to resistance selection since collection. The UCR strain is an insecticide susceptible strain that has been reared in the laboratory for > 40 yr without insecticide exposure. Both strains were maintained and tested under ambient laboratory conditions of 24 ± 2 °C, 30–50% RH, and 12:12 L:D photoperiods. They were provided dog food (Purina Dog Chow, Nestlé Purina Petcare, St. Louis, MO) and distilled water. Adult males were used for all experiments because they have the most consistent physiology among the stages and sexes of *B. germanica* (Appel et al. 1983, Abd-Elghafar and Appel 1992).

Baseline Toxicity of Topically Applied Deltamethrin

A range of six doses causing > 0% and < 100% mortality at 3 d was prepared by diluting technical grade deltamethrin (≥ 98%, Sigma Aldrich Corporation, St. Louis, MO) in acetone. Adult male cockroaches from the Ryan strain were anesthetized with a brief exposure to CO_2 (~10 s). A dose of deltamethrin (0.5 µl) was topically applied to the abdominal sternites using a microapplicator (Instrumentation Specialties, Seward, NE). Controls were treated with acetone only. Treated cockroaches were provided with dog food and water; mortality (inability to move or right itself within 30 s when probed with forceps) was recorded at 3 d. A total of 6 to 12 replicates of ten cockroaches each were conducted for each treatment dose (n = 430) with 7 replicates for the control. Data for the UCR strain was retrieved from Lee et al. 2022a, and the LD_{50}, LD_{20}, and RR_{50} (resistance ratio) at 3 d were generated for the UCR and Ryan strains using PoloPlus (LeOra Software LLC, Petaluma, CA).

Baseline Toxicity of Fipronil and Indoxacarb in Food Source

Crude baits consisting of dog food (same type as rearing) treated with fipronil or indoxacarb were created by crushing dog food to powder in a mortar and adding specific quantities of technical grade fipronil (≥ 96%, Syngenta Crop Protection LLC, Greensboro, NC, USA) or indoxacarb (52.7%, Syngenta Crop Protection LLC, Greensboro, NC, USA) diluted in acetone to create a range of concentrations causing > 0% and < 100% mortality at 5 d. The dog food was mixed thoroughly with insecticide and dried under a fume hood overnight. Ten adult male cockroaches of the UCR or Ryan strains were introduced to arenas (27.5 × 20 × 9 cm) containing a cardboard harborage, a water source, filter paper on the bottom to increase traction, and fluon on the walls to prevent escape. At the start of the trial, ~0.5 g of treated dog food was introduced to the arena in a weigh boat, and mortality (inability to move within 30 s when disturbed with forceps) was recorded at 5 d. Controls received acetone-treated dog food. No alternative food source was provided. Six replicates were conducted for 6 concentrations of fipronil, and 4 to 7 replicates were conducted for 5 to 6 concentrations of indoxacarb (n = 300 to 360). Controls were replicated 6 times. The LC_{50} and RR_{50} at 5 d for fipronil and indoxacarb were calculated for the UCR and Ryan strains using PoloPlus.

Downloaded from https://academic.oup.com/jee/article/118/4/1842/8169665 by University of California, Riverside user on 18 September 2025

Impact of Sublethal Deltamethrin Exposure on Deltamethrin Susceptibility

The LD_{20} deltamethrin for the UCR and Ryan strains (UCR: 2.9 ng/insect; Ryan: 16.6 ng/insect; Table 1) were prepared by diluting technical grade deltamethrin in acetone. Adult males of the UCR or Ryan strains were anesthetized with a brief exposure to CO_2. The strain-specific LD_{20} (0.5 µl) was topically applied to the abdominal sternites using a microapplicator. Controls were treated with acetone. A total of 30 to 34 treatment replications of 10 cockroaches each were performed for each strain, and controls were replicated 27 to 28 times. Cockroaches were provided with dog food and water, and mortality (inability to move or right itself within 30 s when probed with forceps) was recorded at 3 d. Three or seven days after treatment, surviving cockroaches were anesthetized with a brief exposure to CO_2 again. The strain-specific LD_{20} was topically applied to the abdominal sternites using a microapplicator. Controls were treated with acetone. Cockroaches were provided with dog food and water, and mortality was recorded at 3 d. Treatments and controls were conducted with 4 to 8 replicates of 9 to 11 cockroaches each, depending on survivors of the first treatment, for a total $n = 42–79$. Final % mortalities from deltamethrin → deltamethrin, deltamethrin → acetone, and acetone → acetone treatments were corrected (Henderson and Tilton 1955) and compared with Mantel-Haenszel chi-square tests using R version 4.3.1. Only mortalities from the second (final) treatments were compared; dead cockroaches from the first treatment were excluded from % mortality calculations.

Impact of Sublethal Deltamethrin Exposure on Fipronil or Indoxacarb Susceptibility

Adult males of the UCR or Ryan strains were anesthetized with a brief exposure to CO_2. The strain-specific LD_{20} was topically applied (0.5 µl dose) to the abdominal sternites using a microapplicator. Controls were treated with acetone. Treated cockroaches were provided with dog food and water. Three or seven days after treatment, surviving cockroaches were transferred in groups of 10 to arenas (27.5 × 20 × 9 cm) containing a cardboard harborage, a water source, filter paper on the bottom to increase traction, and fluon on the walls to prevent escape. Crude baits consisting of dog food treated with strain-specific LC_{50}'s of fipronil or indoxacarb (see Table 2) for the UCR and Ryan strains were prepared by crushing dog food to powder and adding technical grade fipronil or indoxacarb in acetone. A 0.5 g quantity of fipronil or indoxacarb-treated dog food was introduced to the arenas in weigh boats, and mortality (inability to move within 30 s when disturbed with forceps) was recorded daily for 14 d. Controls received acetone-treated dog food. A total of 5 to 6 replications of 10 cockroaches each were conducted for each treatment combination and control. For treatment groups exposed to fipronil or indoxacarb-treated dog food, survivorship curves and mean survival times were generated using Kaplan–Meier analysis, and survivorship was compared between deltamethrin pre-treated and acetone pre-treated groups using log-rank tests in SPSS 29.0.

