Structural Pest Control Board
Research Advisory Panel

Chow-Yang Lee Proposal

"Improving application of dusts and gel baits for control of insecticide-resistant German cockroaches, a major indoor pest of public health importance."

2025

Represented University:
UC Riverside

Funds Requested: $226,997
Term: January 1, 2026 through September 30, 2028

Research and Economic Development
900 University Avenue
200 University Office Building
Riverside||CA 92521

July 31, 2025

Department of Consumer Affairs, Structural Pest Control Board
2005 Evergreen St.
Suite 1500
Sacramento, CA 95815

RE: UCR Kuali PD #3587

On behalf of The Regents of the University of California, we are presenting for your review a request for support of the following proposal:

Principal Investigator:	Dr. Chow-Yang Lee Entomology Department
Title:	"Improving application of dusts and gel baits for control of insecticide resistant German cockroaches, an important indoor pest of public health importance"
Support Requested:	$226,997
Period of Support:	January 1, 2026 through September 30, 2028
Type of Request:	New Research Grant

Your favorable consideration of this proposal is greatly appreciated. In the event this proposal is selected to be funded, we are committed to providing the appropriate programmatic and administrative personnel as necessary to the project and we are aware of the sponsoring agency's guidelines.

If additional information is required, please contact the undersigned by phone at (951) 827-3815 or via e-mail at lauren.green@ucr.edu.

Sincerely,

DocuSigned by:
Lauren Green

Lauren Green
Sr. Contract & Grant Officer
Sponsored Programs Administration

ATTACHMENT 1

REQUIRED ATTACHMENT CHECKLIST

A complete proposal will consist of the items identified on the list below.

Complete this checklist to confirm that all items are contained with your proposal. Place a check mark or "✓" next to each item that you are submitting to the State. For your proposal to be responsive, in addition to your proposal, all required attachments must be returned. This checklist should be returned along with your proposal.

It is essential that the Cost Proposal be complete, thorough, and comply with content sequence requirements. The proposal must be typed and double-spaced on 8½ X 11 paper. All pages shall be consecutively numbered. All elements shall follow the sequence presented on the following checklist:

✓ Check	Attachment #	Attachment Name/Description	Form Provided	Completion Required
	Attachment 1	Required Attachment Checklist	YES	YES
	Attachment 2	Cost Proposal/Budget Display Sheets	YES	YES
	Attachment 3	Budget Narrative Form and Explanation of Costs	YES	YES
	Attachment 4	Proposer's References	YES	YES
	Attachment 5	Sample Agreement a) Project Summary and Scope of Work b) Schedule of Deliverables c) Key Personnel d) Authorized Representatives and Notices e) Use of Pre-existing Intellectual Property f) Current & Pending Support g) Third Party Confidential Information (if applicable) h) Budget Justification	YES	YES
	Attachment 6	Resumes (Curriculum Vitae) for Proposer, Proposer's staff involved in project, and all Subcontractors	NO	YES
	Attachment 7	Narrative of Research Objectives, as described in Rating/Scoring Criteria	NO	YES
	Attachment 8	Narrative of Project Direction (Work Plan and Work Schedule), as described in Rating/Scoring Criteria	NO	YES
	Attachment 9	Narrative of Qualifications, as described in "Minimum Qualifications for Proposers" and Rating/Scoring Criteria	NO	YES
	Attachment 10	Copy of current business license, professional certificates, or other credentials	NO	YES

ATTACHMENT 2

COST PROPOSAL/BUDGET DISPLAY
RESEARCH PROPOSAL

YEAR 1 – (for first 12 months)

Period of award
(1/1/26 - 9/30/28)
Use separate sheet for each year

Period of award: 1/1/26 – 12/31/26

Contractor: Chow-Yang Lee, UC Riverside

Project Title/Description: Improving application of dusts and gel baits for control of insecticide-resistant German cockroaches, a major indoor pest of public health importance

Description	Hours	Rate	Total
PERSONNEL SERVICES			
1. Post-Doc Researcher	626	34.29	$21,490
2. SRA IV	418	44.78	$18,700
3.			
4.			
		Total Salaries	$40,190
		Total Benefits	$12,638
		Total Personnel Services (A)	$52,828
SUBCONTRACTOR SERVICES			
1. Classification			
		Total Subcontractor Services (B)	
OTHER SERVICES			
1. Classification			
		Total Other Services (C)	
OPERATING EXPENSES			
1. Supplies and Expense			$1,000
2. Travel In-State			$500
3. Travel Out-of-State			
4. Equipment			
5. Other Costs			
		Total Operating Expenses (D)	$1,500
Total Personnel and Operating (Add A through D)		$54,328	
Indirect Costs (detail) (35%)		$19,015	
TOTAL COSTS – Year 1 (for the first 12 months)		$73,343	

ATTACHMENT 2

COST PROPOSAL/BUDGET DISPLAY
RESEARCH PROPOSAL

YEAR 2 – (for months 13 thru 24)

Period of award
(1/1/26 - 9/30/28)
Use separate sheet for each year

Period of award: 1/1/27 – 12/31/27

Contractor: Chow-Yang Lee, UC Riverside

Project Title/Description: Improving application of dusts and gel baits for control of insecticide-resistant German cockroaches, a major pest of public health importance.

Description	Hours	Rate	Total
PERONNEL SERVICES			
1. Post-Doc Researcher	626	35.57	$22,285
2. SRA IV	418	46.11	$19.261
3.			
4.			
		Total Salaries	$41,546
		Total Benefits	$13,045
		Total Personnel Services (A)	$54,591
SUBCONTRACTOR SERVICES			
1. Classification			
		Total Subcontractor Services (B)	
OTHER SERVICES			
1. Classification			
		Total Other Services (C)	
OPERATING EXPENSES			
1. Supplies and Expense			$1,000
2. Travel In-State			$500
3. Travel Out-of-State			
4. Equipment			
5. Other Costs			
		Total Operating Expenses (D)	$1,500
Total Personnel and Operating (Add A through D)		$56,091	
Indirect Costs (detail)		$19,632	
TOTAL COSTS – Year 2 (for 12 months)		$75,723	

ATTACHMENT 2

COST PROPOSAL/BUDGET DISPLAY
RESEARCH PROPOSAL

YEAR 3 – (for months 25 thru 36)

Period of award
(1/1/26 - 9/30/28)
Use separate sheet for each year

Period of award: 1/1/28 – 9/30/28

Contractor: Chow-Yang Lee, UC Riverside

Project Title/Description: Improving application of dusts and gel baits for control of insecticide-resistant German cockroaches, a major indoor pest of public health importance

Description	Hours	Rate	Total
PERONNEL SERVICES			
1. Post-Doc Researcher	626	36.65	$22,953
2. SRA IV	418	47.50	$19,838
3.			
4.			
		Total Salaries	$42,791
		Total Benefits	$13,436
		Total Personnel Services (A)	$56,227
SUBCONTRACTOR SERVICES			
1. Classification			
		Total Subcontractor Services (B)	
OTHER SERVICES			
1. Classification			
		Total Other Services (C)	
OPERATING EXPENSES			
1. Supplies and Expense			$1,000
2. Travel In-State			$500
3. Travel Out-of-State			
4. Equipment			
5. Other Costs			
		Total Operating Expenses (D)	$1,500
Total Personnel and Operating (Add A through D)	$57,727		
Indirect Costs (detail)	$20,204		
TOTAL COSTS – Year 3 (for final 12 months)	$77,931		

ATTACHMENT 2

COST PROPOSAL/BUDGET DISPLAY
RESEARCH PROPOSAL

COMBINED YEARS – (up to 3 years or 36 months)

Period of award
(1/1/26 - 9/30/285)

Use separate sheet for each year

Period of award: 1/1/26 - 9/30/28

Contractor: Chow-Yang Lee, UC Riverside

Project Title/Description: Improving application of dusts and gel baits for control of insecticide-resistant German cockroaches, a major indoor pest of public health importance

Description	Hours	Rate	Total
PERONNEL SERVICES			
1. Post-Doc Researcher	1878		$66,728
2. SRA IV	1254		$57,799
3.			
4.			
		Total Salaries	$124,527
		Total Benefits	$39,119
		Total Personnel Services (A)	$163,646
SUBCONTRACTOR SERVICES			
1. Classification			
		Total Subcontractor Services (B)	
OTHER SERVICES			
1. Classification			
		Total Other Services (C)	
OPERATING EXPENSES			
1. Supplies and Expense			$3,000
2. Travel In-State			$1,500
3. Travel Out-of-State			
4. Equipment			
5. Other Costs			
		Total Operating Expenses (D)	$4,500
Total Personnel and Operating (Add A through D)			$168,146
Indirect Costs (detail)			$58,851
TOTAL COSTS – GRAND TOTAL UP TO 3 YEARS (for up to 36 months)			$226,997

ATTACHMENT 3,

BUDGET NARRATIVE FORM AND EXPLANATION OF COSTS:

Explain the need for individual staff, budgeted travel, equipment, subcontracts and consultants:

(Dennis) Shao-Hung Lee, postdoctoral Researcher, University of California, Riverside, 30% salaried time. The postdoctoral researcher will organize and carry out many of the research activities described in the proposal. The postdoctoral researcher will play a crucial role in the experimental design, data collection, and statistical analysis. He will also assist with the interim reports.

Gregory Kund, Staff Research Associate, will work with the Postdoctoral Researcher and PI to set up experiments described in the proposal, and collect and analyze the data. He will also oversee the rearing operations and establishment of the cockroach colonies.

Dr. Michael Rust is an advisor on this project and will provide technical expertise and guidance. He will not incur any costs but will assist with publishing and presenting the research findings as a conduit to support the pest control industry.

The materials and supplies required for rearing insect colonies and completing the experiments for the 3-year project will cost $3,000. The supplies needed will be trash cans ($300) to house the roaches, dog food (14 bags/year x $50=$700), cotton for water wicks ($100 per case of 12 rolls each year=$300), and quart sized water jars (case of 12 $25).

One-time material expenses:	**Annual expenses:**
4 -44-gal Trash cans $350	Dog food 14 bags x $50=700
Water jars 1 quart (case) $25	Cotton wick rolls (1 case) $100
Barrier double sided tape (5) $50	
Foil duct tape (5) $50	
9V batteries (5 packs) $125	
Subtotal=$600	Subtotal (3 years x $800) =$2,400
Total material expenses=$3,000	

Travel expenses are minimal and will be $1,500 for the partial cost of a leased truck, which will be used for cockroach collection and routine trips for supplies and colony maintenance at different lab locations. These fees are equivalent to leasing a vehicle at 6% interest for $684.16/month or $ 8,209.92 annually, as stipulated by UC Riverside Fleet Services ($0.06 x $ 8,209.92 = $500 per year).

No other direct costs are requested.

Please explain how the costs were arrived at:

Personnel costs were derived from approved UC Riverside rates for both personnel costs and fringe rates, with a cost-of-living increase built in annually. Supplies were estimated on historical figures from similar projects and online pricing. Travel was estimated using the current mileage reimbursement rates, approved by the IRS and current UC Riverside vehicle lease rates.

Please explain why the rates are considered reasonable and/or appropriate in your opinion:

Personnel costs were estimated at the UC Riverside's approved rates, by title. These costs are set by the University. At UC Riverside, we purchase materials, supplies, and other services (gene sequencing) through approved vendors, providing reasonable pricing to the University. Travel was estimated using currently approved federal rates.

Are costs based on industry standard or other basis of measurement? Please explain:

Personnel costs are based on UC Riverside's approved rates by pay title, with the recommended cost of living adjustments. Materials/Supplies are based in industry standards for the University of California system. Travel is based on approved federal reimbursement rates.

ATTACHMENT 4

PROPOSER REFERENCES

1. Please attach three letters of reference on company letterhead.
2. List below three references of similar types of services performed, as described in the description of services, within the last five years. If three references cannot be provided, please explain why on an attached sheet of paper.

REFERENCE 1	
Name of Firm	Rentokil Terminix
Address	305 N Crescent Way, Anaheim, CA 92801
Contact Person	Claudio Salem – DVM – BCE
Telephone Number	800-968-2440
Dates of Service	N/A
Value or Cost of Service	N/A

Brief Description of Service Provided: Rentokil helped to collect field German cockroaches for us in this project: *Resistance monitoring of an isoxazoline compound in Blattella germanica in the United States*. The project is still presently undergoing.

REFERENCE 2	
Name of Firm	IPM4You
Address	9830 Via Leslie, Santee, CA 92071
Contact Person	James Panknin
Telephone Number	844-476-4968
Dates of Service	N/A
Value or Cost of Service	N/A

Brief Description of Service Provided: We collaborated with Mr. Jim Panknin in a project where he assisted with the collection of field populations of the German cockroach. The work resulted in two peer-reviewed papers, where Mr. Panknin's help was acknowledged.

Lee SH, DH Choe, MK Rust & CY Lee. 2022. Reduced susceptibility towards commercial bait insecticides in field German cockroach (Blattodea: Ectobiidae) populations from California. *Journal of Economic Entomology* 115: 259–265.

Lee SH, DH Choe, ME Scharf, MK Rust, & CY Lee. 2022. Combined metabolic and target-site resistance mechanisms confer fipronil and deltamethrin resistance in field-collected German cockroaches (Blattodea: Ectobiidae). *Pesticide Biochemistry and Physiology* 184: 105123.

REFERENCE 3	
Name of Firm	Thrasher Pest Control
Address	8957 Complex Dr, San Diego, CA 92123, United States
Contact Person	Garrett Thrasher
Telephone Number	619-955-5121
Dates of Service	N/A
Value or Cost of Service	N/A

Brief Description of Service Provided: We collaborated with Mr. Garrett Thrasher in a project where he assisted with the collection of Formosan subterranean termites. The work resulted in a peer-reviewed paper where Mr Thrasher's help was mentioned in the acknowledgment.

Tseng SP, S Taravati, DH Choe, MK Rust, & CY Lee. 2022. Genetic evidence for multiple invasions of *Coptotermes formosanus* (Blattodea: Rhinotermitidae) in California. *Journal of Economic Entomology* 115: 1251–1256.

ATTACHMENT 5 – SAMPLE AGREEMENT

STANDARD AGREEMENT

STD 213 (Rev 06/03)

AGREEMENT NUMBER
REGISTRATION NUMBER

1. This Agreement is entered into between the State Agency and the Contractor named below:

STATE AGENCY'S NAME
Department of Consumer Affairs, Structural Pest Control Board

CONTRACTOR'S NAME
TBD

2. The term of this Agreement is: July 1, 2025 (or upon approval, whichever is later) through **TBD**

3. The maximum amount of this Agreement is: $

4. The parties agree to comply with the terms and conditions of the following exhibits which are by this reference made a part of the Agreement.

Exhibit A – **A7**: A–Scope of Work; A1–Deliverables; A2–Key Personnel; A3–Authorized Representatives; A4–Use of Intellectual Property; A5–Resumes; A6–Current & Pending Support; A7-Third Party Confidential Information (if applicable)	page(s)
Exhibit B – B–Budget; B1–Budget Justification; B2– Subawardee Budgets (if applicable); B3–Invoice Elements	page(s)
Exhibit C* – University Terms and Conditions	UTC-220

Check mark additional Exhibits below, and attach applicable Exhibits or provide internet link:

☐ **Exhibit D** – Additional Requirements Associated with Funding Sources	page(s)
☐ **Exhibit E** – Special Conditions for Security of Confidential Information	page(s)
☐ **Exhibit F** – Access to State Facilities or Computing Resources	page(s)
☐ **Exhibit G** – Negotiated Alternate UTC Terms	page(s)

Items shown with an Asterisk (*), are hereby incorporated by reference and made part of this agreement as if attached hereto. These documents can be viewed at http://www.dgs.ca.gov/ols/Resources/ModelContractLanguageUniversities.aspx.

IN WITNESS WHEREOF, this Agreement has been executed by the parties hereto.

CONTRACTOR

California Department of General Services Use Only

CONTRACTOR'S NAME *(if other than an individual, state whether a corporation, partnership, etc.)*

BY *(Authorized Signature)*	DATE SIGNED *(Do not type)*
✍	

PRINTED NAME AND TITLE OF PERSON SIGNING

ADDRESS

STATE OF CALIFORNIA

AGENCY NAME
Department of Consumer Affairs, Structural Pest Control Board

BY *(Authorized Signature)*	DATE SIGNED *(Do not type)*
✍	

PRINTED NAME AND TITLE OF PERSON SIGNING

ADDRESS
1625 N. Market Blvd., Suite S-103
Sacramento, CA 95834

Exhibit A – Scope of Work

Project Summary & Scope of Work

☐ Contract ☐ Grant

PI Name: *Chow-Yang Lee, UC Riverside* .

Project Title: **Improving application of dusts and gel baits for control of insecticide resistant German cockroaches, an important indoor pest of public health importance**

Project Summary/Abstract

The German cockroach, *Blattella germanica* (L.), is a significant indoor public health pest that could mechanically transmits pathogens and produces allergens that cause chronic asthma. Insecticides are the main tool used to control this species, with pyrethroid sprays and gel baits making up most of the formulations used. However, widespread pyrethroid resistance challenges the sustainability of residual sprays, and growing resistance to gel baits over the past decade discourages the overreliance on such formulations as well. Dust insecticides are promising alternatives that possess advantages such as a diverse range of active ingredients (desiccant, contact, oral, etc.), unique modes of action that are effective against resistant cockroaches (e.g., silica gel, boric acid), and long-lasting applications when undisturbed. However, almost no modern information exists on the efficacy of dust products, especially on their repellency, compatibility with gel baits, and effectiveness against resistant cockroach strains. These questions must be answered to responsibly incorporate dusts into IPM programs. Our project aims to address all these shortfalls.

The first step of our project involves screening a representative range of dust products against both insecticide-resistant and susceptible German cockroaches to identify effective treatments under forced exposure (no choice) and choice experiments. We will select seven dust products with active registrations in California that cover all major active ingredient categories: desiccants (e.g., silica gel), oral toxicants (e.g., boric acid, indoxacarb), contact toxicants (e.g., pyrethroid), and combinations (more than one category). Three field-collected resistant cockroach strains and one susceptible strain will be tested. Forced exposure experiments will involve confining adult male cockroaches to dust at label rates in petri dishes without access to food and water, enabling us to measure the maximum toxicity of each treatment. Choice experiments will be conducted in Ebeling choice boxes, which assess efficacy and repellency simultaneously, predicting how treatments perform under field conditions where cockroaches can avoid dust deposits in dark areas. Treatment performance will be compared using Kaplan-Meier survivorship analyses and performance indices (PI), a metric unique to Ebeling choice boxes that combines efficacy with repellency.

Similar to gel baits, dusts are applied under appliances, behind cabinets, in voids, and other less disturbed areas, which means the two formulations may physically overlap when present in the same site. Therefore, understanding their interactions is crucial when using dusts in IPM programs. The next experiments will assess the compatibility of dust and gel bait insecticides when applied together and determine the proper application sequence for both. Up to two of the best-performing dusts from previous experiments and gel baits with common active ingredients (e.g., fipronil and indoxacarb) will be selected for testing. The following treatment combinations will be applied to adult male cockroaches and evaluated in Ebeling choice boxes: (1) dust on top of gel bait, (2) gel bait on top of dust, (3) dust alone, and (4) gel bait alone. The most effective treatment combination(s) will be identified using a proportional hazards model that assesses the contribution of dusts, gel baits, and application order, both separately and together. Once determined, the best combination(s) will be tested against mixed stages and sexes of German cockroaches in choice boxes, and efficacy will be compared across strains using Kaplan-Meier survivorship analyses and PIs.

Finally, the behavioral response of susceptible and resistant German cockroaches to dusts will be examined using video monitoring assays. Individual cockroaches will be placed into arenas with half-treated filter paper covering the bottom. Their position and movement between treated and untreated sides will be tracked with EthoVision XT software and analyzed using F-tests followed by post-hoc analysis.

If Third-Party Confidential Information is to be provided by the State:

☐ Performance of the Scope of Work is anticipated to involve use of third-party Confidential Information and is subject to the terms of this Agreement; ***OR***

☐ A separate CNDA between the University and third-party is required by the third-party and is incorporated in this Agreement as Exhibit A7, Third Party Confidential Information.

Scope of Work

Describe the goals and specific objectives of the proposed project and summarize the expected outcomes. If applicable, describe the overall strategy, methodology, and analyses to be used. Include how the data will be collected, analyzed, and interpreted as well as any resource sharing plans as appropriate. Discuss potential problems, alternative strategies, and benchmarks for success anticipated to achieve the goals and objectives.

This information is fully provided in the Attachment 7 (Research Objectives) and Attachment 8 (Project Direction)

Exhibit A1 – Deliverables

SCHEDULE OF DELIVERABLES

List all items that will be delivered to the State under the proposed Scope of Work. Include all reports, including draft reports for State review, and any other Deliverables, if requested by the State and agreed to by the Parties.

If use of any Deliverable is restricted or is anticipated to contain preexisting Intellectual Property with any restricted use, it will be clearly identified in Exhibit A4, Use of Preexisting Intellectual Property.

Unless otherwise directed by the State, the University Principal Investigator shall submit all Deliverables to the State Contract Project Manager, identified in Exhibit A3, Authorized Representatives.

Deliverable	Description	Due Date
Interim progress report	Provide a brief (2-3 pages) written interim progress report to address progress made, findings to date, and problems encountered.	06/30/2026
Interim progress report	Provide a brief (2-3 pages) written interim progress report to address progress made, findings to date, and problems encountered.	12/31/2026
Present a progress report	Present a progress report at one Board Meeting	TBD (2026)
Interim progress report	Provide a brief (2-3 pages) written interim progress report to address progress made, findings to date, and problems encountered.	06/30/2027
Interim progress report	Provide a brief (2-3 pages) written interim progress report to address progress made, findings to date, and problems encountered.	12/31/2027
Present a progress report	Present a progress report at one Board Meeting	TBD (2027)
Final report	Provide a comprehensive written Final Report	09/30/2028
Present a final report	Present a final report at one Board Meeting	TBD
The following Deliverables are subject to Section 19. Copyrights, paragraph B of Exhibit C		

Exhibit A2 – Key Personnel

KEY PERSONNEL

List Key Personnel as defined in the Agreement starting with the PI, by last name, first name followed by Co-Pis. Then list all other Key Personnel in alphabetical order by last name. For each individual listed include his/her name, institutional affiliation, and role on the proposed project. Use additional consecutively numbered pages as necessary.

Last Name, First Name	Institutional Affiliation	Role on Project
PI:		
Lee, Chow-Yang	*Department of Entomology* *University of California* *Riverside*	As a principal investigator of the project, Lee will be overseeing the project plan, execution, and progress. Lee will be also advising the project team members who will be carrying out the proposed research.
Co-PI(s) – if applicable:		
Choe, Dong-Hwan	*Department of Entomology* *University of California* *Riverside*	As a co-PI, Choe will be providing his expertise in cockroach biology and management for the current project.
Other Key Personnel (if applicable):		
Rust, Michael	*Department of Entomology* *University of California* *Riverside*	As a collaborator, Rust will be providing his expertise in cockroach control using dusts and baits for the current project. He will also provide support for the publishing and presentation of the research findings.
Lee, Shao-Hung	*Department of Entomology* *University of California* *Riverside*	The postdoctoral researcher will be organizing and carrying out much of the research activities described in the proposal. The postdoctoral researcher will be integral to the experimental design and data collection for the project under the guidance of PI (Lee) and other project team members.
Kund, Gregory	*Department of Entomology* *University of California* *Riverside*	As a Staff Research Associate IV, Kund will work with the Postdoctoral Researcher (Lee) and PI to set up experiments described in the proposal and collect and analyze the data.

Exhibit A3 – Authorized Representatives

AUTHORIZED REPRESENTATIVES AND NOTICES

The following individuals are the authorized representatives for the State and the University under this Agreement. Any official Notices issued under the terms of this Agreement shall be addressed to the Authorized Official identified below, unless otherwise identified in the Agreement.

State Agency Contacts	University Contacts
Agency Name: <Agency Name>	University Name: The Regents of the University of California (UC), Riverside
Contract Project Manager (Technical) Name: <Name> <Title> Address: <Department> <Address> <City,State,Zip> Telephone: <Telephone#> Fax: <Fax#, if available> Email: <EmailAddress>	***Principal Investigator*** Name: Chow-Yang Lee / Professor in Urban Entomology Address: Department of Entomology University of California Riverside, CA 92508 Telephone: 951-827-2626 Fax: 951-827-3086 Email: chowyang.lee@ucr.edu Designees to certify invoices under Section 14 of Exhibit C on behalf of PI: N/A
Authorized Official (contract officer) Name: <Name> <Title> Address: <Department> <Address> <City,State,Zip> Telephone: <Telephone#> Fax: <Fax#, if available> Email: <EmailAddress> ***Send notices to (if different):*** Name: <Name> <Title> Address: <Department> <Address> <City,State,Zip> Telephone: <Telephone#> Email: <EmailAddress>	***Authorized Official*** Name: Lauren Green Address: Sponsored Programs Administration University of California, Riverside 245 University Office Bldg. Riverside, CA 92521-0217 Telephone: 951-827-3815 Fax: 951-827-4483 Email: Lauren.Green@UCR.EDU, awards@ucr.edu ***Send notices to (if different):***
Administrative Contact Name: <Name> <Title> Address: <Department> <Address> <City,State,Zip>	Name: Lauren Green Address: Sponsored Programs Administration University of California, Riverside 245 University Office Bldg. Riverside, CA 92521-0217 Telephone: 951-827-3815

Telephone: <Telephone#> Fax: <Fax#, if available> Email: <EmailAddress>	Fax: 951-827-4483 Email: Lauren.Green@UCR.EDU
Financial Contact/Accounting Name: <Name> <Title> Address: <Department> <Address> <City,State,Zip> Telephone: <Telephone#> Fax: <Fax#, if available> Email: <EmailAddress>	***Authorized Financial Contact/Invoicing*** Name: Kimberly Gala Post Award Accounting Manager Address: Extramural Funding 900 University Ave. Riverside, CA 92521 Telephone: 951-827-1953 Fax: Email: EMF@UCR.EDU Designees for invoice certification in accordance with Section 14 of Exhibit C on behalf of the Financial Contact: 1. Patrice Delgado, EMF Accountant 3, patrice.delgado@ucr.edu 2. <Name>, <Title>, <EmailAddress> 3. <Name>, <Title>, <EmailAddress>

Exhibit A4 – Use of Intellectual Property

USE OF INTELLECTUAL PROPERTY

If either Party will be using any third-party or pre-existing intellectual property (including, but not limited to data, copyrighted works, known patents, trademarks, service marks and trade secrets) "IP" with restrictions on use, then list all such IP and the nature of the restriction below. If no third-party or pre-existing IP will be used, check "none" in this section.

A. State: Preexisting IP to be provided to the University from the State or a third party for use in the performance in the Scope of Work.

☒ None or ☐ List:

Owner (Name of State Agency or 3rd Party)	Description	Nature of restriction:

B. University: Restrictions in Preexisting IP included in Deliverables identified in Exhibit A1, Deliverables.

☒ None or ☐ List:

Owner (Name of University or 3rd Party)	Description	Nature of restriction:

C. Anticipated restrictions on use of Project Data.

If the University PI anticipates that any of the Project Data generated during the performance of the Scope of Work will have a restriction on use (such as subject identifying information in a data set) then list all such anticipated restrictions below. If there are no restrictions anticipated in the Project Data, then check "None" in this section.

☒ None or ☐ List:

Owner (University or 3rd Party)	Description	Nature of Restriction:

Exhibit A5 – RÉSUMÉ/BIOSKETCH

RÉSUMÉ/BIOSKETCH

Attach Resume/Biosketch for the PI and other Key Personnel listed in Exhibit A2, Key Personnel.

These items are provided in Attachment 6.

Exhibit A6 – Current & Pending Support

CURRENT & PENDING SUPPORT

University will provide current & pending support information for Key Personnel identified in Exhibit A2 at time of proposal and upon request from State agency. The "Proposed Project" is this application that is submitted to the State. Add pages as needed.

PI: Chow-Yang Lee

Status (currently active or pending approval)	Award # (if available)	Source (name of the sponsor)	Project Title	Start Date	End Date
Proposed Project	N/A	Structural Pest Control Board	Improving application of dusts and gel baits for control of insecticide-resistant German cockroaches, a major pest of public health importance (as PI)	Jan 2026	Jun 2028
Proposed Project	N/A	Structural Pest Control Board	Impact of High Temperature and Chitin Synthesis Inhibitors on Gut Microbial Symbiont Community and Desiccation Tolerance in Western Drywood Termite (as co-PI)	Jan 2026	Jun 2028
PENDING	N/A (TBD)	California Department of Pesticide Regulation	Improvement of silica gel dust and dust mixtures as safer alternatives, and development of a lethal harborage for German cockroach control (as PI)	2025 (exact date TBD)	Jun 2028
PENDING	N/A (TBD)	California Department of Pesticide Regulation	*Reinventing Integrated Pest Management System for Wood Destroying Insects* (as co-PI)	2025 (exact date TBD)	TBD
CURRENT PROJECT		Syngenta Crop Protection, NC	Resistance monitoring of an isoxazoline compound in *Blattella germanica* in United States	Jan 2022	Dec 2025
CURRENT PROJECT		California Celery Research Advisory Board	Insecticide resistance of celery pests focusing on the Lygus bug, Lygus hesperus	Oct 2022	Sep 2025

Dong-Hwan Choe

Status	Award #	Source	Project Title	Start Date	End Date
Proposed Project	N/A	Structural Pest Control Board	Impact of High Temperature and Chitin Synthesis Inhibitors on Gut Microbial Symbiont Community and Desiccation Tolerance in Western Drywood Termite (as PI)	Jan 2026	June 2028
Proposed Project	N/A	Structural Pest Control Board	Improving application of dusts and gel baits for control of insecticide-resistant German cockroaches, a	Jan 2026	June 2028

			major pest of public health importance (as Co-PI)		
PENDING	N/A (TBD)	California Department of Pesticide Regulation	*Reinventing Integrated Pest Management System for Wood Destroying Insects* (as PI)	2025 (exact date TBD)	TBD
PENDING	N/A (TBD)	California Department of Pesticide Regulation	Improvement of silica gel dust and dust mixtures as safer alternatives, and development of a lethal harborage for German cockroach control (as Co-PI)	2025 (exact date TBD)	Jun 2028

Exhibit A6 – Current & Pending Support

CURRENT & PENDING SUPPORT

University will provide current & pending support information for Key Personnel identified in Exhibit A2 at time of proposal and upon request from State agency. The "Proposed Project" is this application that is submitted to the State. Add pages as needed.

Personnel: Michael Rust

Status (currently active or pending approval)	Award # (if available)	Source (name of the sponsor)	Project Title	Start Date	End Date
Proposed Project	N/A	Structural Pest Control Board	Improving application of dusts and gel baits for control of insecticide-resistant German cockroaches, a major pest of public health importance (as PI)	Jan 2026	Jun 2028
Proposed Project	N/A	Structural Pest Control Board	Impact of High Temperature and Chitin Synthesis Inhibitors on Gut Microbial Symbiont Community and Desiccation Tolerance in Western Drywood Termite (as co-PI)	Jan 2026	Jun 2028
PENDING	N/A (TBD)	California Department of Pesticide Regulation	Improvement of silica gel dust and dust mixtures as safer alternatives, and development of a lethal harborage for German cockroach control (as PI)	2025 (exact date TBD)	Jun 2028
PENDING	N/A (TBD)	California Department of Pesticide Regulation	*Reinventing Integrated Pest Management System for Wood Destroying Insects* (as co-PI)	2025 (exact date TBD)	TBD
CURRENT PROJECT		Syngenta Crop Protection, NC	Resistance monitoring of an isoxazoline compound in *Blattella germanica* in United States	Jan 2022	Dec 2025

Exhibit A6 – Current & Pending Support

CURRENT & PENDING SUPPORT

University will provide current & pending support information for Key Personnel identified in Exhibit A2 at time of proposal and upon request from State agency. The "Proposed Project" is this application that is submitted to the State. Add pages as needed.

Personnel: Shao-Hung Lee

Status (currently active or pending approval)	Award # (if available)	Source (name of the sponsor)	Project Title	Start Date	End Date
Proposed Project	N/A	Structural Pest Control Board	Improving application of dusts and gel baits for control of insecticide-resistant German cockroaches, a major pest of public health importance (as PI)	Jan 2026	Jun 2028
Proposed Project	N/A	Structural Pest Control Board	Impact of High Temperature and Chitin Synthesis Inhibitors on Gut Microbial Symbiont Community and Desiccation Tolerance in Western Drywood Termite (as co-PI)	Jan 2026	Jun 2028
PENDING	N/A (TBD)	California Department of Pesticide Regulation	Improvement of silica gel dust and dust mixtures as safer alternatives, and development of a lethal harborage for German cockroach control (as PI)	2025 (exact date TBD)	Jun 2028
PENDING	N/A (TBD)	California Department of Pesticide Regulation	*Reinventing Integrated Pest Management System for Wood Destroying Insects* (as co-PI)	2025 (exact date TBD)	TBD
CURRENT PROJECT		Syngenta Crop Protection, NC	Resistance monitoring of an isoxazoline compound in *Blattella germanica* in United States	Jan 2022	Dec 2025

Exhibit A6 – Current & Pending Support

CURRENT & PENDING SUPPORT

University will provide current & pending support information for Key Personnel identified in Exhibit A2 at time of proposal and upon request from State agency. The "Proposed Project" is this application that is submitted to the State. Add pages as needed.

Personnel: **Gregory Kund**

Status (currently active or pending approval)	**Award # (if available)**	**Source (name of the sponsor)**	**Project Title**	**Start Date**	**End Date**
Proposed Project	N/A	Structural Pest Control Board	Improving application of dusts and gel baits for control of insecticide-resistant German cockroaches, a major pest of public health importance (as PI)	Jan 2026	Jun 2028
Proposed Project	N/A	Structural Pest Control Board	Impact of High Temperature and Chitin Synthesis Inhibitors on Gut Microbial Symbiont Community and Desiccation Tolerance in Western Drywood Termite (as co-PI)	Jan 2026	Jun 2028
PENDING	N/A (TBD)	California Department of Pesticide Regulation	Improvement of silica gel dust and dust mixtures as safer alternatives, and development of a lethal harborage for German cockroach control (as PI)	2025 (exact date TBD)	Jun 2028
PENDING	N/A (TBD)	California Department of Pesticide Regulation	*Reinventing Integrated Pest Management System for Wood Destroying Insects* (as co-PI)	2025 (exact date TBD)	TBD
CURRENT PROJECT		Syngenta Crop Protection, NC	Resistance monitoring of an isoxazoline compound in *Blattella germanica* in United States	Jan 2022	Dec 2025
CURRENT PROJECT		California Celery Research Advisory Board	Insecticide resistance of celery pests focusing on the Lygus bug, Lygus hesperus	Oct 2022	Sep 2025

Exhibit A7

Third Party Confidential Information

Confidential Nondisclosure Agreement

(Identified in Exhibit A, Scope of Work – will be incorporated, if applicable)

If the Scope of Work requires the provision of third-party confidential information to either the State or the Universities, then any requirement of the third party in the use and disposition of the confidential information will be listed below. The third party may require a separate Confidential Nondisclosure Agreement (CNDA) as a requirement to use the confidential information. Any CNDA will be identified in this Exhibit A7.

Not applicable

EXHIBIT B

BUDGET FOR PROJECT PERIOD

(Cost Proposal/Budget Display from selected proposer will be inserted here)

EXHIBIT B-1

BUDGET JUSTIFICATION

The Budget Justification will include the following items in this format.

Personnel

Name. *Starting with the Principal Investigator list the names of all known personnel who will be involved on the project for each year of the proposed project period. Include all collaborating investigators, individuals in training, technical and support staff or include as "to be determined" (TBD).*

Chow-Yang Lee, PI

Dong-Hwan Choe, Co-PI

Michael Rust

Shao-Hung (Dennis) Lee

Gregory Kund

Role on Project. *For all personnel by name, position, function, and a percentage level of effort (as appropriate), including "to-bedetermined" positions.*

Chow-Yang Lee, PI, Professor & Endowed Presidential Chair in Urban Entomology, University of California, Riverside. 5% effort in-kind, no salary requested. Will serve as the PI during the entire project period, and will develop experimental designs, coordinate with co-PI and other project members, and provide academic oversight and guidance.

Dong-Hwan Choe, co-PI, Cooperative Extension Specialist / Professor in Urban Entomology, University of California, Riverside. 1% effort in-kind, no salary requested. Will serve as the co-PI during the entire project period, collaborating with the PI, Chow-Yang Lee

Michael Rust, Distinguished Professor of Entomology, Emeritus, University of California, Riverside. 1% effort in-kind, no salary requested. Will serve as a collaborator during the entire project period, providing advice and useful insights for the PI and co-PI.

Shao-Hung (Dennis) Lee, Postdoctoral Researcher (30% effort). will be organizing and carrying out much of the research activities described in the proposal. The postdoctoral researcher will be integral to the experimental design and data collection for the project under the guidance of PI (Lee) and other project team members.

Gregory Kund, Staff Research Associate IV (20% effort). Kund will work with the Postdoctoral Researcher (Lee) and PI to set up experiments described in the proposal and collect and analyze the data.

Salary. *For all personnel, including "to-be-determined" positions, list the salary per year and the total salary. Additionally, note any in-kind salary contributions.*

Chow-Yang Lee – Year 1 - $0; in-kind; Year 2 - $0; in-kind; Year 3 - $0; in-kind; Total: $0; in-kind

Dong-Hwan Choe – Year 1 - $0; in-kind; Year 2 - $0; in-kind; Year 3 - $0; in-kind; Total: $0; in-kind

Michael Rust – Year 1 - $0; in-kind; Year 2 - $0; in-kind; Year 3 - $0; in-kind; Total: $0; in-kind

Shao-Hung (Dennis) Lee – Year 1 - $21,490; Year 2 - $22,285; Year 3 - $22,953; Total: $66,728

Gregory Kund – Year 1 - $18,700; Year 2 - $19,261; Year 3 - $19,838; Total: $ 57,799

Fringe Benefits.

In accordance with University policy, explain the costs included in the budgeted fringe benefit percentages used, which could include tuition/fee remission for qualifying personnel to the extent that such costs are provided for by University policy, to estimate the fringe benefit expenses on Exhibit B.

Dong-Hwan Choe – Year 1 - $0; in-kind; Year 2 - $0; in-kind; Year 3 - $0; in-kind; Total: $0; in-kind

Chow-Yang Lee – Year 1 - $0; in-kind; Year 2 - $0; in-kind; Year 3 - $0; in-kind; Total: $0; in-kind

Michael Rust – Year 1 - $0; in-kind; Year 2 - $0; in-kind; Year 3 - $0; in-kind; Total: $0; in-kind

Shao-Hung (Dennis) Lee – Year 1 - $4,148; Year 2 - $4,301; Year 3 - $4,430; Total: $12,879

Gregory Kund – Year 1 - $8,490; Year 2 - $8,744; Year 3 - $9,006; Total: $ 26,240

Travel

Itemize all travel requests separately by trip and justify in Exhibit B1, in accordance with University travel guidelines. Provide the purpose, destination, travelers (name or position/role), and duration of each trip. Include detail on airfare, lodging and mileage expenses, if applicable. Should the application include a request for travel outside of the state of California, justify the need for those out-of-state trips separately and completely.

Travel expenses are minimal and will be $1,500 for the partial cost of a leased truck, which will be used for cockroach collection and routine trips for supplies and colony maintenance at different lab locations. These fees are equivalent to leasing a vehicle at 6% interest for $684.16/month or $ 8,209.92 annually, as stipulated by UC Riverside Fleet Services ($0.06 x $ 8,209.92 = $500 per year).

Materials and Supplies

Itemize materials supplies in separate categories. Include a complete justification of the project's need for these items. Theft sensitive equipment (under $5,000) must be justified and tracked separately in accordance with State Contracting Manual Section 7.29.

The materials and supplies required for rearing insect colonies and completing the experiments for the 3-year project will cost $3,000. The supplies needed will be trash cans ($300) to house the roaches, dog food (14 bags/year x $50=$700), cotton for water wicks ($100 per case of 12 rolls each year=$300), and quart sized water jars (case of 12 $25).

One-time material expenses:	**Annual expenses:**
4 -44-gal Trash cans $350	Dog food 14 bags x $50=700
Water jars 1 quart (case) $25	Cotton wick rolls (1 case) $100
Barrier double sided tape (5) $50	
Foil duct tape (5) $50	
9V batteries (5 packs) $125	
Subtotal=$600	Subtotal (3 years x $800) =$2,400
Total material expenses=$3,000	

Equipment

List each item of equipment (greater than or equal to $5,000 with a useful life of more than one year) with amount requested separately and justify each.

N/A

Consultant Costs

Consultants are individuals/organizations who provide expert advisory or other services for brief or limited periods and do not provide a percentage of effort to the project or program. Consultants are not involved in the scientific or technical direction of the project as a whole.

Provide the names and organizational affiliations of all consultants. Describe the services to be performed, and include the number of days of anticipated consultation, the expected rate of compensation, travel, per diem, and other related costs.

Subawardee (Consortium/Subrecipient) Costs

Each participating consortium organization must submit a separate detailed budget for every year in the project period in Exhibit B2 Subcontracts. Include a complete justification for the need for any subawardee listed in the application.

N/A

Other Direct Costs

Itemize any other expenses by category and cost. Specifically justify costs that may typically be treated as indirect costs. For example, if insurance, telecommunication, or IT costs are charged as a direct expense, explain reason and methodology.

N/A

Rent

If the Scope of Work will be performed in an off-campus facility rented from a third party for a specific project or projects, then rent may be charged as a direct expense to the award.

N/A

Indirect (F&A) Costs

Indirect costs are calculated in accordance with the budgeted indirect cost rate in Exhibit B.

Total: $58,851 (IDC rate: 35%)

Exhibit B2 – Subawardee Budgets

Budget Pertaining to Subawardee(s) (when applicable)

Subawardee Name: **Exhibit B2** ______________________

Principal Investigator (Last, First): ______________________

COMPOSITE SUBAWARDEE BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
07/01/2025 to 06/30/2028

From: / To: / BUDGET CATEGORY		7/1/2025 – 6/30/2026 Year 1	7/1/2026 – 6/30/2027 Year 2	7/1/2027 – 6/30/2028 Year 3	TOTAL
PERSONNEL: *Salary and fringe benefits.*		$0	$0	$0	$0
TRAVEL		$0	$0	$0	$0
MATERIALS & SUPPLIES		$0	$0	$0	$0
EQUIPMENT		$0	$0	$0	$0
CONSULTANT		$0	$0	$0	$0
SUBRECIPIENT		$0	$0	$0	$0
OTHER DIRECT COSTS (ODC)	***Subject to IDC Calc***				
ODC #1	***Y***	$0	$0	$0	$0
ODC #2	***Y***	$0	$0	$0	$0
ODC #3	***Y***	$0	$0	$0	$0
ODC #4	***Y***	$0	$0	$0	$0
ODC #5	***Y***	$0	$0	$0	$0
ODC #6	***Y***	$0	$0	$0	$0
TOTAL DIRECT COSTS		**$0**	**$0**	**$0**	**$0**
Indirect (F&A) Costs Rate	**F&A Base** MTDC *	*$0* **$0**	*$0* **$0**	*$0* **$0**	*$0* **$0**
TOTAL COSTS PER YEAR		**$0**	**$0**	**$0**	
TOTAL COSTS FOR PROPOSED PROJECT PERIOD					**$0**

EXAMPLE

* MTDC = Modified Total Direct Cost

JUSTIFICATION. *See Exhibit B1 - Follow the budget justification instructions.*

Project Period Budget Flexibility (lesser of % or Amount)

Prior approval required for budget changes between approved budget categories above the thresholds **%** **10.00***or*

% identified. **Amount** *$10,000*

Exhibit B3 – Invoice Elements

Invoice and Detailed Transaction Ledger Elements

In accordance with Section 14 of Exhibit C – Payment and Invoicing, the invoice, summary report and/or transaction/payroll ledger shall be certified by the University's Financial Contact and the PI (or their respective designees).

Summary Invoice – includes either on the invoice or in a separate summary document – by approved budget category (Exhibit B) – expenditures for the invoice period, approved budget, cumulative expenditures and budget balance available[1]

- Personnel
- Equipment
- Travel
- Subawardee – Consultants
- Subawardee – Subcontract/Subrecipients
- Materials & Supplies
- Other Direct Costs ○ TOTAL DIRECT COSTS (if available from system)
- Indirect Costs
    - TOTAL

Detailed transaction ledger and/or payroll ledger for the invoice period [2]

- Univ Fund OR Agency Award # (to connect to invoice summary)
- Invoice/Report Period (matching invoice summary)
- GL Account/Object Code
- Doc Type (or subledger reference)
- Transaction Reference#
- Transaction Description, Vendor and/or Employee Name
- Transaction Posting Date
- Time Worked
- Transaction Amount

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[1] If this information is not on the invoice or summary attachment, it may be included in a detailed transaction ledger.

[2] For salaries and wages, these elements are anticipated to be included in the detailed transaction ledger. If all elements are not contained in the transaction ledger, then a separate payroll ledger may be provided with the required elements.

Exhibit C – University Terms and Conditions

CMA (AB20) State/University Model Agreement Terms & Conditions 220

https://www.ucop.edu/research-policy-analysis-coordination/_files/cma_documents/exhibit-c_utc220_feb_2020.pdf

Attachment 6 – RÉSUMÉ/BIOSKETCH

RÉSUMÉ/BIOSKETCH

Attach Resume/Biosketch for the PI and other Key Personnel listed in Exhibit A2, Key Personnel.

Chow-Yang Lee

AFFILIATION: Department of Entomology, University of California, Riverside, CA 92521.

POSITION TITLE: Professor & Endowed Presidential Chair in Urban Entomology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Universiti Sains Malaysia, Penang	B.Sc. Ph.D.	07/1989 09/1993	08/1993 09/1996	Biology (Zoology) Entomology (Insect Toxicology)
Entomological Society of America	Board Certified Entomologist (BCE)		07/1997	Specialization: Urban & Industrial (No. B2452)

Personal Statement

My research direction centers around the behavioral, ecological, and physiological adaptations of urban insect pests, especially understanding how these adaptations help them to thrive in the urban environment and their biological trade-offs. I am also interested in the roles of human activities and propagule pressure in invasion history of urban insect pests. Using the research findings obtained, my students and I design, evaluate, and integrate multiple management tactics to provide a system-level approach towards urban pest management. Our recent research activities focus on morphological and biological traits, insecticide resistance and its underlying mechanisms, endosymbiont roles, phylogenetics, population genetics, and environmental physiology of bed bugs, termites, cockroaches, pest ants, and mosquitoes.

PROFESSIONAL APPOINTMENTS (1996–Present)

2019–present: Professor & Endowed Presidential Chair in Urban Entomology, University of California, Riverside (75% Professor of Entomology, 25% Entomologist)

2006–2019: Professor of Entomology, School of Biological Sciences, Universiti Sains Malaysia (50% Research, 50% Teaching)

2010: Acting Dean of Life Science, Universiti Sains Malaysia.

2002–2006: Associate Professor of Entomology, School of Biological Sciences, Universiti Sains Malaysia (50% Research, 50% Teaching)

1996–2002: Lecturer, School of Biological Sciences, Universiti Sains Malaysia (50% Research, 50% Teaching)

SELECTED HONORS AND AWARDS (2000 – 2025)

2025 Entomology Team Work Award, Pacific Branch, Entomological Society of America

2024 Fellow, Entomological Society of America

2022 Recognition Award in Medical, Urban & Veterinary Entomology, Entomological Society of America

2022	Distinguished Achievement Award in Urban Entomology, National Conference on Urban Entomology
2021	Medical, Urban & Veterinary Entomol. Award, Pacific Branch, Entomological Society of America
2012	Top Research Scientists Malaysia Award, awarded by the Academy of Sciences, Malaysia.
2008	The Outstanding Young Malaysian Awards 2008, Junior Chamber International Malaysia.
2003	MSPTM Silver Medal 2003, Malaysian Society of Parasitology and Tropical Medicine.
2002	Fulbright Scholarship 2002, Malaysian-American Commission of Educational Exchange.
2000	National Young Scientist Award 2000. Ministry of Science and Technology, Malaysia.

PUBLICATIONS AND MENTORING (1993 – 2025)

Total peer-reviewed journal articles:	**239**
Total edited books:	**7**
Total books:	**4**
Total book chapters:	**38**
Total number of graduate students mentored:	**17 Ph.D. and 33 M.S.**
Present number of graduate students mentored:	**4 Ph.D.**
Total number of postdoctoral scholars mentored:	**6**

PEER-REVIEWED PUBLICATIONS (2019 – 2025)

Feng X, DH Choe, MK Rust, CY Lee. 2025. Toxicant translocation and colony impact in the Pharaoh ant (*Monomorium pharaonis*) (Hymenoptera: Formicidae) after consumption of gel bait-killed German cockroach (*Blattella germanica*) (Blattodea: Ectobiidae) cadavers. *Journal of Economic Entomology*. https://doi.org/10.1093/jee/toaf199

Feng X, DH Choe, CY Lee. 2025. Effects of over-the-counter aerosol products on sociotomy of the Pharaoh ant, *Monomorium pharaonis* (Hymenoptera: Formicidae). *Journal of Economic Entomology*. https://doi.org/10.1093/jee/toaf185

Lum JY, R Chungsawat, EL Ta, DH Choe, CY Lee. 2025. Differential toxicological responses to commercial gel baits in the brownbanded cockroach and the German cockroach (Blattodea: Ectobiidae). *Journal of Medical Entomology*. https://doi.org/10.1093/jme/tjaf071

Chen JTC, L Nelson, PF Rugman-Jones, SP Tseng, AM Sutherland, DH Choe, MI Haverty, CY Lee. 2025. Description of a new species of subterranean termite in the genus *Reticulitermes* (Blattodea: Heterotermitidae) from Southern California. *Annals of the Entomological Society of America* 118: 315–330.

Lee SHD, M Zhao, CY Lee. 2025. Changes in insecticide susceptibility after sublethal exposure to deltamethrin in the German cockroach (Blattodae: Ectobiidae). *Journal of Economic Entomology.* https://doi.org/10.1093/jee/toaf124

Lee CY, SH Lee. 2025. Termite baiting – how it changed the landscape of the pest management industry and termite research in Southeast Asia. *Journal of Economic Entomology.* https://doi.org/10.1093/jee/toaf081

Lee CY. 2025. Global perspective of insecticide resistance in bed bugs and management options. *Entomological Research* 55: e70038.

Yu JJ, SH Lee, CY Lee, C Wang. 2025. Multiple mechanisms associated with deltamethrin and imidacloprid resistance in field-collected common bed bug, *Cimex lectularius* L. *Pesticide Biochemistry and Physiology.* https://doi.org/10.1016/j.pestbp.2025.106357

Poulos NA, CY Lee, MK Rust, DH Choe. 2025. Toxicity and horizontal transfer of chitin synthesis inhibitors in the western drywood termite (Blattodea: Kalotermitidae). *Journal of Economic Entomology*. https://doi.org/10.1093/jee/toaf064

Kruaysawat P, ME Chen, SH Lee, CY Lee, KB Neoh. 2025. Characterization of insecticide resistance and their mechanisms in field populations of the German cockroach (Blattodea: Ectobiidae) in Taiwan under different treatment regimes. *Journal of Economic Entomology* 118: 307–319.

Ng WK, KT Koay, CY Lee. 2025. Nutrient-enriched live lobster cockroach, *Nauphoeta cinerea*, enhances growth and pigmentation of the pearl arowana, *Scleropages jardini. Journal of Insects as Food and Feed* 11: 581–591.
DOI:10.1163/23524588-00001294

Hellemans S, MM Rocha, M Wang, JR Arias, DK Aanen, A-G Bagnères, A Buček, TF Carrijo, T Chouvenc, C Cuezzo, JP Constantini, R Constantino, F Dedeine, J Deligne, P Eggleton, TA Evans, C Jouault, R Hanus, MC Harrison, M Harry, G Josens, CM Kalleshwaraswamy, E Kaymak, J Korb, CY Lee, F Legendre, HF Li, N Lo, T Lu, K Matsuura, K Maekawa, DP McMahon, N Mizumoto, DE Oliveira, M Poulsen, D Sillam-Dussès, NY Su, G Tokuda, EL Vargo, JL Ware, J Šobotník, RH Scheffrahn, E Cancello, Y Roisin, MS Engel, T Bourguignon. 2024. Genomic data provide insights into the classification of extant termites. 2024. *Nature Communications* 15:6724.

Le B, K Campbell, H Park, S-P Tseng, R Pandey, GS Simmons, R Henderson, C Gispert, MK Rust, CY Lee, R Karimzadeh, YL Park, DH Choe. 2024. Field evaluations of biodegradable boric acid hydrogel baits for the control of Argentine ants: Promising results in vineyards and citrus orchards. *California Agriculture*. Doi: 10.3733/001c.120496.

Poulos NA, CY Lee, MK Rust, DH Choe. 2024. Potential use of pinenes to improve localized insecticide injections targeting the western drywood termite (Blattodea: Kalotermitidae). *Journal of Economic Entomology* 117: 1628–1635.

Lee SH, J So, GS Kund, JY Lum, E Trinh, EL Ta, R Chungsawat, DH Choe, DL Cox, MK Rust, CY Lee. 2024. Toxicity of isocycloseram, an isooxazoline insecticide, against laboratory and field-collected German cockroaches (Blattodea: Ectobiidae). *Journal of Economic Entomology* 117: 1086–1094.

Tseng SP, SH Lee, DH Choe, CY Lee. 2024. Overexpression of cytochrome P450 gene CYP6K1 is associated with pyrethroid resistance in German cockroaches (Blattodea: Ectobiidae) from California. *Journal of Economic Entomology* 117: 1071–1076.

Lee CY, ME Scharf. 2024. Editorial overview: Insecticide resistance mechanisms — from behavior and physiology to microbiome science. Current Opinion in Insect Science 63: 101204.

Scharf ME, CY Lee. 2024. Insecticide resistance in social insects: assumptions, realities, and possibilities. *Current Opinion in Insect Science* 62: 101161.

Lee SH, DH Choe, MK Rust, CY Lee. 2024. Oral toxicity of an artificial sweetener sucralose on the German cockroach (Blattodea: Ectobiidae) and its impact on water balance and gut microbiome. *Journal of Economic Entomology* 117: 268–279.

Rust MK, CY Lee, GW Bennett, WH Robinson. 2024. The emergence and sustainability of urban entomology. *Annual Review of Entomology* 69: 59–79.

Kamimura Y, CY Lee. 2023. Subcortical life, evolution of flattened body, and constrained mating posture in the earwig Platylabia major (Insecta: Dermaptera: “Anisolabididae”). *PloS One* 18: e0293701.

Tseng SP, LJ Nelson, CW Hubble, AM Sutherland, MI Haverty, CY Lee. 2023. Phylogenetic analyses of *Reticulitermes* (Blattodea: Rhinotermitidae) from California and other western states: multiple genes confirm undescribed species identified by cuticular hydrocarbons. *Journal of Economic Entomology* 116: 2135–2145.

Principato S, A Romero, CY Lee, K Campbell, DH Choe, C Schal, Z DeVries. 2023. Histamine excretion in common indoor and hematophagous arthropods. *Journal of Medical Entomology* 60: 1269–1277.

Tisgratog R, C Panyafaeng, SH Lee, MK Rust, CY Lee. 2023. Insecticide resistance and its potential mechanisms in field-collected German cockroaches (Blattodea: Ectobiidae) from Thailand. *Journal of Economic Entomology* 116: 1321–1328.

So J, DH Choe, MK Rust, JT Trumble & CY Lee. 2023. The impact of selenium on insects. *Journal of Economic Entomology* 116:1041–1062.