Results

Baseline Toxicity of Topically Applied Deltamethrin

The topical LD_{50} of deltamethrin at 3 d for UCR strain adult males was 4.6 ng/insect, and the LD_{20} was 2.9 ng/insect (Table 1) (Lee et al. 2022a). For the Ryan strain, the LD_{50} was 42.9 ng/insect, and the LD_{20} was 16.6 ng/insect (Table 1). The Ryan strain was 9.3 times resistant to deltamethrin (RR_{50}) (Table 1).

Baseline Toxicity of Fipronil and Indoxacarb in Food Source

The LC_{50} of fipronil-treated dog food was 0.3 µg/g for UCR strain adult males and 1.1 µg/g for Ryan strain adult males, resulting in an RR_{50} of 3.6 (Table 2). The LC_{50} of indoxacarb-treated dog food was 12.3 µg/g for UCR strain adult males and 40.5 µg/g for Ryan strain adult males, resulting in an RR_{50} of 3.3 (Table 2).

Impact of Sublethal Deltamethrin Exposure on Deltamethrin Susceptibility

The LD_{20} of deltamethrin of the UCR strain (2.9 ng/insect) caused 21.7% mortality at 3 d in adult males (Table 3). When deltamethrin survivors were treated with deltamethrin again 3 d later (deltamethrin → deltamethrin), they experienced significantly greater mortality (49.8%) compared to acetone → deltamethrin (24.9%) ($\chi^2 = 12.2$, df = 1, $P < 0.001$) and acetone → acetone (8.5%) ($\chi^2 = 35.9$, df = 1, $P < 0.0001$) groups (Fig. 1A; Table 3). When the time between treatments was 7 d, deltamethrin → deltamethrin treated cockroaches reached 33.3% mortality, which was significantly greater than acetone → acetone (6.8%) ($\chi^2 = 20.1$, df = 1, $P < 0.0001$) but not acetone → deltamethrin (25.2%) ($\chi^2 = 0.9$,

Table 1. Toxicity of topically applied deltamethrin at 3 d for adult males of the UCR and Ryan strains.

Strain	n	LD_{20} (95% CI) (ng/insect)	LD_{50} (95% CI) (ng/insect)	RR_{50}[b] (95% CI) (ng/insect)	Slope	SE	χ^2 (df)
UCR[a]	820	2.9 (2.5–3.3)	4.6 (4.1–5.1)	-	4.251	0.287	8.7 (6)
Ryan	430	16.6 (7.7–25.4)	42.9 (28.6–65.2)	9.3 (7.8–11.1)	2.047	0.176	11.5 (4)

[a]From bioassays conducted in Lee et al. (2022a).
[b]LD_{50} of the Ryan strain/LD_{50} of the UCR strain.

Table 2. Toxicity of insecticide-treated dog food at 5 d for adult males of the UCR and Ryan strains.

Insecticide	Strain	n	LC_{50} (95% CI) (µg/g)	RR_{50}[a] (95% CI) (µg/g)	Slope	SE	χ^2 (df)
fipronil	UCR	360	0.3 (0.3–0.4)	-	3.678	0.346	7.7 (4)
	Ryan	360	1.1 (0.9–1.4)	3.6 (2.8–4.5)	1.653	0.17	2.6 (4)
indoxacarb	UCR	310	12.3 (10.4–14.8)	-	3.677	0.365	3.0 (3)
	Ryan	300	40.5 (29.6–51.4)	3.3 (2.7–4.0)	2.44	0.288	5.3 (4)

[a]LC_{50} of the Ryan strain/LC_{50} of the UCR strain.

Downloaded from https://academic.oup.com/jee/article/118/4/1842/8169665 by University of California, Riverside user on 18 September 2025

Table 3. Mortality of UCR strain adult males from deltamethrin (topical LD_{20}) or acetone after 3 d or 7 d from the first treatment of deltamethrin (topical LD_{20}) or acetone.

Treatment					
First	Second	Time between treatments	*n*	% mortality 3 d after final treatment[a]	SE
acetone	-	-	270	3.7%	0.01
deltamethrin LD_{20}	-	-	300	21.7%	0.01
acetone	acetone	3 d	47	8.5%	0.6
acetone	deltamethrin LD_{20}	3 d	50	24.9%	0.9
deltamethrin LD_{20}	acetone	3 d	42	6.8%	0.8
deltamethrin LD_{20}	deltamethrin LD_{20}	3 d	59	49.8%	0.9
acetone	acetone	7 d	74	6.8%	0.3
acetone	deltamethrin LD_{20}	7 d	76	25.2%	0.6
deltamethrin LD_{20}	acetone	7 d	61	3.3%	0.3
deltamethrin LD_{20}	deltamethrin LD_{20}	7 d	57	33.3%	0.8

[a]Henderson-Tilton corrected mortalities for deltamethrin LD_{20} → deltamethrin LD_{20} and acetone → deltamethrin LD_{20}