Rust MK, CY Lee, H Park, K Campbell, DH Choe, M Sorenson, A Sutherland, C Hubble, B Nobua-Behremann, J Kabashima, SP Tseng, L Post. The potential of fluralaner as a bait toxicant to control pest yellowjackets in California. *Insects* 14: 311. https://doi.org/ 10.3390/insects14040311

Le B, H Park, K Campbell, MK Rust, CY Lee & DH Choe. 2023. Laboratory evaluations of biodegradable boric acid hydrogel baits for the control of Argentine ant (Hymenoptera: Formicidae). *Journal of Economic Entomology* 116:643– 647.

Kamimura Y, CY Lee, J Yamasako, M Nishikawa. 2023. Identification and reproductive isolation of Euborellia species (Insecta, Dermaptera, Anisolabididae) from East and Southeast Asia. *Zookeys* 1146:115–134.

Doggett SL & CY Lee. 2023. Historical and contemporary control options against bed bugs, *Cimex* spp. *Annual Review of Entomology* 68: 169–190.

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Kai D, SL Doggett & CY Lee. 2022. Performance of pyrethroid-neonicotinoid mixture formulations against field- collected strains of the tropical bed bug (Hemiptera: Cimicidae) on different substrates. *Journal of Economic Entomology* 116: 29–39.

Lee CY, C Wang & NY Su. 2023. Perspective on biology and management of bed bugs: Introduction. *Journal of Economic Entomology* 116:1–4.

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Lee CC, HW Hsu, CY Lin, N Gustafson, K Matsuura, CY Lee & CCS Yang. 2022. First polycipivirus and unmapped RNA virus diversity in the yellow crazy ant, *Anoplolepis gracilipes. Viruses* 14: 2161. https://doi.org/ 10.3390/v14102161

Miki A, R Fukuda, K Takada, A Moriya, Y Kamimura, CY Lee & T Adachi-Yamada. 2022. Differences in energy source storage in eye stalks between two species of stalk-eyed flies, *Sphyracephala detrahens* and *Cyrtodiopsis dalmanni*. *Scientific Reports* 12: 9981.

Tseng SP, S Taravati, DH Choe, MK Rust, & CY Lee. 2022. Genetic evidence for multiple invasions of *Coptotermes formosanus* (Blattodea: Rhinotermitidae) in California. *Journal of Economic Entomology* 115: 1251–1256.

Lee SH, DH Choe, ME Scharf, MK Rust, & CY Lee. 2022. Combined metabolic and target-site resistance mechanisms confer fipronil and deltamethrin resistance in field-collected German cockroaches (Blattodea: Ectobiidae). *Pesticide Biochemistry and Physiology* 184: 105123.

Lee SH, DH Choe, MK Rust & CY Lee. 2022. Reduced susceptibility towards commercial bait insecticides in field German cockroach (Blattodea: Ectobiidae) populations from California. *Journal of Economic Entomology* 115: 259–265.

Lee CY, CCS Yang. 2022. Biology, ecology, and management of the invasive longlegged ant, *Anoplolepis gracilipes*. *Annual Review of Entomology* 67: 43–63.

Dang K, SL Doggett, XY Leong, G Veera Singham & CY Lee. 2021. Multiple mechanisms conferring broad spectrum insecticide resistance in the tropical bed bug (Hemiptera: Cimicidae). *Journal of Economic Entomology* 114: 2473–2484.

Dery M, CY Lee, DH Choe. 2021. Differential responses to aldehyde pheromone blends in two bed bug species (Heteroptera: Cimicidae). *Chemoecology* 31: 397–403.

Hu IH, HY Tzeng, ME Chen, CY Lee, KB Neoh. 2021. Association of *CYP4G19* overexpression with gel bait performance in pyrethroid-resistant German cockroaches (Blattodea: Ectobiidae) from Taiwan. *Journal of Economic Entomology* 114: 1764–1770.

Tseng SP, J Boone, L Boone, N King, S Taravati, DH Choe, CY Lee. 2021. Genetic analysis of Formosan subterranean termite populations in California (Blattodea: Rhinotermitidae). *Journal of Economic Entomology* 114: 1264–1269.

Matsumura Y, Y Kamimura, CY Lee, SN Gorb, H Rajabi. 2021. Penetration mechanics of elongated female and male genitalia of earwigs with a special focus on the bending stiffness. *Scientific Report* 11: 7920. https://doi.org/10.1038/s41598-021-86864-1

Lee SH, DH Choe, CY Lee. 2021. The impact of artificial sweeteners on insects. *Journal of Economic Entomology* 114: 1–13.

Lin CY, CC Lee, YS Nai, HW Hsu, CY Lee, K Tsuji, CCS Yang. 2020. Deformed wing virus in two widespread invasive ants: geographical distribution, prevalence, and phylogeny. *Viruses* 12: 1309.

Dery M, K Arriola, CY Lee, DH Choe. 2020. Ontogenesis of aldehyde pheromones in two synanthropic bed bug species (Heteroptera: Cimicidae). *Insects* 11: 759. Doi:10.3390/insects11110759

Hsu PW, S Hugel, JK Wetterer, SP Tseng, MCS Ooi, CY Lee, CC Yang. 2020. Ant crickets (Orthoptera: Myrmecophilidae) associated with the invasive yellow crazy ant *Anoplolepis gracilipes* (Hymenoptera: Formicidae): evidence for cryptic species and potential co-introduction with hosts. *Myrmecological News* 30: 103–129.

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Hu IH, SM Chen, CY Lee, KB Neoh. 2020. Insecticide resistance and its effects on bait performance in field-collected German cockroaches (Blattodea: Ectobiidae) from Taiwan. *Journal of Economic Entomology* 113: 1389–1398.

Leong XY, G Veera Singham, ASC Chong, SL Doggett, CY Lee. 2020. Influence of exposure time and mortality assessment intervals on bioassay results of insecticide-resistant tropical bed bugs (Hemiptera: Cimicidae). *Insects* 11: 640. Doi: 10.3390/insects11090640.

Hama Y, CY Lee, M Matsuda, Y Kamimura, K Sawamura. 2020. Long-term coexistence of a hybridization -derived population of *Drosophila parapallidosa* with closely related *Drosophila ananassae* (Diptera: Drosophilidae). *Entomological Science* 23: 405–415. Doi: 10.1111/ens.12441

Fujita M, CY Lee, R Machida. 2020. Reproductive biology and embryonic development of *Nocticola* sp. (Insecta: Blattodea, Nocticolidae). *Arthropod Systematics and Phylogeny* 78: 393–403.

Leong XY, DY Kim, K Dang, G Veera Singham, SL Doggett, CY Lee. 2019. Performance of commercial insecticide formulations against different developmental stages of insecticide-resistant tropical bed bugs *Cimex hemipterus* (Hemiptera: Cimicidae). *Journal of Economic Entomology* 113: 353–366.

Tseng SP, JK Wetterer, AV Suarez, CY Lee, T Yoshimura, D Shoemaker, CC Yang. 2019. Invasion and infection: mitochondrial diversity and *Wolbachia* selective sweep of a globally distributed invasive ant. *Frontiers in Genetics* 10: 838. Doi: 10.3389/fgene.2019.00838

Toki W, S Matsuo, PH Thai, P Meleng, CY Lee. 2019. Heads or tails: exaggerated morphologies in relation to the use of large bamboo internodes in two lizard beetles, *Doubledaya ruficollis* and *Oxylanguria acutipennis* (Coleoptera: Erotylidae: Languriinae). *Science of Nature (Naturwissenschaften)* 106: 50. Doi.org/10.1007/s00114-019-1645-6.

Tseng SP, H Darras, CY Lee, T Yoshimura, L Keller, CC Yang. 2019. Isolation and characterization of novel microsatellite markers for a globally distributed invasive ant *Paratrechina longicornis*. *European Journal of Entomology* 116: 253–257.

Tan MK, F Montealegre-Zapata, RH Abdul Wahab, CY Lee, DM Belabut, R Japir, ACY Chung. 2019. Ultrasonic songs and stridulum anatomy of *Asiophlugis* crystal predatory katydids (Tettigonioidea: Meconematinae: Phlugidini). *Bioacoustics*: DOI: 10.1080/09524622.2019.1637783

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Liu K, A Mansor, N Ruppert, CY Lee, NM Azman, N Fadzly. 2019. Rattan litter-collecting structures attract nest- building and defending ants. *Plant Signaling & Behavior*: DOI: 10.1080/15592324.2019. 1621245.

Kamimura Y, CC Yang, CY Lee. 2019. Fitness advantages of the biased use of paired laterally symmetrical penises in an insect. *Journal of Evolutionary Biology* 32: 844–855. DOI: 10.1111/jeb.13486.

Takeda K, J Yamauchi, A Miki, D Kim, XY Leong, SL Doggett, CY Lee, T Adachi-Yamada. 2019. Binucleation of male accessory gland cells in common bed bug *Cimex lectularius*. *Scientific Reports* 9: 6500 doi.org/10.1038/s41598-019-42844-0.

BIOGRAPHICAL SKETCH

NAME: **Choe, Dong-Hwan**
POSITION TITLE: Cooperative Extension Specialist / Professor in Urban Entomology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Department of Entomology University of California, Riverside, CA, USA	PhD	2009	Entomology
Department of Entomology University of California, Riverside, CA, USA	MS	2005	Entomology
Department of Agricultural Biology Korea University, Seoul, Korea	BA	2002	Agriculture

Experience and Research Focus

My research focuses on three major areas: urban entomology, insect behavior, and chemical ecology. In particular, my research has focused on exploring innate and learned behaviors of economically or environmentally important insect species to develop more effective integrated pest management (IPM) programs. I use manipulative laboratory studies to investigate how the behaviors of pest insects can be exploited to improve management and to develop novel management techniques. I also test the feasibility of these new techniques in real-world conditions. I draw upon my expertise in behavioral ecology, experimental design, chemical ecology and analytical chemistry to illuminate the biology of target insects, and to inform the design of new approaches for control. In addition to the bioassays involving chemistry, physiology, behavior, and toxicology, the effort to incorporate the behavioral information of target species into the working IPM program often requires extensive design, manufacturing, and testing of devices in the field.

Positions and Employment

7/2023 – current	CE Specialist and Professor Entomology / UC Riverside
7/2018 – 6/2023	Associate CE Specialist and Associate Professor Entomology / UC Riverside
11/2011 – 6/2018	Assistant CE Specialist and Assistant Professor Entomology / UC Riverside
11/2009 – 10/2011	Postdoctoral Researcher Environmental Science, Policy, and Management / UC Berkeley

Memberships, Honors and Awards

09/2016 – Present	Member. Pi Chi Omega.
01/2005 – Present	Member. Entomological Society of America.
2022	Award for Excellence in Extension, Entomological Society of America, Pacific Branch (PB ESA).
2020	Medical, Urban, and Veterinary Entomology Award. Entomological Society of America, Pacific Branch (PB ESA).

2016	Scientific Teaching Fellow. 2016 Summer Institute on Scientific Teaching for Undergraduate STEM Education.
2013 – 2016	Early Career Chair in Urban Entomology. UCR.

Presentations (selected since 2020)

02/2024	Biology of Termite and UCR Research Update, Target Specialty Product Workshop, Cerritos, CA.
02/2024	Low-impact management for urban pest ants: Two biorational approaches. Pest Insight webinar (organized by Dr. Siavash Taravati). Online.
11/2023	Controlling Argentine ants and fire ants & personal safety. Agricultural Operations, UC Riverside, Riverside, CA.
09/2023	Biodegradable boric acid hydrogel baits for the control of Argentine ant: Case studies in citrus orchard and grape vineyard. Ant Workshop (organized by David Havilland). Temecula, CA.
02/2023	Museum Pests and Their Management. Invited lecture for UCLA Information Studies (Dr. Ellen Pearlstein), Los Angeles, CA.
11/2022	The use of an attractant may improve localized insecticide treatments targeting the western drywood termite, *Incisitermes minor*. Entomological Society of America. Entomological Society of America. Vancouver, Canada.
08/2022	Evaluation of an artificial sweetener as a potential bait toxicant and an insecticide synergist against German cockroaches. CA Department of Pesticide Regulation Pest Management Advisory Committee (PMAC) Meeting. Online.
08/2022	A sustainable boric acid liquid bait delivery system for the management of pest ants in agricultural settings. CA Department of Pesticide Regulation Pest Management Advisory Committee (PMAC) Meeting. Online.
04/2022	The use of an attractant may improve localized insecticide treatments targeting the western drywood termite, *Incisitermes minor*. Pacific Branch Meeting of Entomological Society of America. Santa Rosa, CA.
02/2022	Drywood Termite Biology. PCOC / UC Berkeley Termite Academy. Online.
11/2021	Reducing Risks Associated with Fumigation by Improving Current Heat Treatment and Localized Treatment Technologies. CA DPR Pest Management Advisory Committee (PMAC) Meeting. California Department of Pesticide Regulation (CA DPR). Online.
11/2021	Biology of ants and their management (+UCR research update). Orange County PCOC (Pest Control Operators of California) Meeting. OC PCOC. Online.
11/2021	Delivering boric acid bait via Alginate Hydrogel: field studies with Argentine ants (Hymenoptera: Formicidae). Annual Meeting of Entomological Society of America. Entomological Society of America. Online.
09/2021	Biology of Ants and Their Management. CAPCA 2021 Urban Pest Management Seminar. CAPCA (California Association of Pest Control Advisers). Escondido, CA.
06/2021	Know Your Ants and How to Manage Them. Master Gardener workshop. UCCE Riverside County Master Gardener Program. Online.
05/2021	Developing an effective baiting strategy for yellowjacket management. Small Winegrowers Association California Meeting. Small Winegrowers Association California. Online.
03/2021	Solving current and evolving problems in urban pest management: Argentine ant case studies. Monthly Meetings of the San Francisco IPM Technical Advisory Committee (SF IPM TAC). SF IPM TAC. Online.
03/2021	Update on bed bug research & management. AzPPO (Arizona Pest Professionals Organization) 2021 Great Western Conference. AzPPO. Online.
02/2021	Drywood Termite Biology. Termite Academy 2021. Pest Control Operators California (PCOC) / University of California – Berkeley. Online.
12/2020	UC Riverside Research Update: Advances in Pheromone Research and Novel Detection Methods. Global Bed Bug Summit 2020. National Pest Management Association. Online.

11/2020 The use of an essential oil adjuvant to improve the efficacy of heat treatments targeting the western drywood termite. Annual Meeting of Entomological Society of America. Entomological Society of America. Online.

11/2020 Reduced-risk methods still need to be efficacious – field tests with pheromone and hydrogel. Annual Meeting of Entomological Society of America. Entomological Society of America. Online.

11/2020 Evaluation of an attractant to improve localized insecticide treatments targeting the western drywood termite, *Incisitermes minor*. Annual Meeting of Entomological Society of America. Entomological Society of America. Online.

10/2020 Ants – Nuisance Pests in and Around Buildings. Insects in the Built Environment. HalfMoon Education (https://halfmoonseminars.org). Online.

09/2020 Updates on Formosan subterranean termite infestation in Southern California. Orange County PCOC (Pest Control Operators of California) Meeting. OC PCOC. Online.

03/2020 Reduced-risk IPM strategies for Argentine ant control (2019 field study). Pyrethroids and Water Quality Training. UC IPM. Santa Clarita, CA.

Publications (selected since 2020)

Le, B., K. Campbell, H. Park, S.-P. Tseng, R. Pandey, G. S. Simmons, R.Henderson, C. Gispert, M. K. Rust, C.-Y. Lee, R. Karimzadeh, Y.-L. Park, and D.-H. Choe. 2024. Field Evaluations of Biodegradable Boric Acid Hydrogel Baits for the Control of Argentine Ants: Promising Results in Vineyards and Citrus Orchards. California Agriculture, June. https://doi.org/10.3733/001c.120496

Poulos, N. A, C.-Y. Lee, M. K. Rust, and D.-H. Choe. 2024. Potential use of pinenes to improve localized insecticide injections targeting the western drywood termite (Blattodea: Kalotermitidae). J. Econ. Entomol. 117: 1628–1635. https://doi.org/10.1093/jee/toae101

Lee, S.-H, D.-H. Choe, M. K. Rust, and C.-Y. Lee. 2024. Oral toxicity of an artificial sweetener sucralose on the German cockroach (Blattodea: Ectobiidae) and its impact on water balance and gut microbiome. J. Econ. Entomol. 117: 268–279. https://doi.org/10.1093/jee/toad206

Rust, M. K., C.-Y. Lee, H. E. Park, K. Campbell, D.-H. Choe, M. Sorensen, A. Sutherland, C. Hubble, B. Nobua-Behrmann, J. Kabashima, S.-P. Tseng, and L. Post. 2023. The potential of fluralaner as a bait toxicant to control pest yellowjackets in California. Insects. 14, 311.

Le, B., H. Park, K. Campbell, M. K. Rust, C.-Y. Lee, and D.-H. Choe. 2023. Laboratory evaluations of biodegradable boric acid hydrogel baits for the control of Argentine ant(Hymenoptera: Formicidae). J. Econ. Ent. 116: 643–647.

Dery, M., and D.-H. Choe. 2023. Effect of bed bug (Hemiptera: Cimicidae) aldehydes on efficacy of fungal biopesticides. J. Econ. Entomol. 116: 40–46.

Dery, M., Dinh, B., Budd, R., & Choe, D.-H. 2022. Wash-off potential of pyrethroids after use of total release fogger products. Science of The Total Environment, 847, 157340. https://doi.org/10.1016/j.scitotenv.2022.157340 (Refereed)

Tseng, S.-P., Taravati, S., Choe, D.-H., Rust, M. K., & Lee, C.-Y. 2022. Genetic Evidence for Multiple Invasions of *Coptotermes formosanus* (Blattodea: Rhinotermitidae) in California. Journal of Economic Entomology, 115(4), 1251–1256. https://doi.org/10.1093/jee/toac104

Lee, S.-H., Choe, D.-H., Scharf, M. E., Rust, M. K., & Lee, C.-Y. 2022. Combined metabolic and target-site resistance mechanisms confer fipronil and deltamethrin resistance in field-collected German cockroaches (Blattodea: Ectobiidae). Pesticide Biochemistry and Physiology, 184, 105123. https://doi.org/10.1016/j.pestbp.2022.105123

Lee, S.-H., Choe, D.-H., Rust, M. K., & Lee, C.-Y. 2021. Reduced Susceptibility Towards Commercial Bait Insecticides in Field German Cockroach (Blattodea: Ectobiidae) Populations from California. Journal of Economic Entomology. https://doi.org/10.1093/jee/toab244

Dery, M., C.-Y. Lee, and D.-H. Choe. 2021.Differential responses to aldehyde pheromone blends in two bed bug species (Heteroptera: Cimicidae). Chemoecology. 31: 397–403.

Choe, D.-H., J.-W. Tay, K. Campbell, H. Park, L. Greenberg, and M. K. Rust. 2021. Development and demonstration of lowimpact IPM strategy to control Argentine ants (Hymenoptera: Formicidae) in urban residential settings. J. Econ. Entomol. 114: 1752–1757.

Lee, S.-H., D.-H. Choe, and C.-Y. Lee. 2021. The impact of artificial sweeteners on insects. J. Econ. Entomol. 114: 1-13.

Dery, M., K. Arriola, C.-Y. Lee, and D.-H. Choe. 2020. Ontogenesis of aldehyde pheromones in two synanthropic bed bug species (Heteroptera: Cimicidae). Insects. 11(11), 759.

Ko, A. and D.-H. Choe. 2020. Development of a lateral flow test for bed bug detection. Sci. Rep. 10: 13376. https://doi.org/10.1038/s41598-020-70200-0

Tay, J.-W., D.-H. Choe, A. Mulchandani, and M. K. Rust. 2020. Hydrogels: from controlled release to a new bait delivery for insect pest management. J. Econ. Entomol. 113: 2061–2068.

Perry, D. T. and D.-H. Choe. 2020. Volatile essential oils can be used to improve the efficacy of heat treatments targeting the western drywood termite: evidence from simulated whole house heat treatment trials. J. Econ. Entomol. 113: 2448-2457.

McCalla, K., J.-W. Tay, A. Mulchandani, D.-H. Choe, M. S. Hoddle. 2020. Biodegradable alginate hydrogel bait delivery system effectively controls high-density populations of Argentine ant in commercial citrus. J. Pest. Sci. 93:1031–1042.

Perry, D. T. and D.-H. Choe. 2020. Volatile essential oils can be used to improve the efficacy of heat treatments targeting the western drywood termite: evidence from a laboratory study. J. Econ. Entomol. 113: 1373-1381.

Michael K. Rust – Curriculum Vitae

Residence	**Business**
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Academic Record

M.A. University of Kansas, Lawrence, Kansas. Entomology. 1973. Thesis title: "The Mecoptera of India and Adjacent Regions." Major Professor – Dr. George W. Byers.

Ph.D. University of Kansas, Lawrence, Kansas, 1975. Dissertation title: "An Ethometric Analysis of Sex Pheromone and Associated Behavior in the American Cockroach, *Periplaneta americana*." Major Professor – Dr. William J. Bell.

Positions

Distinguished Professor and Entomologist, Department of Entomology and the Graduate Division, University of California, Riverside — 2012-present

Distinguished Professor and Entomologist, Department of Entomology, University of California, Riverside — 2011-2012

Professor and Entomologist, Department of Entomology University of California, Riverside — 2003-2010

Professor and Entomologist, Department of Entomology University of California, Riverside. Director for Center for Exotic Pest Research; Associate Director University of California Integrated Pest Management Program — 2000-2003

Professor and Entomologist, Department of Entomology University of California, Riverside — 1997-1999

Associate Professor and Associate Entomologist and Head, Division of Economic Entomology, University of California, Riverside — 1983-1986

Assistant Professor and Assistant Entomologist, Department of Entomology, University of California, Riverside — 1975-1982

Professional Societies – Entomological Society of America, Pi Chi Omega, AAAS

Honors and Awards

Orkin Research Award – 1990, 1995, 1997
Outstanding Urban Entomologist Award Recipient; Pacific Branch Entomology Society of America, 1990, 1991 and 1993
Distinguished Achievement Award in Urban Entomology, Entomology Society of America, 1993
W.W. Woodworth Award; Pacific Branch, Entomology Society of America 1994
1994 Excellence in Entomology Award – California Association, American Registry of Professional Entomologists
Book of Professional Services Award, Pi Chi Omega, 1995

UC Presidential Scholar in Entomology, 1999, 2000
Mallis Recognition Award, National Conference on Urban Entomology, 2000
Pest Control Technology's 25 Most Influential People in the Industry, 2000
Fellow of the Entomological Society of America, 2001
Fellow of American Association for the Advancement of Science, 2002
PCT/Zeneca Leadership Award 2002
Pest Control Hall of Fame 2007
Entomological Society of America Recognition Award in Entomology, Pacific Branch 2008
Lifetime Achievement Award – Association of Applied IPM Ecologists 2009
IPM Team Award 2010, Pacific Branch of the Entomological Society of America
IPM Team Award 2010, National Entomological Society of America

<u>Recent Relevant Publications</u>

Tay, J.-W, D.-H. Choe, A. Mulchandani, and M.K. Rust. 2020. Hydrogels: from controlled release to a new bait delivery for insect pest management. J. Econ. Entomol. 11: 2061-6068.

Wang, C., C.-Y. Lee, and M K. Rust. 2021. Biology and Management of the German cockroach. CSIRO, Clayton South, Australia. 308 pp.
Appel, A.G., and M.K. Rust. 2021. Management using baits. In: Biology and Management of the German Cockroach (Wang, C., C.-Y. Lee, M.K. Rust, eds.). CSIRO, Clayton South Australia. Pp. 213-230.

Lee, C.-Y., and M.K. Rust. 2021. Chemical control methods. In: Biology and Management of the German Cockroach (Wang, C., C.-Y. Lee, M.K. Rust, eds.). CSIRO, Clayton South Australia. Pp. 165-212.

Rust, M.K. 2021. Alternative control measures. In: Biology and Management of the German Cockroach (Wang, C., C.-Y. Lee, M.K. Rust, eds.). CSIRO, Clayton South Australia. Pp. 257-268

Lee, S.-H., D.-H. Choe, M.K. Rust, and C.-Y Lee, 2022. Reduced susceptibility towards commercial bait insecticides in field German cockroach (Blattodea: Ectobiidae) populations from California. J. Econ. Entomol. 115: 259-265.

Lee, S.-H., D.-H. Choe, M.E. Scharf, M.K. Rust, and C.-Y. Lee. 2022. Combined metabolic and target-site resistance confer fipronil and deltamethrin resistance in field-collected German cockroaches (Blattodea: Ectobiidae). Pest. Biochem. Physiol. 184. Doi.org/10/1016/j./pestbp.2022. 105123.

Benning, L., H. Park, K. Campbell, M.K. Rust, C.-Y. Lee, and D.-H. Choe. 2023. Laboratory evaluations of biodegradable boric acid hydrogel baits for the control of Argentine ant (Hymenoptera: Formicidae). J. Econ. Entomol. 116: 643-647.

So, J., Choe, D.-H., Rust, M.K., Trumble, J.T., and Lee, C.-Y. 2023. The impact of selenium on insects. J. Econ. Entomol. 116: 1041-1062.

Tisgratog, R., C. Panyafeang, S.-H. Lee, M.K. Rust, and C.-Y. Lee. 2023. Insecticide resistance and its potential mechanisms in field-collected German cockroaches (Blattodea: Ectobiidae) from Thailand. J. Econ. Entomol. 116: 1321-1328.

Lee, S.-H., D.-H. Choe, M.K. Rust, C.-Y. Lee. 2024. Oral toxicity of artificial sweetener sucralose on the German cockroach (Blattodea: Ectobiidae) and its impact on water balance and microbiome. J. Econ. Entomol. Vol. 117: 268-279.

Lee, S.-H., J. So, G.S. Kund, J.Y. Lum, E. Trinh, E.L. Ta, R. Chungsawat, D.-H. Choe, D. L. Cox, M.K. Rust, C.-Y. Lee. 2024. Toxicity of isocycloseram, an 39sooxazoline insecticide, against laboratory and field-collected German cockroaches (Blattodea: Ectobiidae). J. Econ. Entomol.

Poulos, N.A., C.-Y. Lee, M.K. Rust, D.-H. Choe. 2024. Potential use of pinenes to improve localized insecticide injections targeting the western drywood termite (Blattode: Kalotermitidae. J. Econ. Entomol. 10.193/jee/toae 101.

SHAO-HUNG DENNIS LEE
CURRICULUM VITAE

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Riverside, CA 92521

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EDUCATION

2023:	Ph.D., Entomology, University of California, Riverside. GPA 4.00
2018:	BS, Entomology, University of California, Riverside. GPA 3.76

AWARDS AND HONORS

2017–2020, 2022:	Carl Strom and Western Exterminator Scholarship, UC Riverside
2018:	Chancellor's Distinguished Fellowship, UC Riverside

PROFESSIONAL APPOINTMENTS

2023–Present:	Postdoctoral Scholar, University of California, Riverside

PEER-REVIEWED PUBLICATIONS

1. **Lee SHD**, M Zhao, CY Lee. 2025. Increase in insecticide susceptibility after sublethal exposure to deltamethrin in the German cockroach (Blattodea: Ectobiidae). *Journal of Economic Entomology*: https://doi.org/10.1093/jee/toaf124
2. Lee CY, **SHD Lee**. 2025. Termite baiting—how it changed the landscape of the pest management industry and termite research in Southeast Asia. *Journal of Economic Entomology*: https://doi.org/10.1093/jee/toaf081
3. Yu JJ, **SH Lee**, CY Lee, C Wang. 2025. Multiple mechanisms associated with deltamethrin and imidacloprid resistance in field-collected common bed bug, Cimex lectularius L. *Pesticide Biochemistry and Physiology* 210: 106357.
4. Kruaysawat P, ME Chen, **SH Lee**, CY Lee, KB Neoh. 2024. Characterization of insecticide resistance and their mechanisms in field populations of the German cockroach (Blattodea: Ectobiidae) in Taiwan under different treatment regimes. *Journal of Economic Entomology*: https://doi.org/10.1093/jee/toae252
5. **Lee SH**, J So, GS Kund, JY Lum, E Trinh, EL Ta, R Chungsawat, DH Choe, DL Cox, MK Rust, CY Lee. 2024. Toxicity of isocycloseram, an isooxazoline insecticide, against laboratory and field-collected German cockroaches (Blattodea: Ectobiidae). Journal of Economic Entomology: https://doi.org/10.1093/jee/toae079
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8. Tisgratog R, C Panyafaeng, **SH Lee**, MK Rust, CY Lee. 2023. Insecticide resistance and its potential mechanisms in field-collected German cockroaches (Blattodea: Ectobiidae) from Thailand. *Journal of Economic Entomology* 116: 1321–1328.

9. **Lee SH**, DH Choe, ME Scharf, MK Rust, & CY Lee. 2022. Combined metabolic and target-site resistance mechanisms confer fipronil and deltamethrin resistance in fieldcollected German cockroaches (Blattodea: Ectobiidae). *Pesticide Biochemistry and Physiology* 184: 105123.
10. **Lee SH**, DH Choe, MK Rust & CY Lee. 2022. Reduced susceptibility towards commercial bait insecticides in field German cockroach (Blattodea: Ectobiidae) populations from California. *Journal of Economic Entomology* 115: 259–265.
11. **Lee SH**, DH Choe, CY Lee. 2021. The impact of artificial sweeteners on insects. *Journal of Economic Entomology* 114: 1–13.
12. Welzel K, **SH Lee**, AT Dossey, KR Chauhan, DH Choe. 2018. Verification of Argentine ant defensive compounds and their behavioral effects on heterospecific competitors and conspecific nestmates. *Scientific Reports* 8(1), 1477.

RESEARCH FUNDING

2024:	$291,125 California Department of Pesticide Regulation Pest Management Grant
2021:	$110,938 California Department of Pesticide Regulation Pest Management Grant

PRESENTATIONS

Academic/Scientific Presentations

1. Horizontal transfer of a novel isocycloseram bait on German cockroaches (Blattodea: Ectobiidae). Internation Conference on Urban Pests. June 30, 2025.
2. Responses to sublethal insecticide exposure in the German cockroach (Blattodea: Ectobiidae). Entomological Society of American Annual Meeting. November 12, 2024.
3. Toxicity of isocycloseram (Plinazolin) against insecticide-susceptible and -resistant German cockroaches (Blattodea: Ectobiidae). Invited speaker. National Conference on Urban Entomology. May 21, 2024.
4. Insecticide Resistance and Underlying Mechanisms in Field-Collected German Cockroaches (Blattodea: Ectobiidae) From California, and Sucralose as a Potential Bait Toxicant. Invited speaker. National Taiwan University. December 11, 2023.
5. Effect of an artificial sweetener on the gut microbiota of the German cockroach (Blattodea: Ectobiidae). Entomological Society of America Annual Meeting. November 7, 2023.
6. Effects of an artificial sweetener on German cockroach (Blattodea: Ectobiidae) water balance. Entomological Society of America Annual Meeting. November 14, 2022.
7. Investigation of insecticide resistance mechanisms in field-collected German cockroaches (*Blattella germanica* L.) from California. Entomological Society of America Annual Meeting. November 1, 2021.
8. The impact of an artificial sweetener on insecticide resistant and susceptible German cockroaches (*Blattella germanica* L.) Entomological Society of America Annual Meeting. November 11–25, 2020. Virtual.

Presentations at Pest Management Meetings

1. Investigating Artificial Sweeteners as German Cockroach Insecticides. UCR Urban Pest Management Conference. March 25, 2025.
2. How Sweet is it: Investigating Artificial Sweeteners for Cockroach Control. Pest World. May 21, 2024.
3. Insecticide Resistance: Overview, Mechanisms, and Perspectives on Management. Invited speaker. UCR Urban Pest Management Conference. March 26, 2024.
4. Seminar on identification of common urban pests. UCR Urban Pest Management Conference. Co-presenter. November 10, 2020. Virtual.

5. Understanding and managing insecticide resistance in the German cockroach. Invited speaker. UCR Urban Pest Management Conference. March 25, 2020. Virtual.

TEACHING

Guest Lectures at UC Riverside

2024: ENTM 128 Principles of Insect Pest Management (March 12–14, 2024)
2022: ENTM 125 Pesticides, Biological Organisms, and the Environment (Fall 2022)
2020: ENTM 125 Pesticides, Biological Organisms, and the Environment (May 7, 2020)

Teaching Assistant at UC Riverside

2020: ENTM 133 Urban Entomology
2021–2022: ENTM 100 General Entomology

STUDENT MENTORSHIP

Graduate Student

Jin-Jia Yu, visiting student, guided experimental methodology (2023)

Undergraduate Student

Hana Mancia, research course credit (Winter 2025) David Ness, research course credit (Spring 2022)
Justin Luy, research course credit (Fall 2021)

High School Student

Jack Jennings, science fair competition (Fall–Winter 2022)

PROFESSIONAL SERVICE

Reviews for Academic Journals

Journal of Economic Entomology – 6 reviews (2020–2025)
Current Opinion in Insect Science – 1 review (2023)
Physiological Entomology – 2 reviews (2024)
Parasites & Vectors – 1 review (2023)
Pest Management Science – 2 reviews (2025)
Bulletin of Entomological Research – 2 reviews (2024–2025)
Tropical Life Sciences Research – 1 review (2025)

Organizations

Treasurer – Entomological Society of America MUVE Section (2025 – Present)
Hospitality Committee Chair – Entomology Graduate Student Association at UC Riverside (2019–2022)
President – Botany and Entomology Undergraduate Student Association at UC Riverside (2017–2018)
Treasurer – Botany and Entomology Undergraduate Student Association at UC Riverside (2016–2017)

OUTREACH

2018–2019: Outreach coordinator for UC Riverside Entomology

Scheduled outreach events, prepared materials, organized personnel, upkeep of outreach resources

2018–2019: > 200 hours of outreach for UC Riverside Entomology

In person education and events with local community

2016–2025: Booth at Riverside Insect Fair

Interactive education on urban entomology

2019, 2024: Judge for Riverside Unified School District science fair

Interviewed middle school and high school students, scored posters

2023–2025: Riverside Insect Fair Cockroach Race MC

REFERENCES

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EDUCATION

Riverside Community College, 1987, Associate Arts Degree.
University of California at San Diego, Revelle College, 1990, B.A. Biology.
University of California at Riverside,1992, Certificate in Hazardous Materials Management.

PROFESSIONAL EXPERIENCE

Laboratory Helper. University of California, Riverside, Entomology, 1984-1987.
Laboratory Assistant II. University of California, Riverside, Entomology, 1988-1989.
Laboratory Assistant III. University of California, Riverside, Entomology, 1990.
Environmental Technician. IOLAB, a Johnson & Johnson Company, 1990-1992.
Associate Scientist. IOLAB, a Johnson & Johnson Company, 1992-1994.
Staff Research Associate I. University of California Riverside, Entomology, 1995-1997.

Staff Research Associate II. Univ. of California Riverside, Entomology,1997 to 2014.

Staff Research Associate III. Univ. of California Riverside, Entomology, 2014 to present.

Staff Research Associate IV. Univ. of California Riverside, Entomology, 2023 to present.

Publications (peer reviewed):

Lee SH, J So, GS Kund, JY Lum, E Trinh, EL Ta, R Chungsawat, DH Choe, DL Cox, MK Rust, CY Lee. 2024. Toxicity of isocycloseram, an isooxazoline insecticide, against laboratory and field-collected German cockroaches (Blattodea: Ectobiidae). *Journal of Economic Entomology* 117: 1086–1094.

Huang CY, Niu D, Kund G, Jones M, Albrecht U, Nguyen L, Bui C, Ramadugu C, Bowman K, Trumble J, Jin H. 2021. A stable antimicrobial peptide with dual functions of treating and preventing citrus Huanglongbing. *Proceeding of the National Academy of Sciences* 118 (6): 1-10.

Huang, C-Y, D. Niu, G. Kund, M. Jones, U. Albrecht, L. Nguyen, C. Bui, C. Ramadugu, K. Bowman, J. Trumble, and J. Hailing. 2020. Identification of citrus immune regulators involved in defense against Huanglongbing using a new functional screening system. *Plant Biotechniology Journal.* Pp. 1-10. Doi: 10.1111/pbi.13502

Li, Z., G. Kund, D. M. De Jong, X. Feng, M. A. Mutschler, J. T. Trumble. 2019. Effects of high-level acylsugar producing tomato lines on the development of tomato psyllids *(Bactericera cockerelli)*. J. Econ. Entomol. 112: 1926–1931.

Prager, S. M., G. Kund and J.T. Trumble. 2016. Low-input, low-cost IPM program helps manage potato psyllid. California Agriculture 70(2):89-95. DOI: 10.3733/ca.v070n02p89. April-June 2016.

Keremane ML, C Ramadugu, Y Duan, L Zhou, G Kund, J Trumble, and R Lee. 2014. Improved methods for genome sequencing of Liberibacters by BAC library-based metagenomics approach. *Journal of Citrus Pathology* 1: 254.

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Trumble, J. T., W. G. Carson and G. Kund. 1997. Economics and environmental impact of a sustainable integrated pest management program in celery. J. Economic Entomology. 90: 139-146.

Publications (recent non-peer reviewed):
2012

Kund, G., Carson, W.G., Trumble, J.T. 2012. Effect of insecticides on celery insects, 2010. Arthropod Management Tests: Vol 37: E22 (Non-Refereed, Electronic)
Website: http://www.entsoc.org/system/Protected/AMT/members_only/AMT37/E/E22.pdf

2013
Kund, G., Carson, W.G., Trumble, J.T. 2013. Effect of insecticides on pepper insects, 2012. Arthropod Management Tests: Vol 38 (1): E42 (Non-Refereed, Electronic)
Website: DOI: http://dx.doi.org/10.4182/amt.2013.E42.

Kund, G., Carson, W.G., Trumble, J.T. 2013. Effect of insecticides on celery insects, 2011. Arthropod Management Tests: Vol 38 (1): E21 (Non-Refereed, Electronic)
Website: DOI: http://dx.doi.org/10.4182/amt.2013.E21.

2014

Kund, G., Carson, W.G., Trumble, J.T. 2014. Effect of insecticides on pepper insects, 2013. Arthropod Management Tests: Vol 39 (1): E26 (Non-Refereed, Electronic)
Website: DOI: http://dx.doi.org/10.4182/amt.2014.E26.

Carson, W., Kund, G., Trumble, J.T. 2014. Effect of insecticides on tomato insects, 2013. Arthropod Management Tests: Vol 39 (1): E6 (Non-Refereed, Electronic)
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2015
Kund, G., Carson, W.G., Trumble, J.T. 2015. Effect of insecticides on pepper insects, 2014. Arthropod Management Tests: Vol 40 (1): E17 (Non-Refereed, Electronic)
Website: DOI: http://dx.doi.org/10.1093/amt/tsv075.

Carson, W., Kund, G., Trumble, J.T. 2015. Effect of insecticides on tomato insects, 2014. Arthropod Management Tests: Vol 40 (1): E18 (Non-Refereed, Electronic)
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2016
Kund, G., Carson, W.G., Trumble, J.T. 2016. Effect of insecticides on pepper insects, 2015. Arthropod Management Tests: Vol 41 (1): tsw098 (Non-Refereed, Electronic)
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2017

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2019

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Website: DOI: https://doi.org/10.1093/amt/tsz035

Kund, G., Jones, M.B.J., Trumble, J.T. 2019. Effect of insecticides on pepper insects, 2018. Arthropod Management Tests: Vol 44 (1): tsz036 (Non-Refereed, Electronic)
Website: DOI: https://doi.org/10.1093/amt/tsz036

ATTACHMENT 7. NARRATIVE OF RESEARCH OBJECTIVES

Improving application of dusts and gel baits for control of insecticide resistant German cockroaches, a major indoor pest of public health importance

A. Background

The German cockroach, *Blattella germanica* (L.), is one of the most common indoor pest species of major public health concern (Wang et al. 2021). Severe infestations are a key source of pathogens, allergens, and chronic childhood asthma (Schal and DeVries 2021). Although they are primarily controlled by insecticides, insecticide resistance often prevents effective management. Pest management professionals (PMPs) can combat resistance by integrating multiple insecticides in rotation and mixture programs to slow down their development; however, this is only possible if PMPs have an adequate selection of viable and registered insecticides.

Two common methods for controlling German cockroaches are the use of sprays and gel baits. Pyrethroids comprise the majority of the active ingredients in spray formulations; however, widespread pyrethroid resistance in California raises concerns about their future effectiveness (Lee et al. 2022b). In contrast, gel baits have proven to be effective and reliable because they are easy to apply, have lower toxicity, and introduce less insecticide into the environment. However, resistance to these formulations is also increasing, and relying solely on one type of control, regardless of its initial success, can lead to resistance (Lee et al. 2022a).

The decline of pyrethroid sprays and the increasing challenges to gel baits underscore the importance of finding alternative control options, particularly those that can support the use of gel baits. Dusts are a different formulation category, applied as dry powders into harborage sites, cracks, crevices, and other hard-to-reach areas. Unlike sprays and gel baits, modern dust products are rarely used, with a 2025 survey showing less than 2% of PMP use (pctonline.com). However, dusts provide a versatile range of active ingredients, including desiccants, oral toxicants, and contact toxicants. No resistance has been observed to inorganic dust ingredients like silica gel and boric acid, and new neurotoxic insecticides have been introduced recently in dry flowable bait dusts, designed for faster kills. Dusts can also last a long time if applied correctly and left undisturbed.

Despite their versatility and potential to address resistance issues, there is a significant lack of research supporting the implementation of dusts into German cockroach IPM. With the increased pressure on existing tools and the recent innovations in dust technologies, we believe it is time to conduct a thorough examination of their toxicity, behavioral effects, and compatibility with gel baits against insecticide resistant German cockroaches to determine how they can improve existing IPM programs.

B. Existing Knowledge and Knowledge Gaps

There are over a dozen insecticide dust products registered in California, each with different active ingredients. These include desiccants such as silica gel and diatomaceous earth, oral toxicants like boric acid, indoxacarb, and neonicotinoids, and contact toxicants like pyrethroids and pyrethrin. Despite recent market additions, such as Advion Microflow [0.22% indoxacarb], registered in 2022, only one peer-reviewed study in the past decade has involved any dust treatment (boric acid) on German cockroaches (Wang et al. 2019), and none have been conducted with the newer organic bait dusts.

It is often believed that inorganic dusts, such as silica gel and boric acid, are effective against insecticide-resistant cockroaches due to their different modes of action and the lack of documented resistance to their active ingredients (Gondhalekar et al. 2021). However, these formulations have not been recently tested, despite some evidence of decreased boric acid effectiveness in the field (Fardisi et al. 2019). There is also a similar lack of studies on neurotoxic dusts (indoxacarb, pyrethroids, etc.) regarding resistant German cockroaches, but resistance issues in strains collected from California (Lee et al. 2022a) highlight the need for further evaluation of this topic.

Repellency is a critical factor impacting dust performance, but repellency studies have only been conducted on historical inorganic formulations containing silica gel or boric acid (Ebeling et al. 1967, Appel et al. 2004). Minimal information exists on newer formulations containing organic insecticides or mixtures, despite the presence of pyrethroids in several modern products that potentially elicit irritancy/repellency behaviors similar to those of residual sprays (Gaire et al. 2024). The impact of other dust products on behavior is virtually unknown.

German cockroach IPM programs often involve the concurrent application of different insecticide formulations. Gel baits are the most common formulation for cockroach control, and they share comparable application targets with dusts, despite their different physical characteristics. Both are applied behind/under appliances and cabinets, in cracks and crevices, voids, and other areas that may harbor cockroaches. An IPM program utilizing both gel baits and dusts must consider physically overlapping applications that risk co-contamination. Interactions between dusts and gel baits, whether synergistic or antagonistic, have the potential to completely alter how both insecticides are applied. As with the other aforementioned issues, there are no studies examining these mechanisms; however, this must be addressed in order to responsibly consider dusts as IPM tools.

C. **Intended Accomplishments and Research Questions**

We have highlighted the following gaps in German cockroach dust research: Gap #1: lack of resistance studies, Gap #2: unknown repellency and behavioral effects, and Gap #3: unknown interaction between dusts and gel baits. The three main objectives of our project are designed to fill these gaps.

Objective 1 – Determining the most effective dust treatments under forced exposure or choice conditions.

Seven registered dust products (Table 1) covering major active ingredient categories are selected for evaluation. Field-collected German cockroach strains pre-evaluated for insecticide resistance and a susceptible strain are treated with dust products under choice and no-choice conditions.

Table 1. Dust products to be evaluated in this project.

Category	Trade Name	Active Ingredient(s)
Desiccant	CimeXa	92.1% silicon dioxide
Oral	BorActin	99% boric acid
	Avert DF	0.05% abamectin
	Advion MicroFlow	0.22% indoxacarb
Contact	D-fense dust	0.05% deltamethrin
Mixture	Alpine Dust	0.25% dinotefuran, 95% diatomaceous earth
	Tri-Die Aerosol	0.6% pyrethrin, 4.8% PBO, 8% silica gel

Addresses Gap #1 and #2

- Resistant strains studied
- Choice assay measures degree of repellency

Objective 2 – Performance and repellency of dust insecticides in the presence of gel baits.

Effective products from Objective 1 are selected to be tested against insecticide resistant and susceptible German cockroaches along with gel baits in a choice assay.

Addresses Gap #1, #2, #3

- Resistant strains studied
- Choice assay measures degree of repellency
- Combining dusts and gel baits in experiment

Objective 3 – Behavioral response of cockroaches

Effective products and product combinations from Objectives 1 & 2 are provided to susceptible and resistant cockroaches in half-treated, half-untreated arenas. Position, movement, and other behaviors are monitored and quantified with EthoVision XT video tracking software.

Addresses Gap #1 and #2

- Resistant strains studied
- Direct quantification of behavioral response

The objectives will provide data that directly improve German cockroach IPM:

- Identification of dust treatments effective against resistant German cockroaches
- Repellency data on all dust products
- Proper application methods when dusts and gel baits are used together

Completion of our study will be followed by publication of all relevant materials and dissemination to PMPs under appropriate educational venues.

D. **<u>Relevance to the Solicitation Notice Criteria</u>**

The proposed project directly addresses the solicitation criteria described in solicitation notice SPCB-25-01 by improving treatment decision-making and application methodology for German cockroach IPM.

"Proposals should focus on new studies, treatments, or technology methods within the framework of integrated pest management (IPM) for the following structural pests: ants, **cockroaches**, termites, and rodents."

References

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Ebeling W, Reierson DA, Wagner RE. 1967. Influence of repellency on the efficacy of blatticides. II. Laboratory experiments with German cockroaches. J. Econ. Entomol. 60(5):1375–1390. https://doi.org/10.1093/jee/60.5.1375

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(1):8292. https://doi.org/10.1038/s41598-019-44296-y

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.

Gondhalekar AD, Appel AG, Thomas GM, et al. 2021. A review of alternative management tactics employed for the control of various cockroach species (Order: Blattodea) in the USA. Insects. 12(6):550. https://doi.org/10.3390/insects12060550

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(1):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

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Wang C, Eiden A, Cooper R, et al. 2019. Changes in indoor insecticide residue levels after adopting an integrated pest management program to control German cockroach infestations in an apartment building. Insects. 10(9):304. https://doi.org/10.3390/insects10090304

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ATTACHMENT 8. NARRATIVE OF PROJECT DIRECTION

Improving application of dusts and gel baits for control of insecticide-resistant German cockroaches, a major indoor pest of public health importance

OBJECTIVE 1 – DETERMINING THE MOST EFFECTIVE DUST TREATMENTS UNDER FORCED EXPOSURE OR CHOICE CONDITIONS.

Insecticides

Seven products with active ingredients including desiccants, oral toxicants, contact toxicants, and mixtures are selected for testing (Table 1). All products were confirmed to have an active registration in California.

Table 1. Dust products to be evaluated in this project.

Category	Trade Name	Active Ingredient(s)
Desiccant	CimeXa	92.1% silicon dioxide
Oral	BorActin	99% boric acid
	Avert DF	0.05% abamectin
	Advion MicroFlow	0.22% indoxacarb
Contact	D-fense dust	0.05% deltamethrin
Mixture	Alpine Dust	0.25% dinotefuran, 95% diatomaceous earth
	Tri-Die Aerosol	0.6% pyrethrin, 4.8% PBO, 8% silica gel

Cockroach Strains

The German cockroach strains N97, N93, and BIN were collected from residential areas in 2023 and maintained in the laboratory under ambient conditions (24 ± 2°C, 30–50% RH, and 12:12 h L:D) with dog food (Purina Dog Chow, Nestle Purina Petcare, St. Louis, MO), cardboard harborages, and water provided ad libitum. The UCR strain is a susceptible strain reared in laboratory ambient conditions without exposure to insecticides for >40 years. All strains were tested for resistance using topical diagnostic doses of deltamethrin, abamectin, indoxacarb, and dinotefuran.

Table 2. German cockroach strains that will be used in this project.

Strain	Collection Date	Mortality of adult males treated with diagnostic dose*			
		deltamethrin	abamectin	indoxacarb	dinotefuran
UCR	-	100.0%	100.0%	100.0%	100.0%
N97	2023	30.0%	83.3%	66.7%	50.0%
N93	2023	6.7%	90.0%	56.7%	60.0%
BIN	2023	13.3%	96.7%	40.0%	60.0%

* Topical 3 d 2 x LD_{95} of the UCR strain.

All strain colonies will be expanded at the start of the project to ensure an adequate number of insects available for testing. For baseline toxicity screening experiments, adult male cockroaches will be used because they are the most physiologically homogeneous group (Abd-Elghafar and Appel 1992).

- **Expected time needed for strain expansion:** January–June 2026

Experiment 1.1: Toxicity of dust products in forced exposure assays

Dust is applied to a circular piece of filter paper at label rates using a bulb duster or other application equipment suitable for each product. The filter paper is placed on the bottom of a plastic petri dish (95 mm diameter). Ten adult male cockroaches are introduced to the petri dish and the cover is placed on the dish to prevent escape. Controls are introduced to a petri dish with no dust. Mortality is recorded at regular intervals (e.g., every hr for 6 hr, then daily up to 7 d) until control mortality > 20%. Each of the seven treatments and controls are replicated four times across four cockroach strains, requiring a **total of 112 replications and n = 1,120 cockroaches** to complete this experiment. Treatment performance is compared between treatments and strains using Kaplan Meier survivorship analysis and logrank tests.

- **Expected completion:** September 2026

Experiment 1.2: Performance of dust products in Ebeling choice box assays

The seven dust products from Table 1 are evaluated using the Ebeling choice box (Fig. 1), which is an arena designed to measure insecticide efficacy under simulated field conditions where (1) the treatment is located in a dark "void" and (2) the cockroaches can access food and water while avoiding the insecticide deposit (Ebeling et al. 1966). It simultaneously measures efficacy and repellency, and has been adopted to predict treatment performance against field-collected cockroaches (Lee et al. 2022).

Ebeling choice boxes are constructed from white pine drawer siding (30.5 by 9.5 cm) and are divided into equal sized compartments: light and dark. Cockroaches can move freely between compartments through a hole in the divider. The dark side is covered with a Masonite panel during the experiment to prevent light from entering, while the light side is covered with a translucent panel.

Figure 1. Ebeling choice box.

Dust is applied to rectangular panels of unpainted plywood (30.8 by 15.2 by 0.8 cm) using a bulb duster or other equipment suitable for each dust product. The panel is placed into the dark side, and food and water are placed into the light side. Controls receive an untreated panel. Twenty adult male cockroaches are introduced into the light side and allowed to explore freely. Mortality and position (light or dark) of each cockroach is recorded daily for 14 d. Each of the seven treatments and controls are replicated four times across four cockroach strains, requiring a **total of 112 replications and n = 2,240 cockroaches** to complete this experiment.

Treatment performance is compared among treatments and between strains using Kaplan-Meier survivorship analysis and log-rank tests. The performance index (PI), which combines mortality and repellency to predict performance, is calculated for each treatment using the following equation:

$$PI = 1 - \left(\frac{Number\ alive + Number\ alive\ in\ light\ side}{Number\ dead + Initial\ total\ number}\right) \times 100$$

Complete repellency and no mortality: a PI of -100. Complete mortality and no repellency: a PI of +100. No mortality and no repellency: a PI of 0.

- **Expected completion:** March 2027

OBJECTIVE 2 – PERFORMANCE OF DUST INSECTICIDES IN THE PRESENCE OF GEL BAITS.

Experiment 2.1: Determining the best treatment combination and application sequence

The best performing dusts from Objective 1 are selected based on the lowest mean survival times in the choice assays (Experiment 1.2). Up to two gel bait products containing common active ingredients (e.g., fipronil and indoxacarb) are tested together with the dusts in Ebeling choice boxes described in Experiment 1.2.

Rectangular panels of unpainted plywood are treated with one of the following treatment combinations:

(1) Dust applied on top of gel bait

(2) Gel bait applied on top of dust

(3) Dust only

(4) Gel bait only

(5) Untreated control

*The total number of treatment combinations is equivalent to:

$$No. of\ treatments = 2(No. of\ dust \times No. of\ gel\ bait) + No. of\ dust + No. of\ gel\ bait + 1$$

For example, an experiment with two dust products and two gel bait products would result in 13 total treatments combinations:

$$No. of\ treatments = 2(2 \times 2) + 2 + 2 + 1 = \mathbf{13}\ \boldsymbol{total\ treatments}$$

Because each treatment combination is replicated four times across four cockroach strains with twenty cockroaches per replication, this gives us a **total of 208 replications and n = 4,160 cockroaches** to complete this experiment.

Dust is applied using a bulb duster or other equipment suitable for each dust product, and gel bait is applied to the center of the panel according to label amounts. The untreated control panel does not have any insecticide application. The panel is placed into the dark side of an Ebeling choice box, and food and water are placed into the light side. Twenty adult male cockroaches are introduced to the light side and allowed to explore freely. Mortality and position of each cockroach is recorded daily for 14 d.

The performance index (PI) is calculated, and mean survival times are generated for each treatment combination with Kaplan Meier analysis. Hazard ratios will be calculated with a proportional hazards model for each of the following factors:

Factor 1: Dust product

E.g., Dust A, Dust B, None

Factor 2: Gel bait product

E.g., Bait A, Bait B, None

Factor 3: Application sequence

Dust on top of gel bait, bait on top of dust

The hazard ratios will determine which of the factors contribute the most to performance, which dust and gel baits performed the best, and which combination of dust + gel bait + application sequence performed the best.

- **Expected completion:** December 2027

<u>Experiment 2.2: Performance against mixed populations</u>

The most effective treatment combination(s) from Experiment 2.1 are selected based on hazard ratios. Rectangular panels of unpainted plywood are treated with the treatment combination(s) in the same manner as Experiment 2.1. The panel is placed into the dark side of an Ebeling choice box. Ten adult males, ten adult females, and ten mid-instar nymphs are introduced into the light side containing food and water. The mortality and position of each cockroach is monitored daily for 14 d. Four replicates are performed for each strain. Performance indices (PI) and Kaplan Meier survivorship estimates are used to compare between strains and sex/stage.

- **Expected completion:** June 2028

OBJECTIVE 3 – BEHAVIORAL RESPONSE OF COCKROACHES TO DUST INSECTICIDES

<u>Experiment 3.3: Behavioral response of cockroaches to combined treatment</u>

The best treatment combination(s) from Experiment 2.1 are chosen for detailed behavioral analysis. The UCR susceptible strain and the most resistant strain from Objective 2 serve as models to represent baseline and field-adapted cockroach populations.