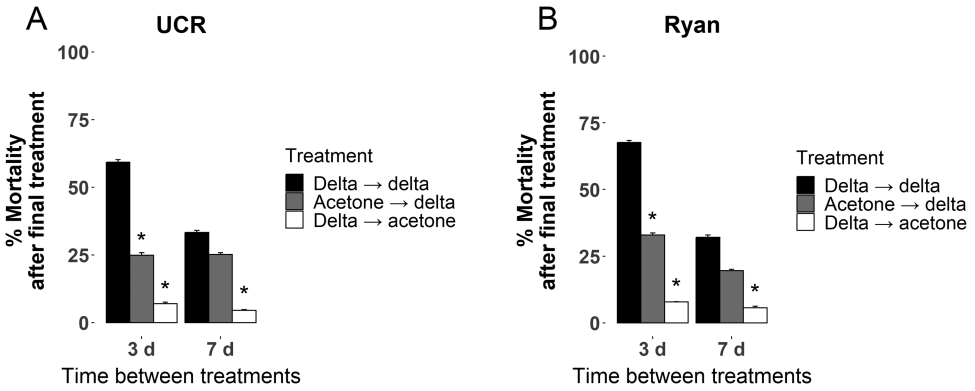

Fig. 1. Mortality of (A) UCR or (B) Ryan strain adult males from deltamethrin (topical 3 d LD_{20}) or acetone administered 3 d or 7 d after a first sublethal exposure to deltamethrin (topical 3 d LD_{20}) or acetone. Asterisks indicate a significant difference from deltamethrin → deltamethrin treatment (Mantel-Haenszel chi-square test; Bonferroni corrected $\alpha = 0.025$).

Table 4. Mortality of Ryan strain adult males from deltamethrin (topical LD_{20}) or acetone after 3 d or 7 d from the first treatment of deltamethrin (topical LD_{20}) or acetone.

Treatment					
First	Second	Time between treatments	n	% mortality 3 d after final treatment[a]	SE
acetone	-	-	280	0.7%	0.01
deltamethrin LD_{20}	-	-	340	22.6%	0.01
acetone	acetone	3 d	60	3.3%	0.1
acetone	deltamethrin LD_{20}	3 d	60	32.9%	0.8
deltamethrin LD_{20}	acetone	3 d	55	7.3%	0.5
deltamethrin LD_{20}	deltamethrin LD_{20}	3 d	60	67.6%	0.8
acetone	acetone	7 d	50	6.3%	0.6
acetone	deltamethrin LD_{20}	7 d	79	19.6%	0.5
deltamethrin LD_{20}	acetone	7 d	61	4.9%	0.4
deltamethrin LD_{20}	deltamethrin LD_{20}	7 d	57	32.1%	0.8

[a]Henderson-Tilton corrected mortalities for deltamethrin LD_{20} → deltamethrin LD_{20} and acetone → deltamethrin LD_{20}

df = 1, $P = 0.35$) (Fig. 1A; Table 3). Acetone control mortality did not exceed 8.5% for the UCR strain (Table 3).

For Ryan strain adult males, the LD_{20} of deltamethrin (16.6 ng/insect) caused 22.6% mortality at 3 d (Table 4). Application of deltamethrin 3 d after a first deltamethrin treatment (deltamethrin → deltamethrin) resulted in significantly greater mortality (67.6%) compared to acetone → deltamethrin (32.9%) ($\chi^2 = 21.6$, df = 1, $P < 0.0001$) and acetone → acetone (3.3%) ($\chi^2 = 84.2$, df = 1, $P < 0.0001$) (Fig 1B; Table 4). A 7 d difference between treatments resulted in 32.1% mortality for deltamethrin → deltamethrin, which was significantly greater than acetone → acetone (6.3%) ($\chi^2 = 20.1$, df = 1, $P < 0.0001$) but not acetone → deltamethrin (25.2%) ($\chi^2 = 0.9$, df = 1, $P = 0.36$) (Fig 1B; Table 4). Acetone control mortality did not exceed 7.3% (Table 4).

Downloaded from https://academic.oup.com/jee/article/118/4/1842/8169665 by University of California, Riverside user on 18 September 2025

Impact of Sublethal Deltamethrin Exposure on Fipronil or Indoxacarb Susceptibility

No significant differences in survivorship within 14 d were found in the UCR or Ryan strain between acetone and deltamethrin (LD_{20}) pre-treated groups when exposed to treated dog food (fipronil or indoxacarb LC_{50}), regardless of when the treatment was started (3 d or 7 d after pre-treatment) (Fig. 2A–D; Fig. 3A–D; Supplementary Table S1). Mean survival times under treatments were 5.5–8.4 d (Supplementary Table S1).

Discussion

The strain-specific LD_{20} of deltamethrin caused 21.7% and 22.6% mortality on the UCR and Ryan strains, respectively (Tables 3 and 4). Based on the stipulations of the probit model, an accurate LD_{20} kills the most susceptible 20% of a population, leaving a surviving 80% that is tolerant of a single LD_{20} treatment. However, when LD_{20} survivors were treated with the LD_{20} again after 3 or 7 d, they experienced 32.1% to 67.6% mortality instead of 3.3% to 7.3% as observed in the deltamethrin → acetone control groups, demonstrating that deltamethrin sublethal exposure significantly increased their susceptibility beyond baseline. Furthermore, deltamethrin → deltamethrin caused greater mortality than acetone → deltamethrin when the second treatment was administered after 3 d, indicating that treated cockroaches were more susceptible than the original untreated population. The majority of deltamethrin-mediated death in the German cockroach occurs within the first 24 to 48 h (Tisgratog et al. 2023) as does most pyrethroid metabolism (Anspaugh et al. 1994), indicating that these changes remain beyond the initial acute toxicity phase and are not due to the combined effects of excessive insecticides in the insect body.

Deltamethrin has a variable detoxification fate in German cockroaches and can be subjected to P450, esterase, and GST-catalyzed reactions (Lee et al. 2022b). P450s are the most important detoxification enzymes, but their utilization is costly because they are only induced upon reception of a substrate (eg an insecticide), and they require NADPH as a high-energy cofactor (Yu 2014). Susceptibility changes from repeated deltamethrin exposure may result from the inability to meet detoxification demand from overburdened resources in a manner functionally similar to starvation, which has been shown to increase susceptibility in German cockroaches (Lee et al. 1996). Significance was detected in both 3 and 7 d groups, though the decrease in magnitude between time points tentatively suggests that the effect is temporary (Fig. 1A–B). An energy or resource-mediated effect may be reversed given enough time with access to food and water, which was provided for treated cockroaches in between treatments. Insects treated sub-lethally earlier than 3 d or later than 7 d were not utilized to avoid the influence of acute toxicity and natural mortality, respectively, on final mortality counts. Future examination of additional time points between 3 and 7 d or beyond can elucidate the existence of a time-dependent relationship.

Unlike the repeated deltamethrin topicals, sublethal exposure to deltamethrin had no impact on indoxacarb or fipronil-treated dog food (Fig. 2; Fig. 3). Because these insecticides belong to different classes and undergo different metabolic fates, impacts limited to deltamethrin-specific pathways would not necessarily influence the toxicity of fipronil and indoxacarb (Yu 2014). For example,

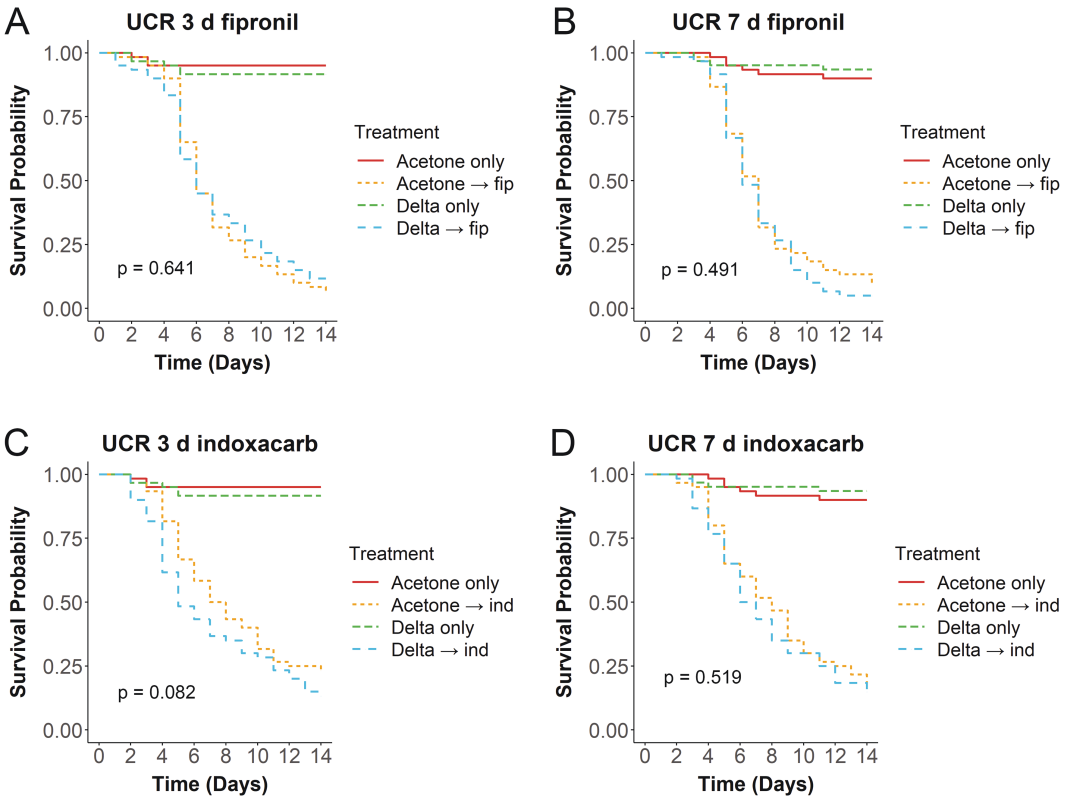

Fig. 2. Survivorship of UCR adult males exposed to fipronil- or indoxacarb-treated dog food (5 d LC_{50}) 3 d or 7 d after a first sublethal exposure to deltamethrin (topical 3 d LD_{20}). Treatment groups are as follows: (A) fipronil treatment started 3 d after deltamethrin treatment, (B) fipronil treatment started 7 d after deltamethrin treatment, (C) indoxacarb treatment started 3 d after deltamethrin treatment, and (D) indoxacarb treatment started 7 d after deltamethrin treatment. Statistical significance indicates a difference in survivorship between deltamethrin pre-treated and acetone pre-treated groups (log-rank test; $\alpha = 0.05$).