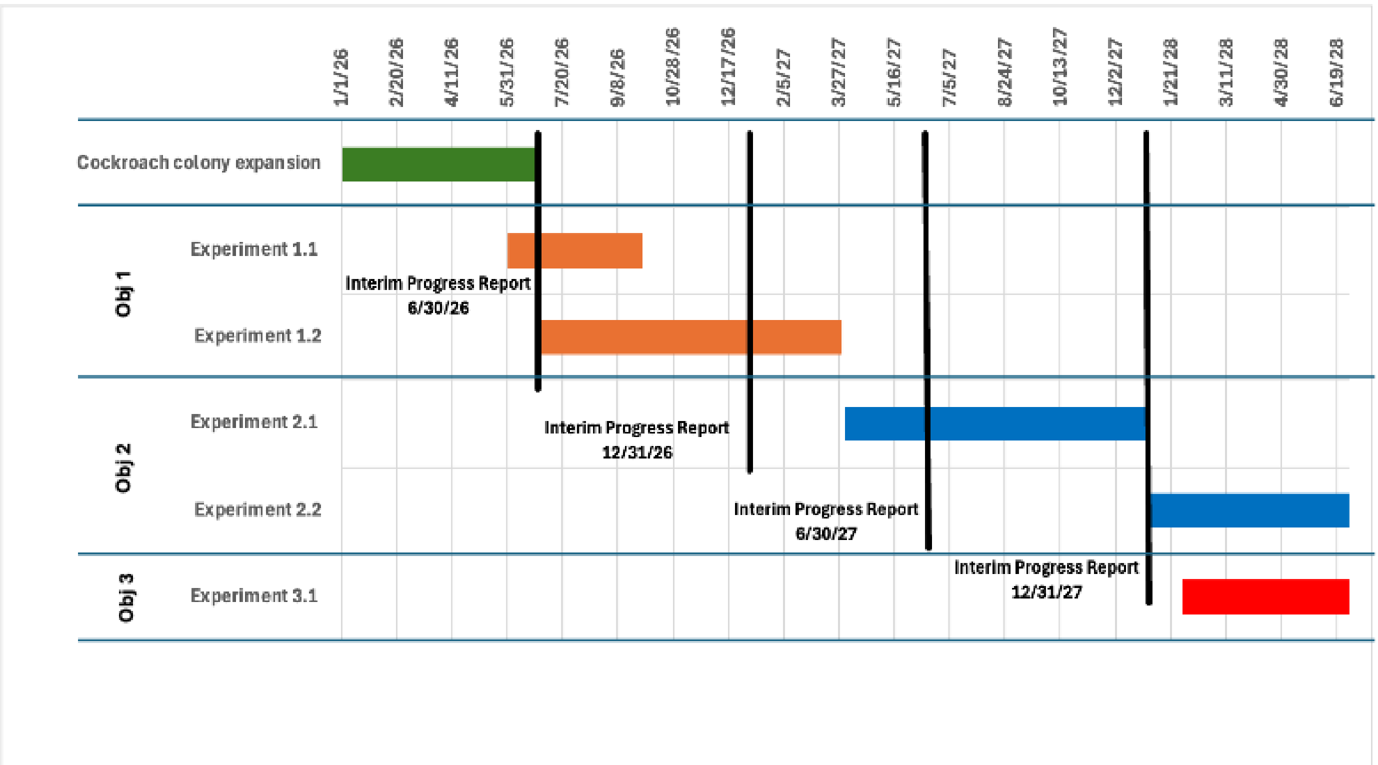
Arenas with half-treated and half-untreated filter paper at the bottom are prepared. An adult male cockroach is placed on the untreated side and allowed to move freely for an appropriate period (e.g., 30 min). The position and movement are tracked using EthoVision XT software and analyzed with F-tests followed by post-hoc tests (e.g., ANOVA and Tukey's).

- **Expected completion:** June 2028

Time allocation and monitoring system

The principal investigator (Chow-Yang Lee) will dedicate 5% of his time to the project. The postdoctoral scholar and staff research associate will dedicate 30% and 20% of their time, respectively, to the project. The principal investigator will oversee all aspects of the project, and the postdoctoral scholar and staff research associate will present their progress during bi-weekly laboratory meetings to discuss short-term and long-term goals.

Timeline of the proposed research



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Abd-Elghafar SF, Appel AG. 1992. Sublethal effects of insecticides on adult longevity and fecundity of German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 85(5):1809–1817. https://doi.org/10.1093/jee/85.5.1809

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Lee S-H, Choe D-H, Scharf ME, et al. 2022. 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

ATTACHMENT 9. NARRATIVE OF QUALIFICATIONS

PI of the project, Chow-Yang Lee, has many years of prior scientific research working with IPM techniques to control German cockroaches and has over 300 publications in the field of entomology. The co-PI, Dong-Hwan Choe, and key participants have all collaborated on multiple projects that have led to numerous publications in structural pest control. The findings are communicated annually at the UC Riverside Urban Pest Conference, which attracts over 150 pest control operators. All these projects were completed on time, and the reprints of the publications are attached.

1. **Publication**:
   Lee SH, DH Choe, MK Rust, CY Lee. 2024. Oral toxicity of an artificial sweetener sucralose on the German cockroach (Blattodea: Ectobiidae) and its impact on water balance and gut microbiome. *Journal of Economic Entomology* 117: 268–279.

   Project:
   Evaluation of an artificial sweetener as a potential bait toxicant and an insecticide synergist against German cockroaches, an important indoor pest of public health, funded by the California Department of Pesticide Regulation, project duration: July 1, 2021–June 30, 2023. PI: Chow-Yang Lee, Co-PI: Dong-Hwan Choe.

2. **Publication**:
   Lee SH, J So, GS Kund, JY Lum, E Trinh, EL Ta, R Chungsawat, DH Choe, DL Cox, MK Rust, CY Lee. 2024. Toxicity of isocycloseram, an isoxazoline insecticide, against laboratory and field-collected German cockroaches (Blattodea: Ectobiidae). *Journal of Economic Entomology* 117: 1086–1094.

   Project:
   Resistance monitoring of an isoxazoline compound in *Blattella germanica* in the United States, funded by Syngenta Crop Protection (US), project duration: September 1, 2022–December 31, 2026. PI: Chow-Yang Lee

3. **Publication**:
   Lee SHD, Z Man, CY Lee. 2025. Increase in insecticide susceptibility after sublethal exposure to deltamethrin in the German cockroach (Blattodea: Ectobiiidae). *Journal of Economic Entomology*. https://doi.org/10.1093/jee/toaf124

   Project:

   UC Riverside Urban Entomology Endowed Chair Research Fund, funded by UC Riverside, project duration: January 1, 2020–present. PI: Chow-Yang Lee

ATTACHMENT 10. COPY OF CURRENT QAC LICENSE (GREGORY KUND) AND LETTERS OF SUPPORT

dpr

DEPARTMENT OF PESTICIDE REGULATION
LICENSING/CERTIFICATION PROGRAM

QAC

QUALIFIED APPLICATOR CERTIFICATE

CERTIFICATE #:84846 EXPIRES: 12/31/2026
Categories: H Issued: 1/1/2025

GREGORY S KUND
DEPT OF ENTOMOLOGY UCR
RIVERSIDE, CA 92521

This Certificate must be shown to any representative of the Director or Commissioner upon request.

This person is qualified to apply or supervise the application of pesticides pursuant to Division 7, Chapter 3.6 of the Food and Agricultural Code in the categories indicated on the face of this card.
This Certificate does not authorize any person to engage for hire in the business of pest control. A DPR Maintenance Gardener Pest Control Business License must be acquired to engage in pest control incidental to a Maintenance Gardener Business in Catagory Q.

SIGNATURE

Certificate Categories

A. Residential, Industrial, and Institutional	H. Seed Treatment
B. Landscape Maintenance	I. Animal Agriculture
C. Right-of-Way	J. Demonstration and Research
D. Plant Agriculture	K. Health Related
E. Forest	L. Soil Fumigation
F. Aquatic	M. Non-Soil Fumigation
G. Regulatory	Q. Maintenance Gardener

IPM4YOU

PEST & TERMITE

July 24, 2025

Department of Consumer Affairs
Structural Pest Control Board
California

Dear Reviewing Committee Members:

RE: Evaluation of commercial dust formulations and behavioral responses of insecticide-resistant German cockroaches, a major indoor public health insect pest of California

I am writing to express my enthusiastic support for Dr. Chow-Yang Lee's research proposal entitled "Evaluation of commercial dust formulations and behavioral responses of insecticide resistant German cockroaches, a major indoor public health insect pest of California," which is being submitted to the Structural Pest Control Board.

The German cockroach is a persistent indoor public health pest in California, particularly in public and low-income housing. Sprays and gel baits have long been used to control German cockroaches, but growing insecticide resistance issues may severely limit their reliability in the future. Viable alternatives that can complement existing methods of cockroach IPM must be identified to ensure proper control. An often overlooked but potentially excellent formulation to fulfill this role are the dust insecticides. Despite their versatility, very little information is known about their effectiveness, especially against insecticide-resistant German cockroaches.

This project offers a safer and more sustainable solution for cockroach management in environments where conventional methods often fail. Dr. Lee's research could provide pest management professionals with more effective tools, leading to better pest control outcomes and reducing the reliance on more toxic chemical treatments. This initiative holds tremendous potential for improving the health and quality of life for residents in low-income housing across the state. The outcomes of this study will benefit the pest control industry, residents, and business owners in California, particularly those in vulnerable communities.

I fully support this project and encourage the Structural Pest Control Board to provide its full backing. The findings from this research will undoubtedly contribute to advancing our structural pest management strategies in a meaningful and impactful way.

Thank you for your consideration.

Yours truly,

James Panknin

James Panknin
President, IPM4YOU Pest and Termite, Inc
OPR 13777

Rentokil
TERMINIX

07/24/2025

To:
Department of Consumer Affairs
Structural Pest Control Board SPCB
Sacramento, CA

Ref: Evaluation of commercial dust formulations and behavioral responses of insecticide-resistant German cockroaches, a major indoor public health insect pest of California.

Dear Reviewing Committee Members,

I am writing to express my strong support for Dr. Chow-Yang Lee's research proposal titled *"Evaluation of commercial dust formulations and behavioral responses of insecticide-resistant German cockroaches, a major indoor public health insect pest of California,"* which will be submitted to the Structural Pest Control Board.

The German cockroach is a persistent indoor public health pest in California, particularly in public and low-income housing. Sprays and gel baits have long been used to control German cockroaches; however, growing issues with insecticide resistance may severely limit their reliability in the future. Viable alternatives that can complement existing methods of cockroach IPM must be identified to ensure proper control. An often-overlooked but potentially excellent formulation to fulfill this role is dust insecticides. Despite their versatility, very little information is known about their effectiveness, especially against insecticide-resistant German cockroaches.

This project provides a safer and more sustainable solution for cockroach management in environments where conventional methods often prove ineffective. Dr. Lee's research could provide pest management professionals with more effective tools, leading to improved pest control outcomes and reduced reliance on more toxic chemical treatments. This initiative holds tremendous potential for improving the health and quality of life for residents in low-income housing across the state. The outcomes of this study will benefit the pest control industry, residents, and business owners in California, particularly those in vulnerable communities.

I fully support this project and encourage the Structural Pest Control Board to provide its full backing. The findings from this study have the potential to advance our pest control strategies significantly and contribute to healthier living environments for those most affected by German cockroach infestations.

Thank you for your consideration.

Sincerely,

Claudio Salem - DVM - BCE
Technical Quality & Compliance Assurance Manager
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Anaheim, CA 92801
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info@ThrasherPest.com
www.ThrasherPest.com

July 24, 2025

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Structural Pest Control Board
California

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Thank you for your consideration.

Yours truly,

Garrett Thrasher

Garrett Thrasher
VP, Thrasher Termite & Pest Control of So Cal, Inc.

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https://doi.org/10.1093/jee/toad206
Advance Access Publication Date: 20 November 2023
Research

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OXFORD

Household and Structural Insects

Oral toxicity of an artificial sweetener sucralose on the German cockroach (Blattodea: Ectobiidae) and its impact on water balance and gut microbiome

Shao-Hung Lee*, Dong-Hwan Choe, Michael K. Rust, Chow-Yang Lee*

Department of Entomology, University of California, 900 University Avenue, Riverside, CA 92521, USA *Corresponding authors, mail: slee228@ucr.edu (S.L.); chowyang.lee@ucr.edu (C.L.)

Subject Editor: Nan-Yao Su

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Artificial or non-nutritive sweeteners are indigestible by most animals. Some sweeteners are orally toxic to insects and have received recent interest as potential safe insecticides due to their low mammalian toxicity. In this study, we investigated the oral toxicity of sucralose on insecticide-susceptible and resistant German cockroaches, *Blattella germanica* (L.). In a nonchoice test, we evaluated 5, 10, and 20% sucralose solutions. Depending on the cockroach strains, mean mortality ranged from 62.5 to 92.5%, 15 to 55%, and 2.5 to 27.5% for 20, 10, and 5% sucralose, respectively. Next, we measured the impact of a 20% sucralose treatment on water loss rates in the cockroach strains. All strains lost 23.0–30.29% of body water by 6 d. Dehydrated cockroaches were more prone to be killed by sucralose than nondehydrated ones. Lastly, we evaluated the effect of 20% sucralose treatment on gut bacterial composition and found the diversity of gut bacteria in treated cockroaches was significantly reduced after 3 days, implicating a rapid change in the alimentary environment.

Key words: non-nutritive sweetener, dehydration, dysbiosis

Introduction

The German cockroach, *Blattella germanica* (L.), is a common urban pest species managed primarily with insecticides. Because it infests indoor environments where safe applications are preferred and insecticide resistance is a pervasive concern, continued innovation is necessary to preempt the overreliance on hazardous treatments (Scharf and Gondhalekar 2021). Certain artificial, non-nutritive, zero-calorie or low-calorie sweeteners are orally toxic to insects and have been investigated for their insecticidal potential due to their inherently low mammalian toxicity (Lee et al. 2021). Sucralose (1,6-dichloro-1,6-dideoxy-*β*-*D*-fructofuranosyl-4-chloro-4-deoxy-*α*-*D*-galactopyranoside) is a synthetic disaccharide ~600× sweeter than sucrose that contains 3 chlorine substitutions at the 4, 1′, and 6′ positions (Glória 2003). Previously, Price et al. (2021, 2022) reported sucralose as an ingested insecticide for *Drosophila suzukii* (Matsumura). The implementation of sucralose as an insecticide for German cockroach control has practical value because oral formulations are already effective (e.g., liquid, gel/paste, and granular baits), and integrating this compound can alleviate the burden on conventional applications that can be harmful to humans or the environment (Schal and DeVries 2021).

Dehydration from increased excretion and regurgitation are common responses after ingesting non-nutritive sweeteners and are probably a primary cause of death (Sampson et al. 2016, Tang et al. 2017, Díaz-Fleischer et al. 2019, Price et al. 2022). Choi et al. (2017) postulated that this is because insects are unable to metabolize the sweeteners, resulting in a buildup in the hemolymph, and subsequent osmotic imbalance. To restore homeostasis, the insect is forced to excrete the sweetener, simultaneously releasing a significant amount of body fluid (Choi et al. 2017, Price et al. 2022). This was supported by the detection of undigested sucralose in the hemolymph and frass, a reduction in glycogen, a decrease in relative body weight, and the desiccated appearance of sucralose-fed *D. suzukii* (Price et al. 2022).

To understand whether German cockroaches experience mortality and dehydration like other insects, we provided 5, 10, or 20% sucralose drinking solutions to susceptible (UCR) and 2 insecticide-resistant (WM and RG386) strains to investigate the concentration-dependent mortality responses. We selected the 20% solution for all subsequent experiments and measured changes in body water and related parameters for up to 6 days of exposure to this treatment. The influence of dehydration severity on sucralose performance and sucralose exposure on dehydration mortality was included to identify any water balance-associated patterns in mortality.

In addition to understanding water loss, there has been a burgeoning interest in identifying methods to disrupt the German cockroach gut microbiome to achieve control (Pan et al. 2020, Zha et al. 2023). The gut microbiome of German cockroaches is putatively involved with many biological processes, including insecticide metabolism; disruptions, such as after antibiotic or insecticide treatment, affect susceptibility towards specific insecticides (Pietri et al. 2018, Chao et al. 2020, Wolfe and Scharf 2022). The alimentary consequences of sucralose ingestion potentially facilitate a substantial change in the gut environment that alters the microbiome. We isolated the whole alimentary tract of German cockroaches and conducted a bacterial 16S rRNA amplicon survey to analyze differences in community composition between untreated and treated cockroaches and determine if shifts in taxa implicate dysbiosis.

Materials and Methods

Cockroach Strains

The populations used in this study were the WM and RG386 strains, insecticide-resistant strains collected from the field and colonized in the laboratory for 4–5 yr, and the UCR susceptible strain (Lee et al. 2022). All strains were provided the same dog food diet (Purina Dog Chow, Nestlé Purina Petcare, St. Louis, MO), distilled water, and reared under conditions of 24 ± 2 °C, 30–50% RH, and 12:12 L:D photoperiods. Randomly selected adult males were used for all experiments due to having the most homogeneous physiology of all the stages of *B. germanica* (Appel et al. 1983, Abd-Elghafar and Appel 1992).

Concentration-Dependent Mortality

Ten cockroaches were introduced into an arena (27.5 × 20 × 9 cm) containing dog food (Purina Dog Chow, Nestlé Purina Petcare, St. Louis, MO), a folded cardboard harborage, a distilled water source, fluon on the walls to prevent escape, and a sheet of filter paper covering the bottom. Sucralose solutions were prepared by diluting pure sucralose (Supplement Partners LLC, Phoenix, AZ) in distilled water (w/v%). At the start of the experiment, the water source was replaced with a 0 (control), 5, 10, and 20% sucralose solution in an 8-ml glass vial with a cotton plug. The solution would permeate through the cotton and allow cockroaches to drink. Mortality was observed daily until the 14th day. All experiments were conducted under 24 ± 2 °C, 40 ± 5% RH, and 12:12 photoperiods. Each concentration was replicated 5 times per strain. Survivorship was compared with Kaplan–Meier analysis and log-rank tests in SPSS version 28 (IBM Corporation, Armonk, NY).

Impact of Dehydration on Sucralose Susceptibility

Ten cockroaches were placed in an arena (27.5 × 20 × 9 cm) containing dog food and a cardboard harborage. The walls of the arena were coated with fluon to prevent escape. Cockroaches were kept without a water source for 0, 1, and 2 days before introducing a 20% sucralose solution delivered in an 8 ml glass vial with a cotton plug. Mortality was recorded daily until 14 d. Mortality that occurred before the introduction of sucralose was ≤10% and was not counted for analysis. Similar to the treated cockroaches, the control cockroaches were kept without a water source for 0, 1, and 2 d, but distilled water was provided instead of sucralose. All experiments were conducted under 24 ± 2 °C, 40 ± 5% RH, and 12:12 photoperiods. Each treatment was replicated 3–5 times. Survivorship was compared with Kaplan–Meier analysis and log-rank tests in SPSS version 28 (IBM Corporation, Armonk, NY).

Impact of Sucralose Pre-Exposure on Dehydration Mortality

Ten cockroaches were placed in an arena (27.5 × 20 × 9 cm) containing dog food, a cardboard harborage, a water source, and fluon on the walls to prevent escape. At the start of the trial, the water source was replaced with a 20% sucralose solution for 0, 1, or 2 days. Then, the sucralose solution/water source was removed, and mortality was recorded for 14 d. Mortality that occurred during sucralose exposure was ≤10% and not counted for analysis. Controls were offered sucralose solutions for 0, 1, and 2 days but provided a clean water source during the remainder of the trial period. All experiments were conducted under 24 ± 2 °C, 40 ± 5% RH, and 12:12 photoperiods. Each treatment was replicated 3–5 times. Survivorship was compared with Kaplan–Meier analysis and log-rank tests in SPSS version 28 (IBM Corporation, Armonk, NY).

Water Loss

Cockroaches were placed in arenas with 20% sucralose solutions as the sole water source, dog food, and cardboard harborages under conditions of 24 ± 2 °C, 40 ± 5% RH, and 12:12 photoperiods. Cockroaches were collected after 0, 3, or 6 d of exposure, killed with a ~20-min exposure to HCN gas, and weighed with a micro balance (Sartorius AG, Göttingen, Germany) to get the total body weight. Sample collection was discontinued after 6 days due to excessive mortality in all the strains. Samples were dried in desiccation chambers containing anhydrous Drierite (W.A. Hammond Co., Xenia, OH) to maintain the humidity at ~0% RH until successive daily weights did not differ by > 0.1 mg (~10–12 d). Weights were measured again to get the dry body weight. Dried individual cockroaches were cut into 4 parts and submerged in a 2:1 chloroform: methanol mixture for 24 h to extract lipids. The solvent was discarded, and the cockroach pieces were dried in the desiccation chamber before weighing to get the lipid-extracted weights. The difference between total and dry body weights was used as the water weight. The difference between dry body weight and lipid-extracted weight was regarded as the weight of lipids lost. Between ~30 and 60 individuals were used for each strain-time combination. Differences between exposure times were compared using pairwise Wilcoxon rank sum tests in R version 4.2.3.

Treatment and Gut Dissections

Cockroaches were provided with 20% sucralose solutions prepared in sterilized water for 3 days, along with dog food, and a cardboard harborage. The food was removed 1 d before collection to reduce the presence of unstable diet-associated microbiota. The cockroaches were chilled on ice, surface cleaned with bleach and ethanol, and dissected to remove the entire alimentary tract. The whole guts of 3 cockroaches were pooled for each replicate to adjust for individual variation and ensure sufficient DNA yield in treated samples. Controls were prepared in the same manner but provided with untreated sterile water. A total of 24 whole guts (8 separate pools of 3 guts) were prepared for each strain and treatment.

DNA Extraction, Amplification of Bacterial 16S, and Library Preparation

The bacterial 16S rRNA gene library was prepared following the method by Shahi et al. (2020) with slight modifications due to differences in equipment and samples. DNA was extracted with the

DNeasy PowerSoil Pro kit (Qiagen LLC, Germantown, MD) following the manufacturer's protocols and spectrophotometrically measured to confirm concentration and quality. Primers for the V3–V4 regions of the bacterial 16S rRNA gene with Illumina overhang adapters, 5′-TCGTCGGCAGCGTCAGATGTGTATAACCTACGG GNGGCWGCAG-3′ (forward) and 5′-GTCTCGTGGGCTCGGA GATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′ (reverse), were used in the first PCR step (Klindworth et al. 2013). Reactions were carried out with cycling parameters of 95 °C for 3 min, 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension of 72 °C for 5 min. An additional PCR with cycle settings of 95 °C for 3 min, 8 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension of 72 °C for 5 min was conducted to attach indices and sequencing adapters using the Nextera XT Index Kit (Illumina Inc., San Diego, CA). Samples were cleaned with AMPure XP reagents (Beckman Coulter Life Sciences, Indianapolis, IN), and equivalent amounts of each sample were pooled. Library quality and concentration were confirmed with gel electrophoresis and Qubit fluorometry (Thermo Fisher Scientific, Waltham, MA) before submission for Illumina MiSeq sequencing (2 × 300 cycle run) at the UC Riverside Genomics Core Facility.

Sequence Filtering and Analysis

Sequences were demultiplexed and imported into QIIME 2 (Bolyen et al. 2019). Primers were trimmed with Cutadapt, and DADA2 was used to denoise, merge, and remove chimeras (Martin 2011, Callahan et al. 2016). Reads shorter than 240 bp (forward) and 220 bp (reverse) were discarded. Alpha rarefaction curves were plotted to confirm sufficient sequencing coverage. Diversity metrics were computed in QIIME 2 at a sampling depth of 41,500, sufficient to include all samples. Community richness and diversity were estimated with Chao1 and Shannon indices, respectively, and compared between all groups with pairwise Kruskal–Wallis tests. Beta-diversity was measured with Jaccard and Bray–Curtis metrics, statistically compared with PERMANOVA, and plotted with the principal coordinate analysis (PCoA) method using Emperor (Anderson 2001, Vázquez-Baeza et al. 2013). Taxa were assigned using a Naïve Bayes classifier trained on the Greengenes 99% OTU reference dataset (Bokulich et al. 2018, Robeson et al. 2021). Reads matching the *Blattabacterium* genus were filtered out before analyses, assuming that this was due to contamination from the small amounts of the fat body attached to dissected guts during sample preparation.

Results

Concentration-Dependent Mortality

Sucralose solutions caused decreased survivorship across all strains of German cockroaches, with higher concentrations having a faster effect (Fig. 1, Supplementary Table S1). The mean survival time was 6.4–10.0 d under the 20% sucralose treatment, 9.5–13.1 d for 10%, and 11.6–13.8 d for 5% (Table 1). Total mortality was 62.5–92.5% for 20% and 15–55% for 10% on day 14. The 5% solution resulted in 2.5–20% total mortality for the WM and RG386 strains and was not significant compared to the water-only control (2.5–7.5% total mortality). However, the effect of 5% sucralose solution was significant for the UCR strain, causing 27.5% total mortality (Fig. 1, Table 1).

Impact of Dehydration on Sucralose Susceptibility

An increase in time without access to water resulted in decreased survivorship when exposed to 20% sucralose solutions (Fig. 2, Table 2). There was a significant difference ($P < 0.05$) in survivorship for the UCR and WM strains at 1 d and 2 d without water compared to no dehydration (Fig. 2A and B). However, this difference was insignificant ($P > 0.05$) in the RG386 strain (Fig. 2C). Without initial water stress, the mean survival time across strains ranged from 6.3–8.7 days, while 2 days without water lowered this to 3.4–6.7 days. Total mortality at 14 d was 86.7–96.7% for the UCR strain, 83.3–100.0% for the WM strain, and 80.0–85.0% for the RG386 strain.

Impact of Sucralose Pre-Exposure on Dehydration Mortality

Pre-exposure to 20% sucralose was followed by earlier dehydrative death in all strains (Fig. 3, Table 3, Supplementary Fig. S1). The mean survival time after 2 d pre-exposure was 2.4–3.5 days versus 3.9–5.4 days for groups without exposure to sucralose, with the 1 d treatment resulting in an intermediate range of 3.7–5.4 days (Table 3). Total mortality at 14 days was ≥ 97.5% across all treatment groups (Table 3).

Water Loss

Body weight measurements associated with water content decreased sequentially with increasing time exposed to 20% sucralose (Fig. 4, Table 4). Total body weights decreased from 47.28 to 37.82 mg in the UCR strain, 52.34 to 37.55 mg in the WM strain, and 50.74 to 40.87 mg in the RG386 strain (Fig. 4A, Table 4). Most of the weight loss was water, which decreased from 33.71 to 25.62 mg in the UCR strain, 37.44 to 26.1 mg in the WM strain, and 36.7 to 28.26 mg in the RG386 strain (Fig. 4C, Table 4). The percent body water of healthy cockroaches (0 days) started at 71.18–72.12% and dropped by 23.0–30.29% at 6 days (Table 4). The weight of extracted lipids decreased from 2.74 to 1.64 mg in the UCR strain, 3.38 to 2.44 mg in the WM strain, and 4.82 to 2.39 mg in the RG386 strain (Fig. 4D, Table 4).

Bacterial Community Composition

The treatment of 3 d 20% sucralose significantly ($P < 0.05$) decreased Chao1 richness and Shannon alpha diversity indices of all strains (Fig. 6, Supplementary Tables S2 and S3). Samples clustered based on treatment and strain in the Jaccard distance PCoA plot (Fig. 7A), which explained ~29% of variance ($F = 3.517$; $R^2 = 0.29$; $P < 0.001$). There was a significant difference in Jaccard similarity coefficients between treated and untreated UCR ($F = 2.825$; $P < 0.001$), WM ($F = 4.128$; $P < 0.01$), and RG386 ($F = 2.403$; $P < 0.001$) strains (Supplementary Table S4). The Bray–Curtis dissimilarity PCoA separated samples depending on treatment status, though clustering was looser, and untreated strains were insignificant ($P > 0.05$) with each other (Fig. 7B). Treatment and strain explained ~34% of variance in Bray–Curtis dissimilarity ($F = 4.252$; $R = 0.34$; $P < 0.001$). Distance between treated and untreated groups was significant for the UCR ($F = 5.869$; $P < 0.001$) and WM strains ($F = 5.860$; $P < 0.001$), but the difference was insignificant for the RG386 strain ($F = 2.157$; $P = 0.085$) (Supplementary Table S5).

The relative abundance of Proteobacteria increased after sucralose treatment from 39.81 to 66.37% in the UCR strain, 37.41 to 47.36% in the WM strain, and 62.65 to 72.55% in the RG386 strain (Fig. 8A). Bacteroidetes dropped from 16.17–28.71% to 5.86–11.13%. There was a near-complete loss of Fusobacteria (1.45–8.43% to 0.01–0.06%), Planctomycetes (0.39–1.03% to 0.00–0.04%), and Verrucomicrobia (0.92–2.48% to 0.04–0.08%) (Fig. 8A). The proportion of other taxa found at <1% relative abundance also decreased after treatment with sucralose (Fig. 8A). At the family level, there

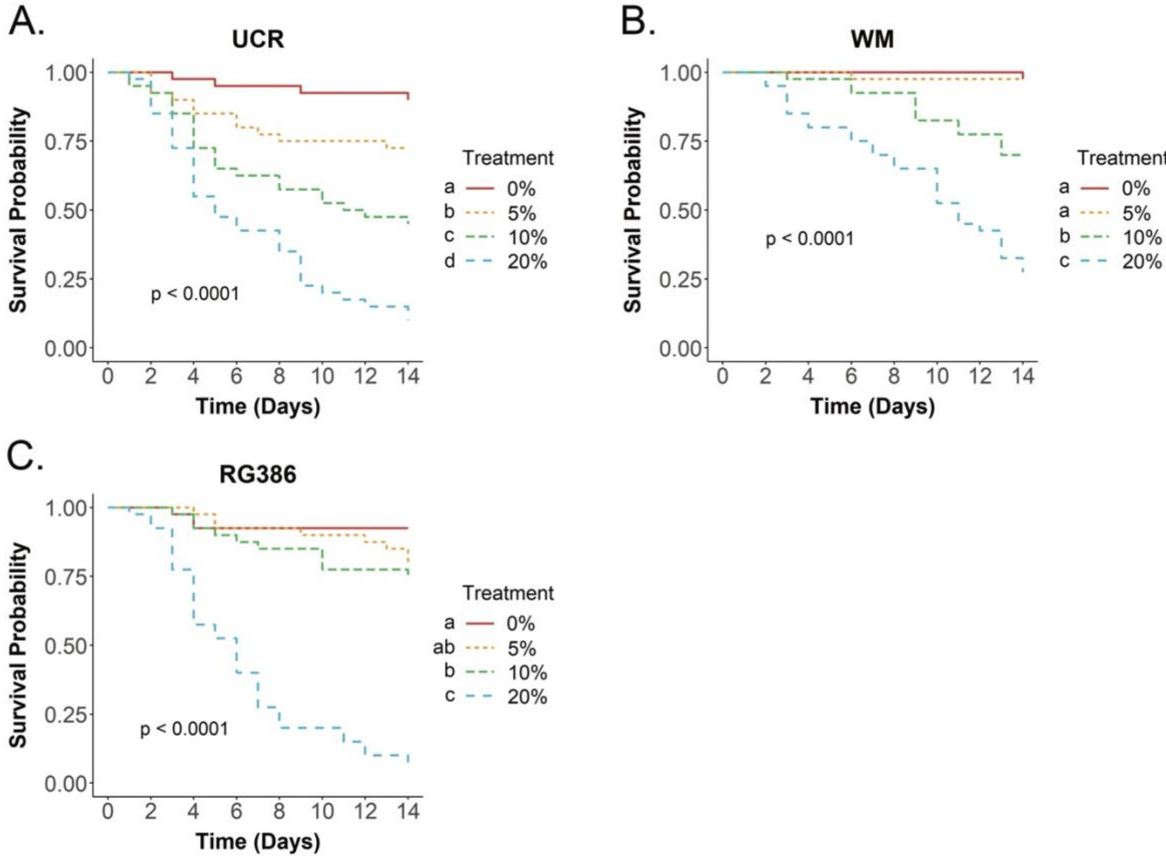

Fig. 1. Survivorship of A) UCR, B) WM, and C) RG386 strains exposed to 20%, 10%, 5%, or 0% (distilled water) sucralose solutions. Different letters by the figure legend denotes significant differences between treatments (Log-rank test; $\alpha = 0.05$).

Table 1. Mean survival time and mortality of UCR, WM, and RG386 strains exposed to 0–20%, sucralose solutions

Strain	Treatment	Mean survival time (days)	95% CI	% Mortality at 14 days
UCR	20%	6.7	5.3–7.9	90.0%
	10%	9.5	7.9–11.0	55.0%
	5%	11.6	10.2–12.9	27.5%
	0%	13.4	12.5–14.1	10.0%
WM	20%	10.0	8.6–11.3	62.5%
	10%	13.1	12.3–13.8	15.0%
	5%	13.8	13.4–14.1	2.5%
	0%	14	–	2.5%
RG386	20%	6.4	5.2–7.5	92.5%
	10%	12.3	11.2–13.4	25.0%
	5%	13.1	12.2–13.9	20.0%
	0%	13.2	12.3–14.0	7.5%

was a relative increase in Coxiellaceae (21.92–51.87% to 45.91–67.96%) and Enteroccocaceae (0.43–5.86% to 4.75–27.53%), but the proportion of a majority of the remaining taxa were decreased (43.96–72.46% to 23.33–26.15%) as did the remaining < 1% relative abundance taxa (3.57–5.19% to 0.59–1.77%) (Fig. 8B).

Accession Numbers

All sequences used in this study were submitted to the NCBI SRA database under BioProject number PRJNA994123.

Discussion

Average body weights of healthy adult male cockroaches were strain-dependent and ranged from 47.28 to 52.34 mg with body water comprising of 71.18–72.48% of total weight, which corroborated with the previous studies (Appel et al. 1983, Appel 1993, Wu and Appel 2017). Body weight decreased sequentially in all strains after exposure to 20% sucralose solutions, most of which was water weight (Fig. 4C, Table 4). The UCR strain was the earliest affected since there was no significant difference in water weight between 3 days and 6 days, whereas the WM and RG386 strains continued dehydrating after 3 days. The latter strains were collected from field sites within the past 5 yr, and the discrepancy possibility owed to an unspecified greater vigor that is sometimes observed in field-adapted populations, although the exact reason is unknown (Fardisi et al. 2019). Because cockroaches lost 23.0–30.29% of their initial body water on average by 6 d and most insects cannot survive after losing 30–40% of water, this demonstrates a severe dehydrative mechanism of sucralose (Hadley 1994).

Under normal circumstances, cockroaches lose water through defecation, excretion, respiration, and cuticle permeation (Appel 2021). In this regard, dehydration can occur with exposure to physical insecticides such as dust that disrupt the cuticular membrane and expedite water loss, although these materials only work when dry (Lee and Rust 2021). In the present study, sucralose was provided exclusively as a drinking solution to ensure an oral route of exposure, and no data shows the contact activity of any sweeteners

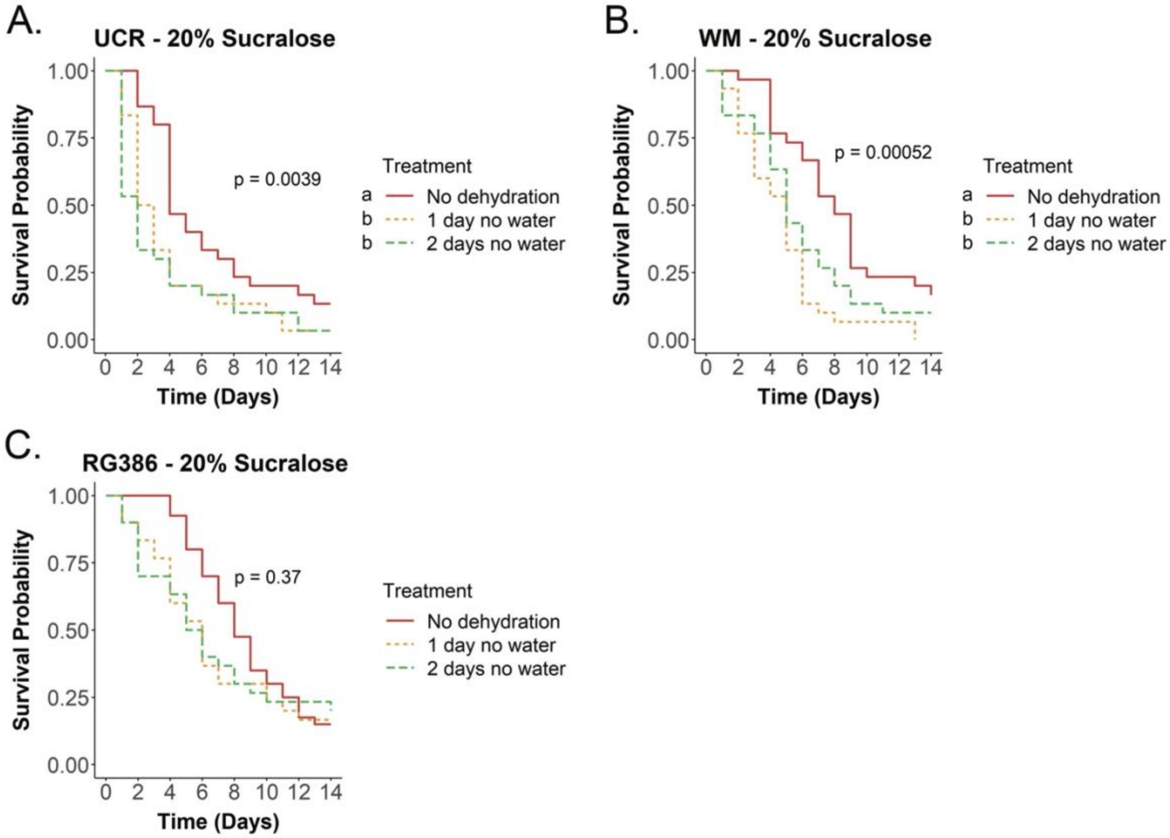

Fig. 2. Survivorship of A) UCR, B) WM, and C) RG386 strains treated with 20% sucralose solutions after 2, 1, or 0 days without water. Different letters by the figure legend denote significant difference, and the *P*-value represents an overall difference between all treatments (Log-rank test; $\alpha = 0.05$).

Table 2. Survival times and mortality of German cockroaches treated with 20% sucralose solutions after 0, 1, and 2 d of water deprivation

Strain	Treatment[a]	Mean survival time (d)	95% CI	% Mortality at 14 d
UCR	2 d no water	3.4	2.1–4.7	96.7
	1 d no water	3.8	2.6–5.0	96.7
	0 d no water	6.3	4.9–7.7	86.7
	2 d control	–	–	3.3
	1 d control	–	–	0.0
	0 d control	–	–	0.0
WM	2 d no water	5.5	4.9–6.0	90.0
	1 d no water	4.7	3.7–5.8	100.0
	0 d no water	8.3	6.9–9.6	83.3
	2 d control	–	–	0.0
	1 d control	–	–	3.3
	0 d control	–	–	0.0
RG386	2 d no water	6.7	5.0–8.4	80.0
	1 d no water	6.7	5.1–8.2	83.0
	0 d no water	8.7	7.7–9.7	85.0
	2 d control	–	–	0.0
	1 d control	–	–	3.3
	0 d control	–	–	0.0

[a]Controls were not treated with sucralose.

towards insects (Lee et al. 2021). Therefore, interference with the cuticle was highly unlikely to have caused the accelerated water loss. More probable is the putative fluid expulsion caused by indigestible sweeteners recorded across several insect species. When fed erythritol, increased regurgitation or excretion was observed in *D. suzukii* and *Anastrepha* spp., and *Drosophila* killed by erythritol had a 'mummified' appearance implicating a desiccating effect (Sampson et al. 2016, Tang et al. 2017, Díaz-Fleischer et al. 2019). *Drosophila suzukii* fed a mixture of sucralose and erythritol excreted more, lost weight, and adopted a dried appearance (Price et al. 2022). Unmetabolized erythritol in the hemolymph and the feces of treated flies led Choi et al. (2017) to hypothesize that an osmotic imbalance resulting from the buildup of indigestible compounds forces the insect to expel the sweeteners through substantial recruitment of body water, resulting in desiccation (Choi et al. 2017, Tang et al. 2017). We made several anecdotal observations during the experiments that indicate a similar response, including an increase in liquid staining on the basin of test arenas, a lack of solid feces, and a lack of solid material in the alimentary system (Fig. 5). Quantifying the excretive rate of cockroaches and the metabolic fate of sucralose would better elucidate any other similarities.

In addition to water, dry weight decreased by 1.37–3.45 mg by 6 days (Fig. 4B, Table 4). While cockroaches were provided food during the exposure period, the effects of sucralose intoxication may have simultaneously interfered with normal food consumption and digestion. The dissected guts of 3 day-treated cockroaches were comparatively lacking in (assumed) digestive material, which would partially explain the lower weight due to reduced intake, or retaining of food (Fig. 5). Although starvation can contribute to morbidity, adult male German cockroaches can survive longer than a week without food, reducing the possibility of starvation as the primary

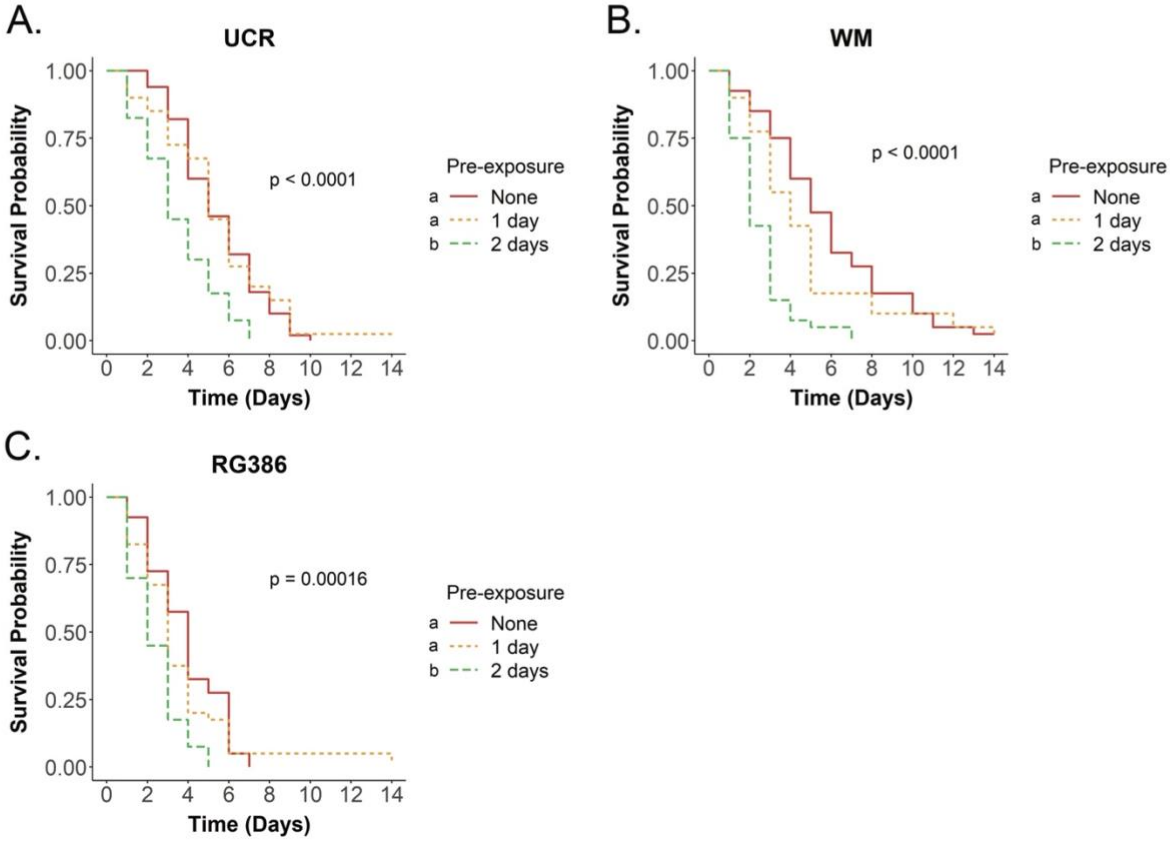

Fig. 3. Survivorship of A) UCR, B) WM, and C) RG386 strains without water after 2, 1, or 0 days exposure to 20% sucralose solution. Different letters by the figure legend denote significant differences, and the *P*-value represents an overall difference between all treatments (Log-rank test; $\alpha = 0.05$).

Table 3. Dehydration survival time and mortality of German cockroaches after 0, 1, and 2 days exposure to 20% sucralose solution

Strain	Treatment[a]	Mean survival time (d)	95% CI	% Mortality at 14 d
UCR	2 d 20% sucralose	3.5	2.9–4.1	100.0
	1 d 20% sucralose	5.4	4.5–6.2	97.5
	0 d 20% sucralose	5.4	4.9–6.0	100.0
	2 d control	-	–	13.3
	1 d control	–	–	6.7
	0 d control	–	–	6.7
WM	2 d 20% sucralose	2.5	2.1–3.0	100.0
	1 d 20% sucralose	4.7	3.6–5.7	97.5
	0 d 20% sucralose	5.8	4.8–6.8	97.5
	2 d control	–	–	3.3
	1 d control	–	–	6.7
	0 d control	–	–	3.3
RG386	2 d 20% sucralose	2.4	2.0–2.8	100.0
	1 d 20% sucralose	3.7	2.8–4.5	97.5
	0 d 20% sucralose	3.9	3.3–4.4	97.5
	2 d control	–	–	0.0
	1 d control	–	–	0.0
	0 d control	–	–	3.3

[a]Controls were provided with water after sucralose exposure.

cause of mortality (Willis and Lewis 1957). Alternatively, part of the dry mass loss was measured to be lipids, providing evidence that fat body hydrolysis for the production of metabolic water may have also contributed to the decrease in dry weight (Danks 2000).

The impacts on water balance were reflected in increased susceptibilities to sucralose and dehydration when cockroaches were water-stressed or pretreated with sucralose, respectively. Cockroaches of the UCR and WM strains kept without water

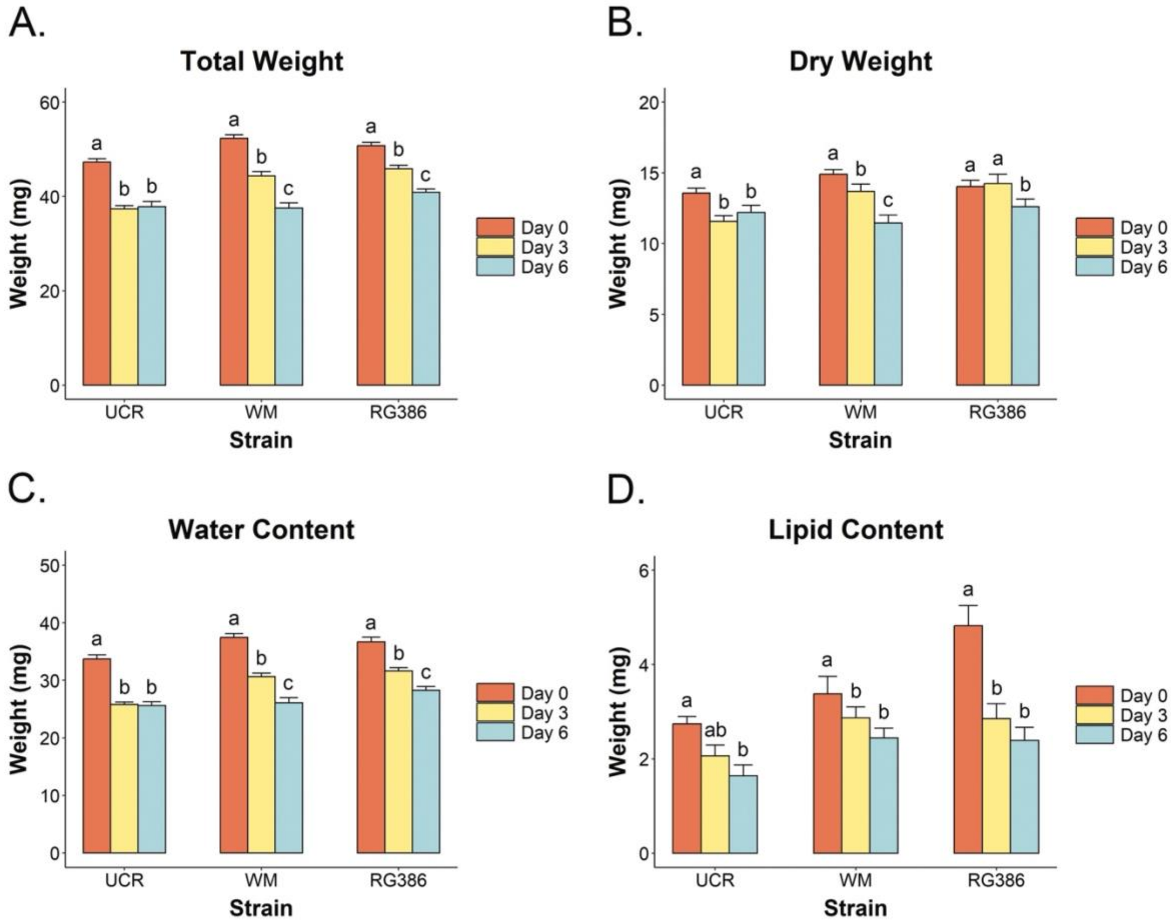

Fig. 4. A) Total weight, B) dry weight, C) water content, and D) lipid content of UCR, WM, and RG386 strains after 0, 3, or 6 days exposure to 20% sucralose solution. Different letters indicate significant difference between days (Wilcoxon rank sum test; α = 0.05).

Table 4. Water loss parameters of German cockroaches exposed to 20% sucralose solutions for 3 and 6 days compared to unexposed (0 days) cockroaches

Strain	Time (days)	Total weight (mg)	Dry weight (mg)	Water weight (mg)	% Water loss[a]	Lipid weight (mg)
UCR	0	47.28 a	13.57 a	33.71 a	–	2.74 a
	3	37.37 b	11.56 b	25.81 b	23.44%	2.06 ab
	6	37.82 b	12.2 b	25.62 b	24.0%	1.64 b
WM	0	52.34 a	14.9 a	37.44 a	–	3.38 a
	3	44.33 b	13.69 b	30.64 b	18.16%	2.87 b
	6	37.55 c	11.45 c	26.1 c	30.29%	2.44 b
RG386	0	50.74 a	14.04 a	36.7 a	–	4.82 a
	3	45.87 b	14.24 a	31.63 b	13.81%	2.85 b
	6	40.87 c	12.61 b	28.26 c	23.0%	2.39 b

[a](Water weight at 0 d—Water Weight)/Water Weight at 0 d × 100.
Different letters indicate significant difference between days (Wilcoxon rank sum test; α = 0.05).

for 1 or 2 days experienced expedited sucralose-associated mortality of up to ~3 days (Table 1). The initial dehydration would have compounded with sucralose-mediated water loss or caused cockroaches to consume more solution, resulting in faster death. Similarly, all strains exposed to 20% sucralose solutions for 2 days succumbed to earlier dehydration (Fig. 3). In the field, German cockroaches depend on consistent water for survival, evidenced by their common occurrence in areas with a local water source, such as kitchens and bathrooms (Wang 2021). Unlike in laboratory rearing conditions where water is provided ad libitum nearby, field populations are more likely to encounter water scarcity. The association of sucralose activity with water relations is advantageous under field treatment conditions where cockroaches may be consistently water challenged.

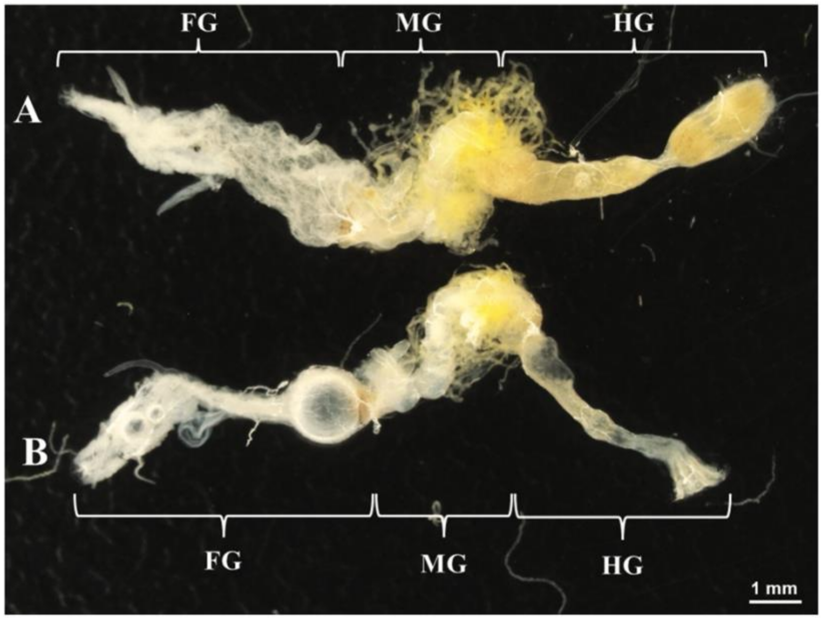

Fig. 5. Alimentary tracts of German cockroaches provided A) sterile water for 3 days and B) 20% sucralose solution for 3 days. FG—foregut; MG—midgut; HG—hindgut.

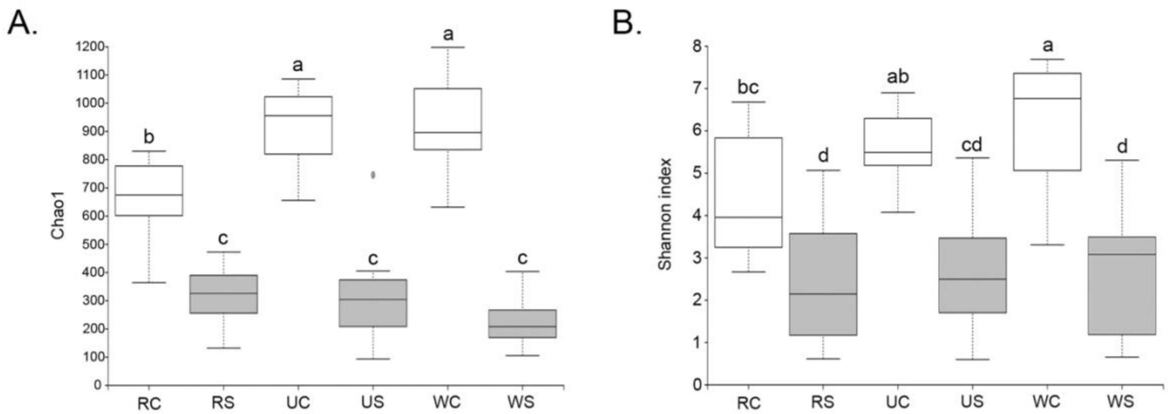

Fig. 6. Boxplots of richness, A) Chao1 and alpha diversity, (B) Shannon, indices. RC and RS are RG386 untreated and treated, respectively. UC and US are UCR untreated and treated, respectively. WC and WS are WM untreated and treated, respectively. Treated groups are shaded and untreated groups are unshaded. Different letters indicate a significant difference between strain-treatment groups (pairwise Kruskal–Wallis test; $\alpha = 0.05$).

The gut microbiome of the German cockroach is highly variable and dependent on multiple factors, especially diet, environment, and host physiology (Pietri and Kakumanu 2021). While German cockroaches from natural infestations are expected to have a different composition of gut bacteria compared to laboratory populations because of these factors, rearing both under similar conditions may cause the communities to converge (Pérez-Cobas et al. 2015, Kakumanu et al. 2018). Nonetheless, some differences can persist due to their association with stable physiological heterogeneity, such as life-history rates and xenobiotic metabolism (Pietri et al. 2018, Zhang and Yang 2019). We report slight differences in the initial diversity of whole guts of adult males between field-collected (WM and RG386) and a laboratory strain (UCR) that have been raised under identical conditions for ~4 yr (Fig. 6). The community richness of the UCR and WM strains was similar, whereas RG386 was significantly lower (Fig. 6A). Shannon diversity decreased sequentially, with WM being the highest, followed by UCR and RG386 (Fig. 6B). These differences may be associated with insecticide susceptibility, as UCR is a susceptible population and WM and RG386 are resistant to multiple insecticides, but further conclusions require additional investigations of the microbiome function (Lee et al. 2022).