Downloaded from https://academic.oup.com/jee/article/118/4/1842/8169665 by University of California, Riverside user on 18 September 2025

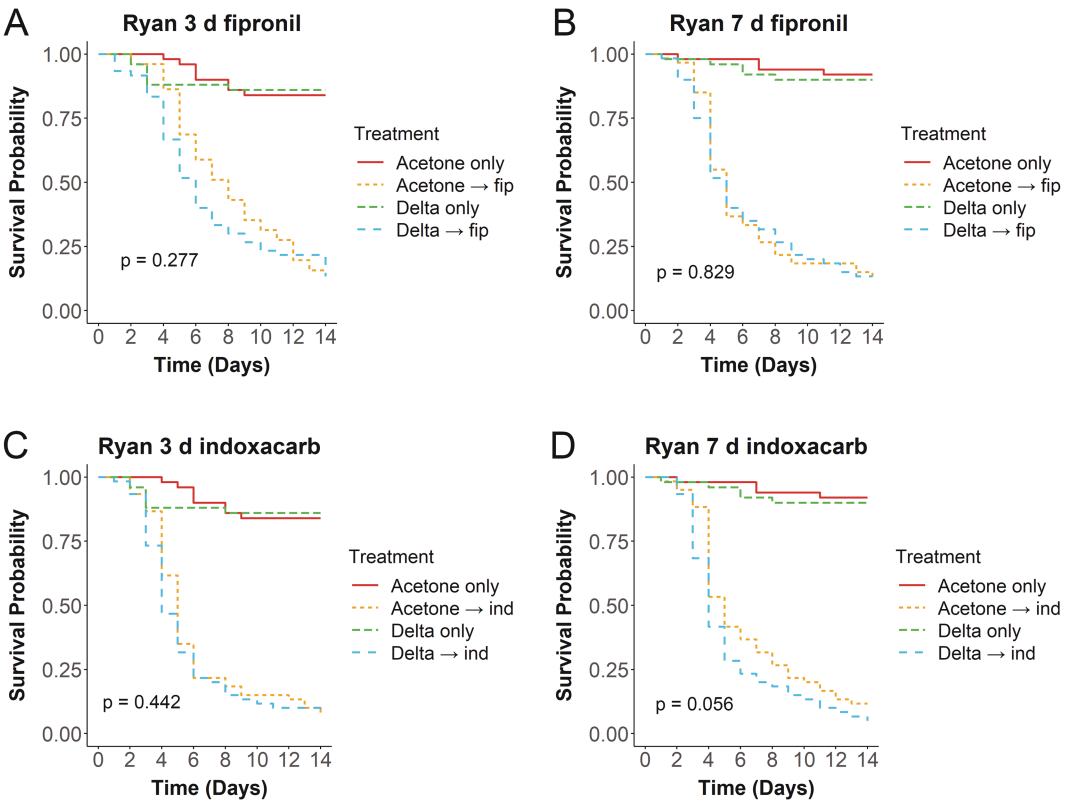

Fig. 3. Survivorship of Ryan adult males exposed to fipronil- or indoxacarb-treated dog food (5 d LC_{50}) 3 d or 7 d after a first sublethal exposure to deltamethrin (topical 3 d LD_{20}). Treatment groups are as follows: (A) fipronil treatment started 3 d after deltamethrin treatment, (B) fipronil treatment started 7 d after deltamethrin treatment, (C) indoxacarb treatment started 3 d after deltamethrin treatment, and (D) indoxacarb treatment started 7 d after deltamethrin treatment. Statistical significance indicates a difference in survivorship between deltamethrin pre-treated and acetone pre-treated groups (log-rank test; $\alpha = 0.05$).

deltamethrin is commonly detoxified via carboxylesterase cleavage, but ester linkages are not present in fipronil, and indoxacarb is bioactivated instead (Gondhalekar et al. 2016, Wolfe and Scharf 2022). Similarly, there is also evidence of strain-level detoxification specificity for deltamethrin that would limit cross-tolerance potential. For example, while the P450 isoform *CYP4G19* has been linked to both pyrethroid and fipronil resistance (Hu et al. 2021), overexpression of this isoform was absent in all pyrethroid-resistant strains of a different study (Tseng et al. 2024). Because the general P450 inhibitor piperonyl butoxide has been shown to affect indoxacarb and fipronil toxicity in the German cockroach, the lack of impact of deltamethrin sublethal exposure on indoxacarb or fipronil-treated dog food implies a more specific mechanism at play (Gondhalekar et al. 2016, Lee et al. 2022b). Specificity may be exacerbated if these changes are also highly localized, as deltamethrin was administered topically while fipronil and indoxacarb were delivered through food.

Alternatively, providing insecticides in freely accessible food sources introduces behavioral factors that may obscure purely physiological responses. In bees and other agricultural insects, nonlethal exposure to insecticides has been shown to impact critical feeding behaviors such as consumption rate, foraging, and locomotion (Haynes 1988, Müller 2018). Because of decreased feeding, German cockroaches that experience similar symptoms when pre-treated with deltamethrin would be less exposed to fipronil or indoxacarb. This superficially demonstrates a lack of impact on susceptibility but does not accurately reflect the physiological state. While we intentionally provided fipronil and indoxacarb in a food source to reflect a bait product with insecticide in a food-based matrix, forced exposure involving these insecticides may provide a more direct evaluation of their physiology. Nonetheless, the lack of increased tolerance indicates that behavioral changes had a negligible impact on mortality even if present.

All the insecticide doses used were strain-specific, but the relative differences in sublethal effect between the UCR susceptible and Ryan resistant strains were minimal. The Ryan strain was collected from the field and was moderately resistant to all insecticides used in this study, which can be attributed to different metabolic and target-site insensitivity responses (Table 1; Table 2) (Lee et al. 2022b). The consistency in response despite pre-existing phenotypic difference contrasts with Khan and Matsumura (Khan and Matsumura 1972), who documented an increase in tolerance to carbaryl after dieldrin exposure in a resistant strain but not a susceptible strain. Possible explanations include the fact that the Ryan strain has been reared in the laboratory without any insecticide exposure for ~5 yr at the time of this study, potentially making it physiologically closer to the UCR strain than a true field population. Further reasons for this discrepancy cannot be inferred until additional resistant/recently collected strains are evaluated using comparable insecticides and methodology.