Exposure to 20% sucralose solution for 3 days severely impacted the diversity of bacteria in the guts of all strains. After treatment, both Chao1 richness and Shannon diversity indices plummeted, and strains were statistically indistinguishable, indicating a consistent detrimental impact of sucralose (Fig. 6A and B). All strains and treatment groups were clustered separately based on Jaccard similarity, showing a low degree of community overlap (Fig. 7A). Furthermore, because untreated strains were densely grouped while treated strains were more

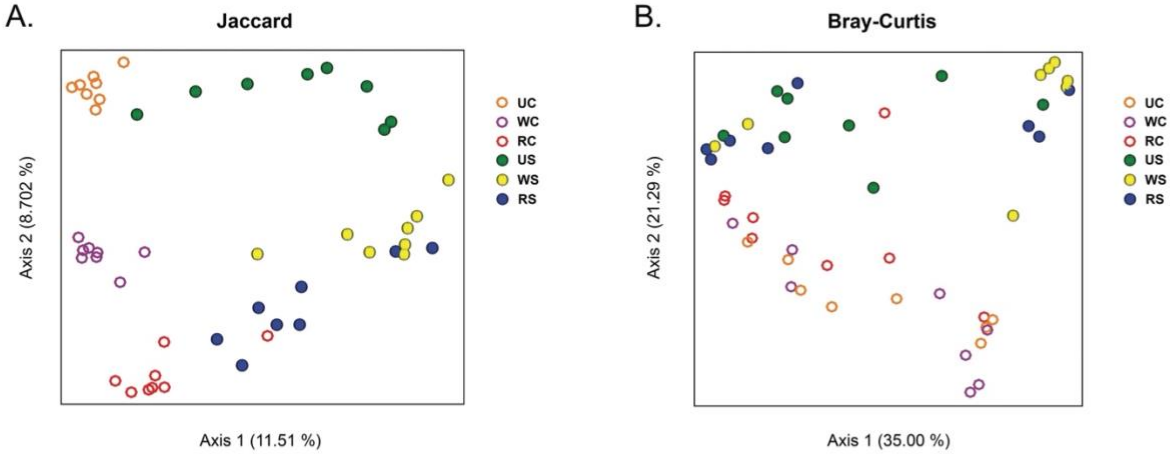

Fig. 7. Principal coordinate analysis plots of beta-diversity metrics A) Jaccard and B) Bray–Curtis. UC and US are UCR untreated and treated, respectively. WC and WS are WM untreated and treated, respectively. RC and RS are RG386 untreated and treated, respectively. Untreated groups are represented by rings and treated groups by solid circles.

spread, sucralose treatment had a diverse effect on the presence of unique reads. Despite being significant, separation was weaker among untreated strains when plotted using Bray–Curtis distances (Fig. 7B, Supplementary Table S5). In contrast, treated strains were not statistically different, suggesting that their discrepancies mainly depended on bacteria found in relatively low abundances (Supplementary Table S5). A significant alteration of bacterial communities was evident in both analyses, as treated cockroaches had minimal overlap with untreated controls across all strains.

The bacterial phyla Bacteroidetes, Proteobacteria, Firmicutes, and Fusobacteria were the dominant groups in all healthy strains, which was consistent with previous studies (Fig. 8A) (Carrasco et al. 2014, Kakumanu et al. 2018, Rosas et al. 2018). Although the abundance of these phyla may vary with respect to collecting location, dietary history, and age, their stability suggests that they constitute the core bacteria involved in the survival of Blattodea (Pietri and Kakumanu 2021). The proportion of these groups was altered after treatment with sucralose, noticeably with the near-complete elimination of Fusobacteria, and the increase in Proteobacteria, a shift associated with dysbiosis in omnivorous animals (Shin et al. 2015). With few exceptions, the other bacterial phyla of lower relative abundances were reduced with sucralose treatment, for example, Verrucomicrobia and Planctomycetes, resulting in an overall loss in diversity (Fig. 8A).

Similar changes between untreated and treated samples were reflected at the family level. Other than an increase in Coxiellaceae and Enterococcaceae, which composed, on average, most of the taxa found in treated guts (72.72–74.90%), the relative abundance of other taxa dropped from 47.54–77.65% to <30%. Many of these families are putatively involved in biological processes, such as Fusobacteriaceae in protein metabolism (Potrykus et al. 2008), Desulfovibrionaceae in nitrogen fixation (Postgate and Kent 1985), and Bacteroidaceae in polysaccharide degradation (Hooper et al. 2002). While the abundances of these groups naturally fluctuate in response to nutritional deficiencies and are otherwise found in healthy cockroaches, an indiscriminate reduction, as observed here, likely reflects decreased host health (Pérez-Cobas et al. 2015). Furthermore, the increase of Coxiellaceae in all strains containing the entomopathogenic *Ricketsiella* spp. implicates a shift toward increased pathogenicity (Jurat-Fuentes and Jackson 2012).

These impacts on the microbial community occurred after only 3 days of exposure to 20% sucralose, demonstrating that sucralose can rapidly affect the gut microbiome. Because only live cockroaches were used for this 16S community survey, the exposure period of 3 days was selected to maximize the number of living cockroaches (<20% mortality for all strains) to prevent excessive selection bias. However, by examining the morbidity and mortality patterns in the previous experiments (Figs. 1–4), the 3-day exposure was insufficient to cause a maximum level of impact in most cockroaches as health-related measurements continued to deteriorate past this point. Thus, we suspect a more significant microbial disruption can be observed with more prolonged exposure periods.

While chronic sucralose consumption has been shown to alter the gut microbiome in mammals (Méndez-García et al. 2022, Zheng et al. 2022), this is the first explicit demonstration of sucralose-induced dysbiosis in insects. The reported experiments do not address the exact mechanism of the microbe disruption; the hindguts of treated cockroaches appeared to be translucent or empty, suggesting a lack of material in the alimentary tract (Fig. 5). Coupled with the co-occurrence of water loss, we provide some considerations for future investigations:

(1) Microbiota may be lost through the expulsion of alimentary fluids; cockroaches disseminate gut bacteria through regurgitation and defecation, which may be expedited via the water loss mechanism (Kakumanu et al. 2018).
(2) Sucralose may have some antimicrobial properties, and its persistence as an indigestible compound creates an inhospitable environment for many bacterial species (Yu and Guo 2022).
(3) Cockroaches may starve due to interrupted digestion; although poorly understood, starvation has been shown to reduce the insect gut microbiome diversity (Blum et al. 2013, Yang et al. 2021, Zhang et al. 2021).

Although the functional impact of sucralose-mediated dysbiosis requires further study, dysbiosis through antibiotics has been shown to shorten the lifespan of cockroaches, reduce fecundity, and cause them to be more susceptible to certain insecticides (Bracke et al. 1978, Pietri et al. 2018). For example, dysbiosis can attenuate the antimicrobial defenses of cockroaches, increasing susceptibility to entomopathogenic agents such as *Metarhizium anisopliae*

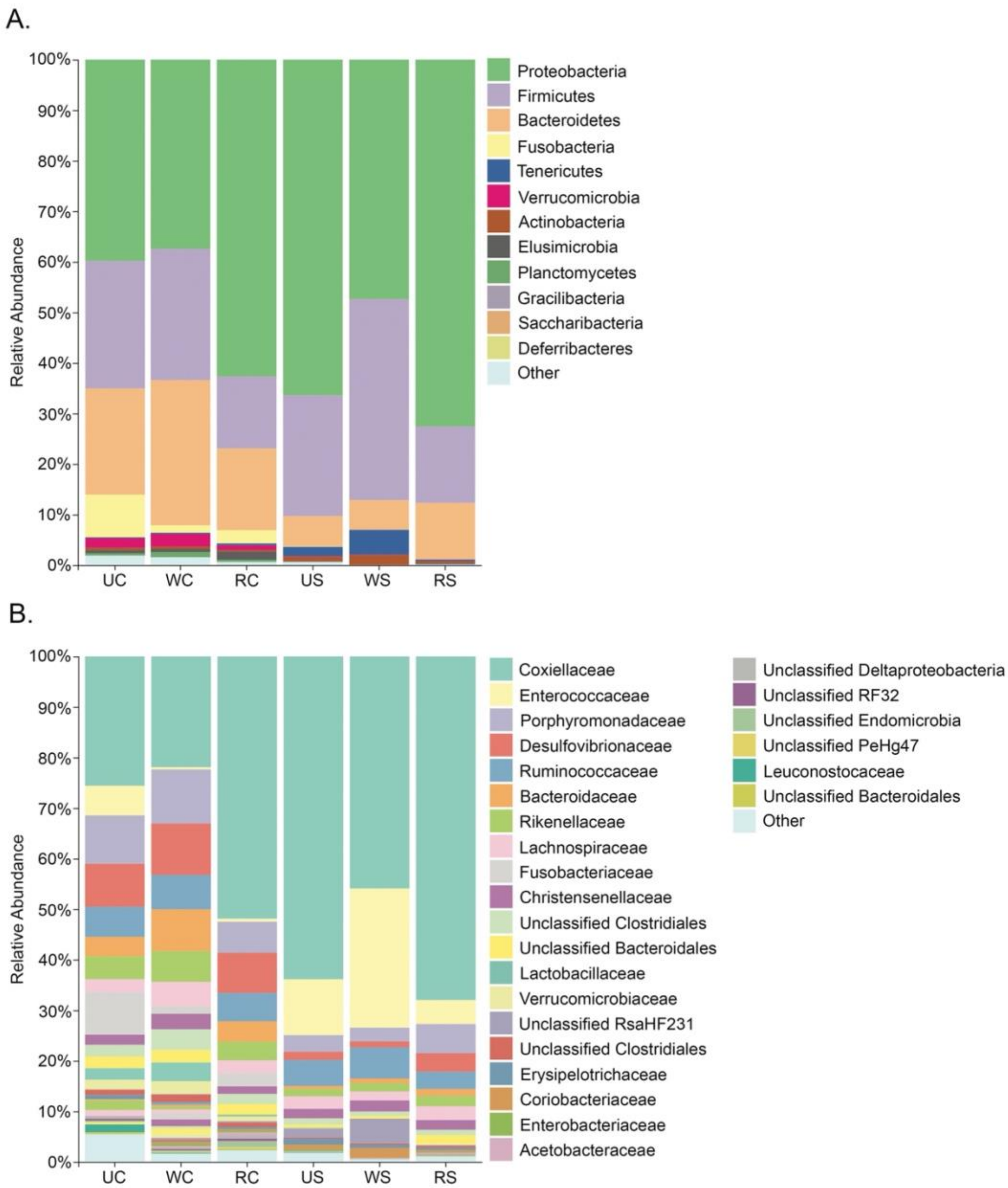

Fig. 8. Relative abundance of bacterial taxa at the A) phylum and B) family level. Taxa detected at <1% relative abundance are grouped in Other. UC and US are UCR untreated and treated, respectively. WC and WS are WM untreated and treated, respectively.

(Metchnikoff) Sorokin, or interfere with neurotoxic pathways to increase the toxicity of indoxacarb (Pietri et al. 2018, Zhang et al. 2018). However, using antibiotics in field treatments is practically and environmentally inadvisable. Sucralose serves as a promising safe alternative to disrupt the microbiome, and future work should be carried out to identify any consequences that sucralose exposure has on the performance of other insecticides.

In conclusion, we demonstrated that orally delivered sucralose is associated with multiple alimentary detriments in German cockroaches. The severe water loss and immediate increase in performance against water-stressed cockroaches suggest that dehydration is a primary mechanism of mortality. While functionally inconclusive, the simultaneous dysbiosis potentially synergizes with other insecticides and warrants further investigation. These impacts were recorded in both susceptible and resistant strains of cockroaches to demonstrate that sucralose has a conserved effect across different resistance phenotypes and has merit to be evaluated against field populations. However, the exclusive usage of a pure water-based solution in no-choice experiments necessitates examining sucralose as a standalone bait formulation in proximity to competing resources. In addition, other mechanisms may contribute to the mode of action, such as disruption of the gut epithelium observed with other gut poisons (Lee and Rust 2021). Otherwise, in combination with its availability and low mammalian toxicity, the current data reveal promising properties of sucralose as a tool for cockroach control.

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Author Contributions

Shao-Hung Lee (Conceptualization [Equal], Data curation [Lead], Formal analysis [Lead], Investigation [Lead], Methodology [Lead], Software [Lead], Validation [Lead], Visualization [Lead], Writing—original draft [Lead], Writing—review & editing [Lead]), Chow-Yang Lee (Conceptualization [Equal], Funding acquisition [Lead], Project administration [Lead], Resources [Lead], Supervision [Lead], Writing—original draft [Supporting], Writing—review & editing [Supporting]), Dong-Hwan Choe (Project administration [Supporting], Resources [Supporting], Supervision [Supporting], Writing—review & editing [Supporting]), and Michael Rust (Methodology [Supporting], Project administration [Supporting], Resources [Supporting], Supervision [Supporting], Writing—review & editing [Supporting])

Supplementary material

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

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Household and Structural Insects

Toxicity of isocycloseram, an isoxazoline insecticide, against laboratory and field-collected German cockroaches (Blattodea: Ectobiidae)

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Isocycloseram is a new insecticide in the isoxazoline class that targets insect GABA-gated chloride channels. In this study, we evaluated a cockroach gel bait formulation containing 1% isocycloseram against a susceptible strain (UCR) and 5 field-collected strains (WM, RG386, Ryan, CDR, and SY) of the German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae), and compared it with several commercial insecticide baits in the laboratory. Using the Ebeling choice box method, we also tested a residual deposit of an SC formulation of isocycloseram against the UCR, RG386, and Ryan strains. The isocycloseram bait was among the fastest-performing treatments against adult males (mean survival time: 0.9–2.7 days) and mixed stages and sexes (mean survival time: 1.4–5.4 days) across all strains. Secondary transfer effects of the bait were demonstrated in the UCR strain by exposing new adult males to individuals killed by direct bait treatment. Physiological resistance was not detected in the WM, CDR, and RG386 strains with topical treatment of a diagnostic dose (3× LD_{95}) of isocycloseram developed using the UCR strain. However, topical assays revealed resistance ratios (RR_{50}) of 1.6 and 3.0× in the Ryan and SY strains, respectively. The performance of a 0.05% isocycloseram residual application against the Ryan strain was improved with the addition of piperonyl butoxide.

Key words: Plinazolin, insecticide resistance, piperonyl butoxide, secondary transfer

Introduction

The German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae), is one of the most important indoor insect pests of public health importance worldwide (Lee and Wang 2021). Two significant health risks are associated with German cockroach infestation in homes: transmission of pathogenic microbes (also as vectors of antibiotic resistance genes) and producers of metabolites that could trigger allergies and asthma (Schal and DeVries 2021). The control of this species has relied heavily on the use of insecticides. Frequent usage and heavy reliance on insecticides have led to the development of widespread resistance in the German cockroach (Scharf and Gondhalekar 2021, Lee et al. 2022a).

Adopting new insecticides for German cockroach management requires caution because of widespread resistance in the species. Resistance management strategies such as rotations and mixtures are often recommended to ensure proper control and to preempt further resistance development (Scharf and Gondhalekar 2021). Because implementing these methods depends on the availability of insecticides across different modes of action, introducing compounds with unique mechanisms is vital to continue using common resistance management strategies.

Isocycloseram, also known as Plinazolin, belongs to the novel class of isoxazolines (Cassayre et al. 2021). It is categorized under the IRAC Mode of Action Group 30 along with meta-diamides (GABA-gated chloride channel allosteric modulators). It is active at low rates against a broad spectrum of arthropod pests such as Lepidoptera, Coleoptera, Thysanoptera, and Diptera (Blythe et al. 2022, Palumbo 2022a, 2022b, Buzza and Alyokhin 2023). To date, no commercial insecticides for *B. germanica* share the same mode of action as isocycloseram.

However, even the judicial introduction of compounds with categorically different modes of action can lead to resistance issues due to the German cockroach's preexisting or fast-developing cross-resistance mechanisms (Fardisi et al. 2019). For example, despite having decades separating their usages, the *Rdl* mutation of the GABA chloride channel was a source of early fipronil resistance because of prior selection from cyclodiene treatment (Kristensen et al. 2005). Broad families of detoxifying enzymes, such as the P450 monooxygenases and esterases, collectively play a crucial role in resistance toward many insecticides and can be a source of preexisting resistance or quickly develop in response to exposure (Hawkins et al. 2019, Lee et al. 2022b, Scharf et al. 2022, Tisgratog et al. 2023). Thus, evaluating isocycloseram against strains of German cockroaches that are resistant to different bait toxicants is necessary to predict its potential in the field.

In this study, we conducted 4 experiments: (i) Evaluation of a 1% isocycloseram bait formulation developed by Syngenta against adult males and mixed stages + sexes of the susceptible UCR strain and 5 field-collected strains (WM, RG386, Ryan, CDR, and SY) in comparison with commercial bait products in laboratory assays. (ii) Investigation of the secondary transfer effects using the UCR strain by exposing adult males to cockroaches killed by the bait. (iii) Contact toxicity of isocycloseram with topical applications on the UCR strain and determine the diagnostic dose to apply to field-collected strains to monitor for resistance. Lastly, (iv) the efficacy of isocycloseram residual spray was assessed using Ebeling choice boxes, and the performance of isocycloseram on the Ryan strain was further investigated with the addition of piperonyl butoxide (PBO).

Materials and Methods

Cockroach Strains

The strains WM, RG386, Ryan, CDR, and SY originated from field populations collected from 2018 to 2020 in California and kept in laboratory conditions of 24 ± 2 °C, 30%–50% RH, and 12:12 L:D photoperiod (Lee et al. 2022a). The WM, RG386, Ryan, CDR, and SY strains are resistant to insecticides including deltamethrin, fipronil, clothianidin, indoxacarb, DDT, and dieldrin (Lee et al. 2022a, 2022b), and have not been selected with insecticides while in the laboratory. The UCR strain, originally from the Orlando-normal strain, is a laboratory-susceptible strain that has been reared for >40 years without insecticide exposure. All strains were reared in 121-liter garbage bins equipped with electrical barriers and provided dog food (Purina Dog Chow, Nestlé Purina Petcare, St. Louis, MO, USA), cardboard harborages, and water ad libitum.

Insecticides and Chemicals

The following baits (% active ingredient) were used in bait assays: isocycloseram bait provided by Syngenta (1%, Syngenta Crop Protection LLC, Greensboro, NC, USA), Advion Cockroach Gel Bait (0.6% indoxacarb, Syngenta Crop Protection LLC, Greensboro, NC, USA), Advion Evolution Cockroach Gel Bait (0.6% indoxacarb, Syngenta Crop Protection LLC, Greensboro, NC, USA), Alpine Cockroach Gel Bait Rotation 1 (0.5% dinotefuran, BASF Corporation, Research Triangle Park, NC, USA), Maxforce FC Magnum Roach Gel Bait (0.05% fipronil, Bayer Environmental Science, Research Triangle Park, NC, USA), Siege 2% Gel Bait (2% hydramethylnon, BASF Corporation, Research Triangle Park, NC, USA), Optigard Cockroach Gel Bait (0.1% emamectin benzoate, Syngenta Crop Protection LLC, Greensboro, NC, USA), and Vendetta Cockroach Gel Bait (0.05% abamectin, MGK Company, MN, USA). Isocycloseram technical material (≥90% to <100%, Syngenta Crop Protection LLC, Greensboro, NC, USA) was used in topical assays. An isocycloseram residual formulation (SC 400) (Syngenta Crop Protection LLC, Greensboro, NC, USA) and piperonyl butoxide (PBO) (90% tech., Sigma–Aldrich, St Louis, MO, USA) were used in the choice box assay.

Experiment 1a: Performance of Cockroach Gel Baits

The performance of the following cockroach gel baits described above was evaluated: 1% isocycloseram, Advion Evolution Cockroach Gel Bait, Alpine Cockroach Gel Bait Rotation 1, Maxforce FC Magnum Roach Gel Bait, Optigard Cockroach Gel Bait, and Siege 2% Gel Bait. Ten adult male cockroaches were acclimatized for 2 days in the test arena (27.5 cm × 20 cm × 9 cm) with dog food, a cardboard harborage, a distilled water source, fluon on the inner arena wall surface to prevent escape, and a sheet of filter paper covering the arena bottom (to increase the traction of cockroach locomotion). A 0.3 g placement of bait was placed on a weigh boat and introduced into the arena, establishing a choice test. Controls were not provided with a bait application. Each bait was replicated 3 times. Mortality was observed every 2 h for 2 days and then every 12 h until 14 days. Cockroaches were considered dead when they could not move or turn upright within 2 min when touched with forceps (Lee et al. 1996, Chai and Lee 2010). Survivorship was calculated using the Kaplan–Meier method and compared with log-rank tests in SPSS version 28 (IBM Corporation, Armonk, NY, USA).

Experiment 1b: Performance of Isocycloseram Bait Against Mixed Stages and Sexes

Ten adult males, 10 nongravid adult females, and 20 nymphs (third–fourth instar, mixed sex) of a cockroach strain (UCR, RG386, or Ryan) were acclimatized for 2 days in a test arena (30.5 cm × 47 cm × 30.5 cm) with dog food, a cardboard harborage, distilled water, and petroleum jelly on the walls to prevent escape. A 1.0 g bait application (1% isocycloseram, Advion Cockroach Gel Bait, Alpine Cockroach Gel Bait Rotation 1, Maxforce FC Magnum Roach Gel Bait, or Vendetta Cockroach Gel Bait) in a weigh boat was introduced into individual arenas, establishing a choice test of an insecticide bait and dog food. Each bait was tested independently. Controls were not provided with a bait application. Each bait was replicated 4 times. Mortality was recorded at selected time intervals for 5 days (susceptible strain) or 14 days (resistant strains). The UCR strain was observed for a shorter period due to faster responses to the treatments. Cockroaches were considered dead when they were unable to move or turn upright within 2 min after being gently probed with a pair of forceps. Mortality of all stages and sexes was pooled, and survivorship was calculated with the Kaplan–Meier method and compared with log-rank tests in SPSS version 28.

Experiment 2: Secondary Transfer Effects of Cockroach Gel Baits

The secondary transfer effects of 5 cockroach gel baits were evaluated using the UCR strain: 1% isocycloseram, Advion Evolution Cockroach Gel Bait, Alpine Cockroach Gel Bait Rotation 1, Maxforce FC Magnum Roach Gel Bait, and Vendetta Cockroach Gel Bait. Ten adult males were introduced into an arena (28 cm × 15 cm × 11 cm) with a water source, a folded cardboard harborage, dog food, and petroleum jelly on the inner walls to prevent escape. After 24 h of acclimatization, 1 g of bait was introduced, and mortality was recorded every 12 h up to 7 days. The cockroaches were considered dead when they were immobile and unresponsive when gently touched with forceps to ensure that the slight movement from moribundity would not affect the secondary exposure group. Controls were not provided bait. Each treatment was replicated 5 times. When all cockroaches were dead, the bait was removed, and a new set of ten adult males was introduced into the same arena containing a water source, a folded cardboard harborage, dog food, and the dead cockroaches. As defined above in this section, mortality was recorded every 12 h up to 7 days using the same criterion.

Experiment 3: Topical Toxicity of Isocycloseram

The topical toxicity of isocycloseram on the UCR, Ryan, and SY strains was measured using a range of doses, causing ~5%–95% mortality. The doses applied to the UCR strain were 0.01, 0.013, 0.019, 0.025, 0.037, and 0.05 μg/insect. The doses applied to the Ryan strain were 0.01, 0.015, 0.024, 0.0375, and 0.05 μg/insect. The doses applied to the SY strain were 0.01, 0.015, 0.024, 0.0375, 0.05, and 0.075 μg/insect. These doses were prepared by serially diluting technical-grade isocycloseram in acetone. Adult males were briefly (<30 s) anesthetized with CO_2, and a 0.5 μl of the known dose of isocycloseram was applied to the abdominal sternites using an Isco Model M microapplicator (Instrumentation Specialties, Seward, NE, USA). Cockroaches were provided food, water, and harborage after the treatment, and mortality was scored at 72 h. Cockroaches were considered dead when they were immobile and unresponsive when touched with forceps. Ten individuals were used per replicate, and each dose was replicated 8–12 times. Controls were treated with acetone. The data were fitted to a probit model using PoloPlus (LeOra Software LLC, Petaluma, CA, USA) to generate the lethal doses: LD_{50} and LD_{95}. Adult males of the UCR, Ryan, and SY strains (n = 30–36) were killed with HCN gas (~20-min exposure) and weighed with a microbalance (Sartorius AG, Göttingen, Germany) to determine whether average mass significantly affected the proportional doses received per strain.

The LD_{95} of the UCR strain was 0.047 μg/insect (see Table 4). A diagnostic dose using the 3× LD_{95}, 0.14 μg/insect, was used to assess the susceptibility of field strains based on the method described by Lee et al. (2022a) (Georghiou and Mellon 1983, Mota-Sanchez et al. 2008). The UCR strain was also tested to confirm that the diagnostic dose causes complete mortality in the susceptible strain. Adult males were anesthetized with CO_2, and the diagnostic dose was applied to the first or second abdominal sternites with the above-mentioned microapplicator. Cockroaches were kept with food, water, and harborage, and mortality was recorded at 72 h posttreatment. Strains that had ≥10% survivors (Ryan and SY strains) were further examined by conducting topical assays and probit analysis to determine their LD_{50} values in an identical manner as the UCR strain.

Experiment 4: Performance of Isocycloseram Residual Formulation (400 SC) Using Ebeling Choice Boxes

The performance and potential field efficacy of residual treatment of isocycloseram were assessed with the Ebeling choice boxes (Ebeling et al. 1966). The boxes were constructed from white pine drawer siding (30.5 cm × 9.5 cm) with a tempered Masonite floor. The tops were covered in plexiglass and divided into 2 equal-sized compartments, light and dark, with the dark side covered with a piece of Masonite to prevent light from entering. A small hole at the top center of the divider allowed the cockroaches to move between the light and dark compartments. Panels of unpainted wood (30.8 cm × 15.2 cm × 0.8 cm) were sprayed with 3 ml of 0.05% isocycloseram aqueous preparation (0.011 mg/cm^2) using an airbrush (Master Hi-Flow AllPurpose, TCP Global, San Diego, CA, USA) and dried for 24 h. Another set of panels was sprayed with 3 ml of 0.05% isocycloseram and allowed to dry for 1 h, then sprayed with 3 ml of 0.5% PBO in acetone solution (0.11 mg/cm^2). Isocycloseram-PBO treated panels were allowed to dry for 24 h.

An isocycloseram-treated panel was placed on the floor of the dark compartment, and 20 adult male cockroaches of the UCR, RG386, or Ryan strains were confined in the light compartment for 5–6 h before being allowed to move freely throughout the box. Three replicates per strain were conducted for each treatment and untreated control. The Ryan strain was tested with isocycloseram + PBO treated panels and isocycloseram alone. Experiments were run under a photoperiod of 12:12 h (L:D), and the number of dead and alive cockroaches in the light and dark compartments was recorded for 14 days. The performance index (PI) was calculated to assess the combined effects of mortality and repellency, with a PI of 100 indicating complete mortality and no repellency, a PI of 0 indicating no mortality and no repellency, and a PI of −100 indicating no mortality and complete repellency (Rust and Reierson 1978):

$$\mathrm{PI} = \left\{1 - \left(\frac{\text{Total number alive} + \text{Number alive in light side}}{\text{Total number dead} + \text{Total number}}\right)\right\} \times 100$$

Results

In 5 of 6 strains, the 1% isocycloseram bait performed comparably or superior to the other baits against adult males. No difference in survivorship was detected in the UCR strain between 1% isocycloseram and Maxforce FC Magnum, Advion Evolution, and Optigard. However, Alpine performed faster, and Siege performed slower than isocycloseram (Fig. 1A). For the WM, RG386, Ryan, and CDR strains, 1% isocycloseram, along with Advion Evolution and Optigard (except for CDR), had the greatest impact on survivorship (Fig. 1B–E). For the SY strain, Alpine caused a comparable decrease in survivorship compared to 1% isocycloseram, although Alpine treatments resulted in incomplete mortality (90%), and 1% isocycloseram killed all insects (Fig. 1F; Table 1). There were significant differences ($P < 0.05$) in survivorship between the UCR strain and resistant strains treated with the 1% isocycloseram bait, with a mean survival time of 0.9 days for the UCR strain and 1.2–2.7 days for the resistant strains (Table 1; Supplementary Fig. S1). Except for the Ryan strain, which ended at 96.7% mortality by 14 days, the 1% isocycloseram bait completely killed the adult males of every strain within the 14-day evaluation period (Table 1).