From a management perspective, the potential impacts of sublethal exposure ranged from moderately beneficial to negligible. Although pyrethroids are commonly perceived as problematic insecticides due to widespread resistance, the results showed that repeated exposures to deltamethrin, even after the initial acute toxicity window, compounded greater mortality levels beyond what was expected. A single LD_{20} treatment caused ~20% mortality, but following up with an additional dose several days later caused a final 46.7 to 74.1% mortality based on the cumulative total. While

Downloaded from https://academic.oup.com/jee/article/118/4/1842/8169665 by University of California, Riverside user on 18 September 2025

single exposures are often used to quantify treatment efficacy in laboratory studies, situations that ensure multiple repeated exposures can improve treatments that may not have desirable efficacy after a single round. Simultaneously, the lack of significance with fipronil and indoxacarb follow-up treatment indicates that these impacts do not apply to all insecticides or forms of exposure. Still, no increase in tolerance was observed in any bioassay, suggesting no within-generation toxicological detriment to applying treatments concurrently. It is important to emphasize, however, that treatments that only kill part of a population are prone to select for insecticide resistance if no effort is made to reach a higher level of control (ffrench-Constant et al. 2004). German cockroaches repeatedly treated with moderate exposures have been shown to reach resistance levels far beyond what has been observed in the field (Ko et al. 2016). Deliberately using ineffective insecticides solely to take advantage of sublethal effects is inadvisable.

Further caution must be exercised because of differences between the experimental design and actual field treatments. If the goal were to document the dynamics of sublethal exposure strictly for its control implications, we would ideally use commercial products in all the experiments. However, label-accurate usage of commercial products typically causes very fast mortality, making precise observations and comparisons of sublethal mechanisms impractical, especially for susceptible strains such as UCR. Acquiring strains simultaneously moderately resistant toward pyrethroids and all other insecticides desired for testing to allow this type of observation using commercial products is an improbable endeavor. Therefore, we developed exposure methods that cause moderate strain-specific mortality to guarantee observable windows over multiple-day periods (ie 5 d LC_{50}). The LD_{20} of deltamethrin was used as the sublethal exposure for all experiments to simulate a resistant population surviving a pyrethroid treatment. This specific value was selected because it is sufficiently low to leave most treated subjects alive for further testing while high enough to practically observe significant differences in the results. Additional ingredients found in pyrethroid formulations, such as synergists, carriers, and solvents, may influence sublethal effects but were out of the scope of this study. Similarly, bait insecticides contain active ingredients at much higher concentrations (0.05 to 2% vs < 0.0001% in this study) in addition to attractants, phagostimulants, preservatives, etc. Evaluations using actual products will be required to make more robust interpretations.

Ultimately, sequential exposure to multiple treatments is not guaranteed. Despite the opportunity often being present, there is no conclusive information about the likelihood of multiple visitations to the same treatment. While German cockroaches forage repeatedly in the same areas and have been shown to contact pyrethroid treatments multiple times, this may be negated through avoidance behavior from toxicant association (Silverman 1986, Ross 1998, Gaire et al. 2024). Furthermore, a multitude of factors may affect sublethal effects, including strain, insecticide choice, dose, sex, and exposure method, which may even cause opposite effects when modified (Khan and Matsumura 1972, Abd-Elghafar and Appel 1992, Lee et al. 1998). Until these dynamics are better understood, the influence of sublethal effects on management should only be regarded conceptually. Nonetheless, this study highlights some previously undocumented efficacy of pyrethroids under conventional management conditions where treatment survival is inevitable.

Supplementary material

Supplementary material is available at *Journal of Economic Entomology* online.

Acknowledgments

The work reported here was supported by UC Riverside Urban Endowed Chair Research Fund. MZ was supported by Henan Agricultural University and China Scholarship Council for her sabbatical leave at UC Riverside.

Author contributions

Shao-Hung Dennis Lee (Conceptualization [Lead], Data curation [Lead], Formal analysis [Lead], Funding acquisition [Supporting], Investigation [Lead], Methodology [Lead], Project administration [Supporting], Resources [Supporting], Supervision [Supporting], Writing - original draft [Lead], Writing - review & editing [Lead]), Man Zhao (Conceptualization [Equal], Data curation [Equal], Formal analysis [Supporting], Funding acquisition [Supporting], Investigation [Equal], Methodology [Equal], Project administration [Supporting], Resources [Supporting], Supervision [Supporting], Writing - original draft [Equal], Writing - review & editing [Equal]), and Chow-Yang Lee (Conceptualization [Supporting], Data curation [Supporting], Formal analysis [Supporting], Funding acquisition [Lead], Investigation [Supporting], Methodology [Supporting], Project administration [Lead], Resources [Lead], Supervision [Lead], Writing - original draft [Supporting], Writing - review & editing [Supporting])

Conflicts of interest. The authors declare that no conflict of interest exists.

Data availability

The data that supports the findings of this study are available in the supplementary material of this article.

References

Abd-Elghafar SF, Appel AG. 1992. Sublethal effects of insecticides on adult longevity and fecundity of German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 85:1809–1817. https://doi.org/10.1093/jee/85.5.1809

Anspaugh DD, Rose RL, Koehler PG, et al. 1994. Multiple mechanisms of pyrethroid resistance in the German cockroach *Blattella germanica* (L.). Pestic. Biochem. Physiol. 50:138–148. https://doi.org/10.1006/pest.1994.1066

Appel AG, Rust MK. 2021. Management using baits. In: Wang C, Lee C-Y, Rust MK, editors. Biology and management of the German cockroach. CABI. p. 213–230.

Appel AG, Reierson DA, Rust MK. 1983. Comparative water relations and temperature sensitivity of cockroaches. Comp. Biochem. Physiol. Part A 74:357–361. https://doi.org/10.1016/0300-9629(83)90615-1

Baek JH, Clark JM, Lee SH. 2010. Cross-strain comparison of cypermethrin-induced cytochrome P450 transcription under different induction conditions in diamondback moth. Pestic. Biochem. Physiol. 96:43–50. https://doi.org/10.1016/j.pestbp.2009.08.014

Boné E, González-Audino PA, Sfara V. 2020. Spatial repellency caused by volatile pyrethroids is olfactory-mediated in the German cockroach *Blattella germanica* (Dictyoptera: Blattellidae). Neotrop. Entomol. 49:275–283. https://doi.org/10.1007/s13744-019-00739-9

Boné E, Roca Acevedo G, Sterkel M, et al. 2022. Characterization of the pyrethroid resistance mechanisms in a *Blattella germanica* (Dictyoptera: Blattellidae) strain from Buenos Aires (Argentina). Bull. Entomol. Res. 112:21–28. https://doi.org/10.1017/S000748532100050X

Cui L, Yuan H, Wang Q, et al. 2018. Sublethal effects of the novel cis-nitromethylene neonicotinoid cycloxaprid on the cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae). Sci. Rep. 8:8915. https://doi.org/10.1038/s41598-018-27035-7

Deans C, Hutchison WD. 2022. Hormetic and transgenerational effects in spotted-wing Drosophila (Diptera: Drosophilidae) in response to three commonly-used insecticides. PLoS One 17:e0271417. https://doi.org/10.1371/journal.pone.0271417

Downloaded from https://academic.oup.com/jee/article/118/4/1842/8169665 by University of California, Riverside user on 18 September 2025

DeVries ZC, Santangelo RG, Crissman J, et al. 2019. Pervasive resistance to pyrethroids in German cockroaches (Blattodea: Ectobiidae) related to lack of efficacy of total release foggers. J. Econ. Entomol. 112:2295–2301. https://doi.org/10.1093/jee/toz120

Fardisi M, Gondhalekar AD, Ashbrook AR, et al. 2019. Rapid evolutionary responses to insecticide resistance management interventions by the German cockroach (*Blattella germanica* L.). Sci. Rep. 9:8292. https://doi.org/10.1038/s41598-019-44296-y

ffrench-Constant RH, Daborn PJ, Goff GL. 2004. The genetics and genomics of insecticide resistance. Trends Genet. 20:163–170. https://doi.org/10.1016/j.tig.2004.01.003

Gaire S, Sierras A, Morgan HL, et al. 2024. Behavioral responses of field-collected German cockroaches to pyrethroids and pyrethroid-formulated insecticides. Pest Manag. Sci. 80:433–441. https://doi.org/10.1002/ps.7774

Gondhalekar AD, Nakayasu ES, Silva I, et al. 2016. Indoxacarb biotransformation in the German cockroach. Pestic. Biochem. Physiol. 134:14–23. https://doi.org/10.1016/j.pestbp.2016.05.003

Gordon JM, Eva MJ, Gaire S, et al. 2024. Common consumer residual insecticides lack efficacy against insecticide-susceptible and resistant populations of the German cockroach (Blattodea: Ectobiidae). Rust M, editor. J. Econ. Entomol. 117:2051–2059. https://doi.org/10.1093/jee/toae158

Guedes RNC, Walse SS, Throne JE. 2017. Sublethal exposure, insecticide resistance, and community stress. Curr. Opin. Insect Sci. 21:47–53. https://doi.org/10.1016/j.cois.2017.04.010

Haynes KF. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Annu. Rev. Entomol. 33:149–168. https://doi.org/10.1146/annurev.en.33.010188.001053

Henderson CF, Tilton EW. 1955. Tests with acaricides against the brown wheat mite. J. Econ. Entomol. 48:157–161. https://doi.org/10.1093/jee/48.2.157

Hu I-H, Chen S-M, Lee C-Y, et al. 2020. Insecticide resistance, and its effects on bait performance in field-collected German cockroaches (Blattodea: Ectobiidae) from Taiwan. J. Econ. Entomol. 113:1389–1398. https://doi.org/10.1093/jee/toaa053

Hu I-H, Tzeng H-Y, Chen M-E, et al. 2021. Association of *CYP4G19* expression with gel bait performance in pyrethroid-resistant German cockroaches (Blattodea: Ectobiidae) from Taiwan. J. Econ. Entomol. 114:1764–1770. https://doi.org/10.1093/jee/toab104

Khan MAQ, Matsumura F. 1972. Induction of mixed-function oxidase and protein synthesis by DDT and dieldrin in German and American cockroaches. Pestic. Biochem. Physiol. 2:236–243. https://doi.org/10.1016/0048-3575(72)90026-0

Ko AE, Bieman DN, Schal C, et al. 2016. Insecticide resistance and diminished secondary kill performance of bait formulations against German cockroaches (Dictyoptera: Blattellidae). Pest Manag. Sci. 72:1778–1784. https://doi.org/10.1002/ps.4211

Kruaysawat P, Chen M-E, Lee S-H, et al. 2024. Characterization of insecticide resistance and their mechanisms in field populations of the German cockroach (Blattodea: Ectobiidae) in Taiwan under different treatment regimes. J. Econ. Entomol. 118:307–319. https://doi.org/10.1093/jee/toae252

Lee C-Y, Wang C. 2021. German cockroach infestations in the world and their social and economic impacts. In: Wang C, Lee C-Y, Rust MK, editors. Biology and management of the German cockroach. CABI. p. 1–16.

Lee C-Y, Yap H-H, Chong N-L. 1996. Insecticide toxicity on the adult German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). Malays. J. Sci. 17A:1–9.

Lee CY, Yap HH, Chong NL. 1998. Sublethal effects of deltamethrin and propoxur on longevity and reproduction of German cockroaches, *Blattella germanica*. Entomol. Exp. Appl. 89:137–145. https://doi.org/10.1046/j.1570-7458.1998.00392.x

Lee S-H, Choe D-H, Rust MK, et al. 2022a. Reduced susceptibility towards commercial bait insecticides in field German cockroach (Blattodea: Ectobiidae) populations from California. J. Econ. Entomol. 115:259–265. https://doi.org/10.1093/jee/toab244

Lee S-H, Choe D-H, Scharf ME, et al. 2022b. Combined metabolic and target-site resistance mechanisms confer fipronil and deltamethrin resistance in field-collected German cockroaches (Blattodea: Ectobiidae). Pestic. Biochem. Physiol. 184:105123. https://doi.org/10.1016/j.pestbp.2022.105123

Miller DM, Smith EP. 2020. Quantifying the efficacy of an assessment-based pest management (APM) program for German cockroach (L.) (Blattodea: Blattellidae) control in low-income public housing units. J. Econ. Entomol. 113:375–384. https://doi.org/10.1093/jee/toz302

Müller C. 2018. Impacts of sublethal insecticide exposure on insects — Facts and knowledge gaps. Basic Appl. Ecol. 30:1–10. https://doi.org/10.1016/j.baae.2018.05.001

Poupardin R, Reynaud S, Strode C, et al. 2008. Cross-induction of detoxification genes by environmental xenobiotics and insecticides in the mosquito *Aedes aegypti*: Impact on larval tolerance to chemical insecticides. Insect Biochem. Mol. Biol. 38:540–551. https://doi.org/10.1016/j.ibmb.2008.01.004

Riaz MA, Poupardin R, Reynaud S, et al. 2009. Impact of glyphosate and benzo[a]pyrene on the tolerance of mosquito larvae to chemical insecticides. Role of detoxification genes in response to xenobiotics. Aquat. Toxicol. 93:61–69. https://doi.org/10.1016/j.aquatox.2009.03.005

Rix RR, Ayyanath MM, Christopher Cutler G. 2016. Sublethal concentrations of imidacloprid increase reproduction, alter expression of detoxification genes, and prime *Myzus persicae* for subsequent stress. J. Pest Sci. 89:581–589. https://doi.org/10.1007/s10340-015-0716-5

Ross MH. 1998. Response of behaviorally resistant German cockroaches (Dictyoptera: Blattellidae) to the active ingredient in a commercial bait. J. Econ. Entomol. 91:150–152. https://doi.org/10.1093/jee/91.1.150

Rust M, Appel A, Lee S-H, et al. 2022. Impact of sprays and baits in controlling German cockroaches, *Blattella germanica*. Proceedings of the Tenth International Conference on Urban Pests, Barcelona, Spain. p. 148–151. https://www.icup.org.uk/conferences/2022/papers/impact-of-sprays-on-baits-in-controlling-german-cockroaches-blattella-germanica/

Scharf ME, Gondhalekar A. 2021. Insecticide resistance: perspectives on evolution, monitoring, mechanisms and management. In: Wang C, Lee C-Y, Rust MK, editors. Biology and management of the German cockroach. CABI. p. 231–255.

Silverman J. 1986. Adult German cockroach (Orthoptera: Blattellidae) feeding and drinking behavior as a function of density and harborage-to-resource distance. Environ. Entomol. 15:198–204. https://doi.org/10.1093/ee/15.1.198

Tisgratog R, Panyafeang C, Lee S-H, et al. 2023. Insecticide resistance and its potential mechanisms in field-collected German cockroaches (Blattodea: Ectobiidae) from Thailand. J. Econ. Entomol. 116:1321–1328. https://doi.org/10.1093/jee/toad117

Tseng S-P, Lee S-H, Choe D-H, et al. 2024. Overexpression of cytochrome P450 gene CYP6K1 is associated with pyrethroid resistance in German cockroaches (Blattodea: Ectobiidae) from California. J. Econ. Entomol. 117:1071–1076. https://doi.org/10.1093/jee/toae057

Ullah F, Gul H, Tariq K, et al. 2020. Thiamethoxam induces transgenerational hormesis effects and alteration of genes expression in *Aphis gossypii*. Pestic. Biochem. Physiol. 165:104557. https://doi.org/10.1016/j.pestbp.2020.104557

Wolfe ZM, Scharf ME. 2022. Microbe-mediated activation of indoxacarb in German cockroach (*Blattella germanica* L.). Pestic. Biochem. Physiol. 188:105234. https://doi.org/10.1016/j.pestbp.2022.105234

Yang Y, Yu N, Zhang J, et al. 2018. Induction of P450 genes in *Nilaparvata lugens* and *Sogatella furcifera* by two neonicotinoid insecticides. Insect Sci. 25:401–408. https://doi.org/10.1111/1744-7917.12440

Yu SJ. 2014. The toxicology and biochemistry of insecticides. 2nd ed. CRC Press, https://doi.org/10.1201/b18164

Zha C, Wang C, Buckley B, et al. 2018. Pest prevalence and evaluation of community-wide integrated pest management for reducing cockroach infestations and indoor residues. J. Econ. Entomol. 111:795–802. https://doi.org/10.1093/jee/tox356

Downloaded from https://academic.oup.com/jee/article/118/4/1842/8169665 by University of California, Riverside user on 18 September 2025