When treating mixed stages and sexes of the UCR strain, 1% isocycloseram showed comparable performance to Alpine and Maxforce FC Magnum but was more efficacious than the Advion Evolution and Vendetta baits (Fig. 2A). The 1% isocycloseram bait was the fastest performing bait against RG386 mixed stages (Fig. 2B; Table 2). The 1% isocycloseram bait, Advion Evolution, and Maxforce FC Magnum were most efficacious against the Ryan mixed stages based on survivorship (Fig. 2C). None of the treatments caused complete mortality in any strain, and mortality from 1% isocycloseram ranged from 82.6% to 95.7% (Table 2).

In the secondary transfer effect experiment, the 1% isocycloseram bait resulted in the fastest decrease in survivorship for the UCR strain adult males under direct exposure, resulting in a mean survival time of 1.3 days (Fig. 3A; Table 3). Survivorship under secondary transfer conditions was also lowest for isocycloseram-exposed cockroaches, with a mean survival time of 1.5 days vs. 1.8–2.9 days with the other baits (Fig. 3B; Table 3).

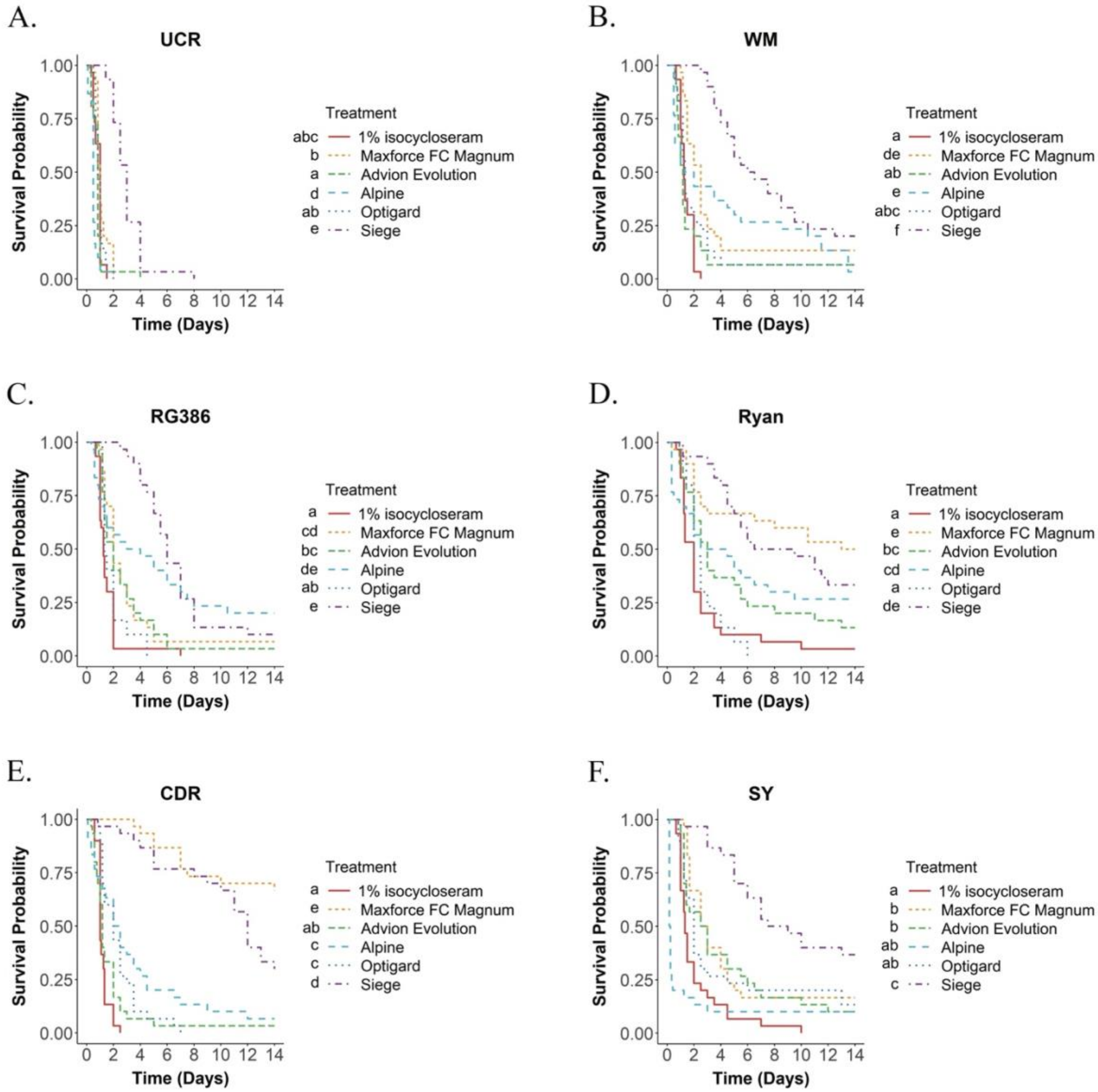

Fig. 1. Survivorship of adult male cockroaches of the A) UCR, B) WM, C) RG386, D) Ryan, E) CDR, and F) SY treated with baits. Different letters by the figure legend indicate significant differences between treatments (log-rank test; α = 0.05).

Topical applications of isocycloseram on the UCR adult males resulted in a 72 h LD_{50} of 0.015 (0.013–0.017) µg/insect and LD_{95} of 0.047 (0.040–0.059) µg/insect (Table 4). Mortality of the field strains from topical applications of the diagnostic dose (3 × LD_{95}) (0.14 µg/insect) was 97.5%–100% for WM, CDR, and RG386 strains and 90% for the Ryan and SY strains. The LD_{50} of isocycloseram for Ryan strain adult males was 0.022 (0.019–0.024) µg/insect, resulting in a resistance ratio (RR_{50}) of 1.6 (1.4–1.7) (Table 4). The LD_{50} of isocycloseram for SY strain adult males was 0.042 (0.036–0.048) µg/insect, resulting in an RR_{50} of 3.0 (2.6–3.6) (Table 4).

The UCR and RG386 strains had similar slopes in the choice box assay. They reached PIs of 100, indicating complete kill (Fig. 4). Isocycloseram alone did not cause complete mortality for the Ryan strain, with a PI of ~69.4. At the same time, the combination of PBO and isocycloseram resulted in complete mortality (Fig. 4). The lack of negative PI suggests that there was no repellency detected in the isocycloseram residual treatment (Supplementary Table S1).

Discussion

When tested against adult male German cockroaches of susceptible and field-collected strains, the 1% isocycloseram bait showed comparable performance to the other the gel baits evaluated. Despite overt resistance toward some baits, such as Siege, Alpine, and Maxforce FC Magnum, the performance of the isocycloseram bait was not similarly compromised, indicating a lack of cross-resistance to the isocycloseram bait, supporting similar findings of previous studies on other insect species (Sun et al. 2023). The strain-wise comparison

Table 1. Survival time and mortality of adult male cockroaches treated with baits

Strain	Treatment	Mean survival time (days)	95% CI	% Total mortality
UCR	1% isocycloseram	0.9	0.8–1.0	100.0
	Maxforce FC Magnum	1.1	1.0–1.3	100.0
	Advion Evolution	0.9	0.6–1.1	100.0
	Alpine	0.5	0.4–0.7	100.0
	Optigard	1.0	0.8–1.1	100.0
	Siege	3.0	2.6–3.4	100.0
	Control	—	—	0.0
WM	1% isocycloseram	1.4	1.2–1.6	100.0
	Maxforce FC Magnum	3.7	2.3–5.2	86.7
	Advion Evolution	2.1	1.0–3.3	93.3
	Alpine	4.4	2.7–6.2	96.7
	Optigard	2.4	1.3–3.6	93.3
	Siege	7.6	6.1–9.0	80.0
	Control	—	—	0.0
RG386	1% isocycloseram	1.5	1.2–2.0	100.0
	Maxforce FC Magnum	3.1	2.0–4.2	93.3
	Advion Evolution	2.8	1.9–3.7	96.7
	Alpine	5.4	3.6–7.2	80.0
	Optigard	1.9	1.5–2.3	100.0
	Siege	6.7	5.6–7.8	90.0
	Control	—	—	2.5
Ryan	1% isocycloseram	2.7	1.7–3.7	96.7
	Maxforce FC Magnum	9.3	7.3–11.2	50.0
	Advion Evolution	5.0	3.4–6.6	86.7
	Alpine	5.7	3.8–7.7	73.3
	Optigard	2.7	2.2–3.1	100.0
	Siege	8.6	7.0–10.3	66.7
	Control	—	—	5.0
CDR	1% isocycloseram	1.2	1.0–1.3	100.0
	Maxforce FC Magnum	11.7	10.3–13.1	33.3
	Advion Evolution	1.8	1.0–2.7	96.7
	Alpine	3.6	2.2–5.0	93.3
	Optigard	2.4	1.8–2.9	100.0
	Siege	10.4	8.9–11.9	70.0
	Control	—	—	7.5
SY	1% isocycloseram	2.1	1.4–2.9	100.0
	Maxforce FC Magnum	4.6	3.1–6.2	83.3
	Advion Evolution	4.4	2.9–5.9	90.0
	Alpine	1.8	0.3–3.3	90.0
	Optigard	4.3	2.6–6.0	86.7
	Siege	9.0	7.4–10.6	63.3
	Control	—	—	5.0

revealed a temporal delay in mortality between the resistant strains and the UCR strain, indicating that slower performance may be observed when treating field populations (Table 1; Supplementary Fig. S1). Nonetheless, complete mortality was consistently reached within 14 days for all strains (except 96.7% for Ryan).

Tests on mixed stages and sexes of the UCR, RG386, and Ryan strains showed that the isocycloseram bait retained its comparative efficacy relative to commercial competitor baits as one of the fastest-acting treatments (Fig. 2). However, unlike in the bioassays solely treating adult males, there were survivors at the end of all the exposure periods irrespective of strain or bait. For the UCR strain, this is likely due to the 5-day observational period instead of 14 days for the other strains. For the RG386 and Ryan strains, reoccurring incomplete mortality runs a risk of resistance propagation and treatment failure. Recommended reapplication intervals for conventional baits ranges from 2 to 4 weeks, which would contribute to eliminating the remaining cockroaches (Appel and Rust 2021). The tendency to have a fraction of survivors was not a unique issue for the isocycloseram bait alone and was observed for all bait treatments.

When tested against adult males of UCR strain, the isocycloseram bait showed secondary transfer effects as with the other bait products, with every treatment exerting complete kill within 7 days via secondary exposure. Because cockroaches are not guaranteed to interact directly with bait applications in the field, demonstrating that they can be killed through exposure to treated conspecifics provides additional utility (Appel and Rust 2021). Also, in both direct treatment and secondary transfer experiments, the isocycloseram bait maintained its advantage as the fastest-acting bait, suggesting a high level of bioavailability from carcasses, physically transferred residues, feces, and/or vomitus of poisoned cockroaches (Buczkowski et al. 2001, 2008).

The LD_{50} of the UCR strain at 72 h was 0.015 μg/insect, making isocycloseram a moderately active compound compared to other conventional neurotoxic insecticides, being less toxic than deltamethrin and fipronil (LD_{50} = 0.0046 and 0.0013 μg/insect, respectively), but

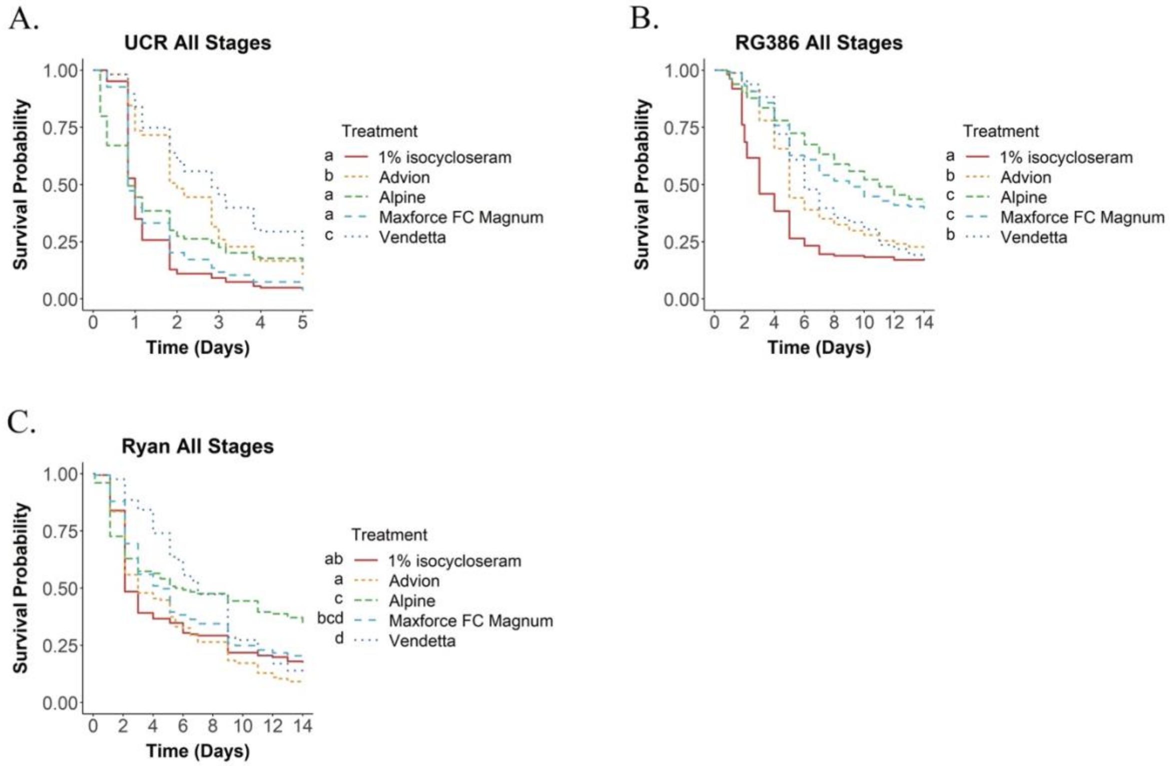

Fig. 2. Survivorship of mixed stages and sexes of the A) UCR, B) RG386, and C) Ryan strains treated with baits. Different letters by the figure legend indicate significant differences between treatments (log-rank test; α = 0.05).

Table 2. Survival time and mortality of mixed stages and sexes treated with baits

Strain	Treatment	Mean survival time (days)	95% CI	% Total mortality[a]
UCR	1% isocycloseram	1.4	1.2–1.5	95.7
	Advion	2.5	2.2–2.7	77.3
	Alpine	1.7	1.5–2.0	86.6
	Maxforce FC Magnum	1.5	1.3–1.7	96.3
	Vendetta	3.0	2.7–3.2	89.5
	Control	–	–	10.0
RG386	1% isocycloseram	5.1	4.5–5.8	83.0
	Advion	7.1	6.4–7.8	77.1
	Alpine	9.5	8.8–10.2	57.7
	Maxforce FC Magnum	9.0	8.2–9.7	60.9
	Vendetta	7.7	7.0–8.3	83.2
	Control	–	–	4.4
Ryan	1% isocycloseram	5.4	4.5–6.1	82.6
	Advion	5.3	4.6–5.9	90.8
	Alpine	7.5	6.5–8.5	65.3
	Maxforce FC Magnum	6.3	5.5–7.0	79.6
	Vendetta	7.6	7.0–8.3	88.5
	Control	–	–	2.6

[a]Mortality at 14 days for RG386 and Ryan, 5 days for UCR.

more toxic than indoxacarb and clothianidin (LD_{50} = 0.1100 and 0.0199 μg/insect, respectively) (Lee et al. 2022a). Topical treatments with the 3× LD_{95} of the UCR strain (diagnostic dose) caused high mortality (>97.5%) in the WM, CDR, and RG386 strains, suggesting a lack of contact resistance towards isocycloseram (ffrench-Constant and Roush 1990). The Ryan and SY strains had 10% survivors from the diagnostic dose and were 1.6 and 3.0× less sensitive based on topical application, respectively (Table 4). Although low levels of insensitivity do not necessarily cause observable problems with treatment efficacy, it is a sign that alleles that decrease susceptibility are present in a subset of the population (ffrench-Constant and Roush 1990).

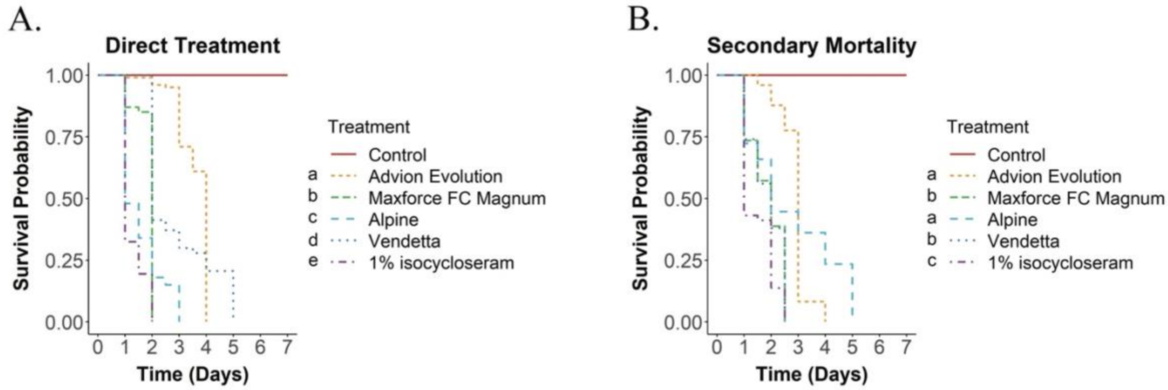

Fig. 3. Survivorship of UCR adult males from A) direct bait treatment and B) secondary mortality exposure. Different letters by the figure legend indicate significant differences between treatments (log-rank test; $\alpha = 0.05$).

Table 3. Survival time and mortality of the UCR strain adult males from direct bait treatment and secondary mortality exposure

Exposure type	Treatment	Mean survival time (days)	95% CI	% Total mortality at 7 days
Direct	Control	—	—	0.0%
	Advion Evolution	3.6	(3.5–3.7)	100.0%
	Maxforce FC Magnum	1.9	(1.8–1.9)	100.0%
	Alpine	1.6	(1.4–1.7)	100.0%
	Vendetta	2.9	(2.6–3.1)	100.0%
	1% isocycloseram	1.3	(1.2–1.4)	100.0%
Secondary	Control	—	—	0.0%
	Advion Evolution	2.9	(2.7–3.0)	100.0%
	Maxforce FC Magnum	1.9	(1.7–2.0)	100.0%
	Alpine	2.7	(2.3–3.2)	100.0%
	Vendetta	1.8	(1.7–2.0)	100.0%
	1% isocycloseram	1.5	(1.3–1.7)	100.0%

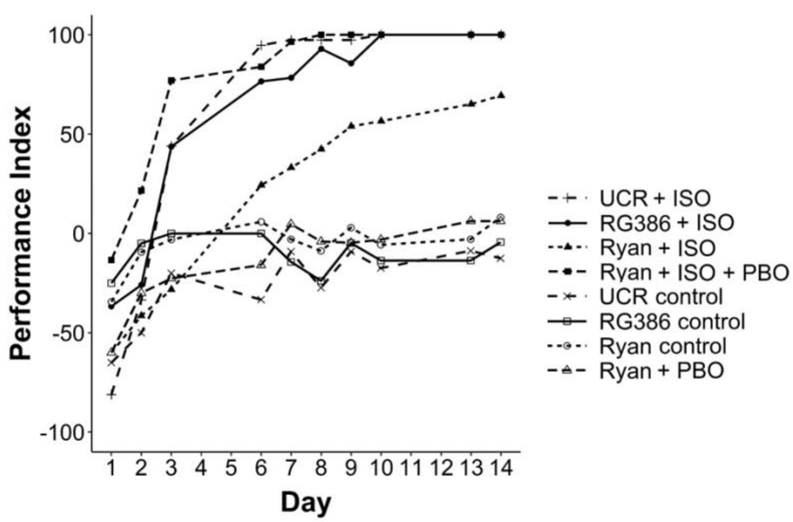

Fig 4. Response of the UCR, RG386, and Ryan adult males exposed to treated panels (0.05% isocycloseram [ISO], 0.5% piperonyl butoxide [PBO], and/or no treatment [control]) in the choice box assay.

Variation in susceptibility to isocycloseram among field-collected populations with known resistance to various classes of insecticides suggests that establishing proper rates to control all individuals in cockroach populations will minimize the chance for the escape of more tolerant individuals, which may lead to the development of a resistant population. Such variations in susceptibility were most clearly reflected in the choice box assay, in which the UCR and RG386 strains reached PIs of 100 when exposed to panels treated

Table 4. Topical toxicity of isocycloseram on the UCR, Ryan, and SY adult males at 72 h

Strain	*n*	LD_{50} (95% CI) (μg/insect)[a]	LD_{95} (95% CI) (μg/insect)	Slope	SE	χ^2 (df)	RR_{50}[b] (95% CI)
UCR	601	0.015 (0.013–0.017)	0.047 (0.040–0.059)	3.317	0.322	1.250 (4)	—
Ryan	290	0.022 (0.019–0.024)	0.065 (0.053–0.091)	3.456	0.403	2.515 (3)	1.6 (1.4–1.7)
SY	310	0.042 (0.036–0.048)	0.175 (0.127–0.288)	2.637	0.313	0.590 (4)	3.0 (2.6–3.6)

[a]Average mass of adult males: UCR (52.37 ± 1.0 mg), Ryan (53.18 ± 0.8 mg), SY (53.73 ± 0.8 mg); $P = 0.61$, Kruskal–Wallis test.
[b]Resistance ratio: LD_{50} (μg/insect) of field-collected strain divided by LD_{50} (μg/insect) of the UCR strain.

with isocycloseram alone, but the Ryan strain was only partially killed with ~70% mortality (Fig. 4). Because the Ryan strain did not reach a PI of 100, it was additionally tested using PBO + isocycloseram (Fig 4). The combination of insecticide and synergist increased the potency of the isocycloseram-treated panel, implying the negation of P450 activity in the Ryan strain (Fig. 4). We were unable to collect enough cockroaches of the SY strain to conduct similar comparisons due to the slow development of this strain. Appropriate doses should be identified to ensure that all field strains can be killed to prevent resistance selection when using isocycloseram.

Isocycloseram is classified under IRAC Group 30, which designates GABA-gated chloride channel modulation as its primary mode of action. GABA receptors are reportedly affected by 2 other registered insecticide classes for German cockroach control: phenylpyrazoles (fipronil), for which GABA receptors are the primary target, and avermectins, for which some lesser interactions with the channel can occur (Yu 2014, Casida and Durkin 2015). Although all resistant strains used in the present study have evolved high frequencies of the *Rdl* mutation (A302S) on the GABA chloride channel that confers fipronil resistance (Lee et al. 2022b), they remained mostly susceptible to isocycloseram. This was similar to the findings of Blythe et al. (2022) in which unaltered isocycloseram susceptibility was documented in *Drosophila melanogaster* Meigen expressing the A301S mutation. This phenomenon was attributed to the distinct binding sites of fipronil and isocycloseram. In contrast, *D. melanogaster* with L280C mutations (generated via gene editing) were less sensitive to topically applied isocycloseram, but this mutation has not been found in field populations (Ozoe et al. 2024). Unlike fipronil, target-site alterations associated with avermectin resistance have never been documented in *B. germanica*. Nonetheless, an abamectin resistance-conferring mutation in *Plutella xylostella* (L.) did not have an effect on isocycloseram toxicity, providing some evidence that target-site insensitivity has not developed simultaneously toward both compounds through currently identified mechanisms (Sun et al. 2023).

The impact of PBO on the performance of isocycloseram-treated panels for the Ryan strain provides evidence for P450-mediated detoxification pathways for this insecticide in *B. germanica* and may explain the slightly reduced susceptibilities of Ryan and SY (Table 4). This result supports the documentation of PBO increasing the sensitivity of *D. melanogaster* to topically applied isocycloseram (Ozoe et al. 2024). Because isocycloseram had never been used to control German cockroaches before these strains were collected from field sites (circa 2020), this is not a result of isocycloseram selection. The diverse P450 enzymatic family in *B. germanica* detoxifies a wide range of insecticides, and preexisting pathways may have been co-opted to target isocycloseram through pleiotropic mechanisms (Harrison et al. 2018, Hawkins et al. 2019). Because these mechanisms already exist, improper use of such compounds may quickly lead to resistance through additional elevation or alteration of P450 activity (Scharf et al. 2022). Like other insecticides, resistance management strategies such as rotating or mixing will theoretically benefit the longevity of isocycloseram by preventing continuous selection pressure.

The present study demonstrates that 1% isocycloseram bait is an effective new formulation against German cockroaches when directly compared to other bait products. Although bait performance cannot solely be attributed to the active ingredient in the formulation due to other vital inert components such as phagostimulants, the topical applications revealed negligible differences in response between the UCR (susceptible), WM, CDR, and RG386 strains toward isocycloseram. While the Ryan and SY strains were less sensitive to topical application, the bait formulation remained among the most effective for these strains (Fig 1D and F). With its consistent performance across different strains, isocycloseram is a promising bait toxicant for the control of the German cockroach.

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Conflict of Interest

David Cox is affiliated with Syngenta Crop Protection. Syngenta Crop Protection provided the isocycloseram bait and residual insecticide and partially funded the study.

Author Contributions

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[supporting], Methodology [supporting], Project administration [supporting], Resources [supporting], Writing—review & editing [equal]), Michael Rust (Data curation [supporting], Formal analysis [supporting], Investigation [equal], Methodology [equal], Resources [supporting], Supervision [supporting], Writing—review & editing [supporting]), and Chow-Yang Lee (Conceptualization [lead], Funding acquisition [lead], Methodology [equal], Project administration [lead], Resources [lead], Software [equal], Supervision [lead], Writing—original draft [equal], Writing—review & editing [equal])

Supplementary Material

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

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

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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 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.

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.

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}

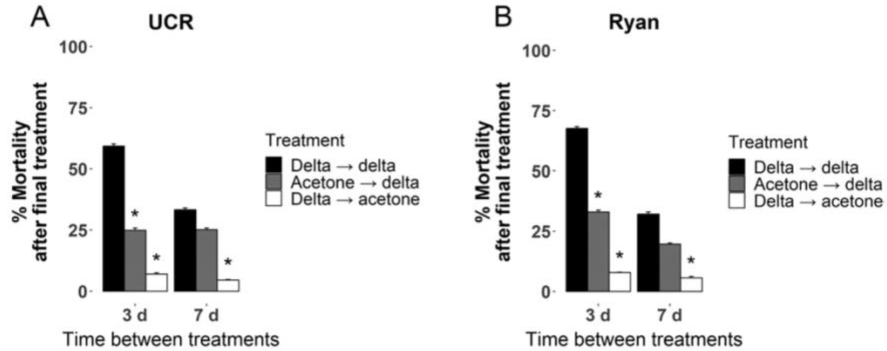

Fig. 1. Mortality of (A) UCR or (B) Ryan strain adult males from deltamethrin (topical 3 d LD_{20}) or acetone administered 3 d or 7 d after a first sublethal exposure to deltamethrin (topical 3 d LD_{20}) or acetone. Asterisks indicate a significant difference from deltamethrin → deltamethrin treatment (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).

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,

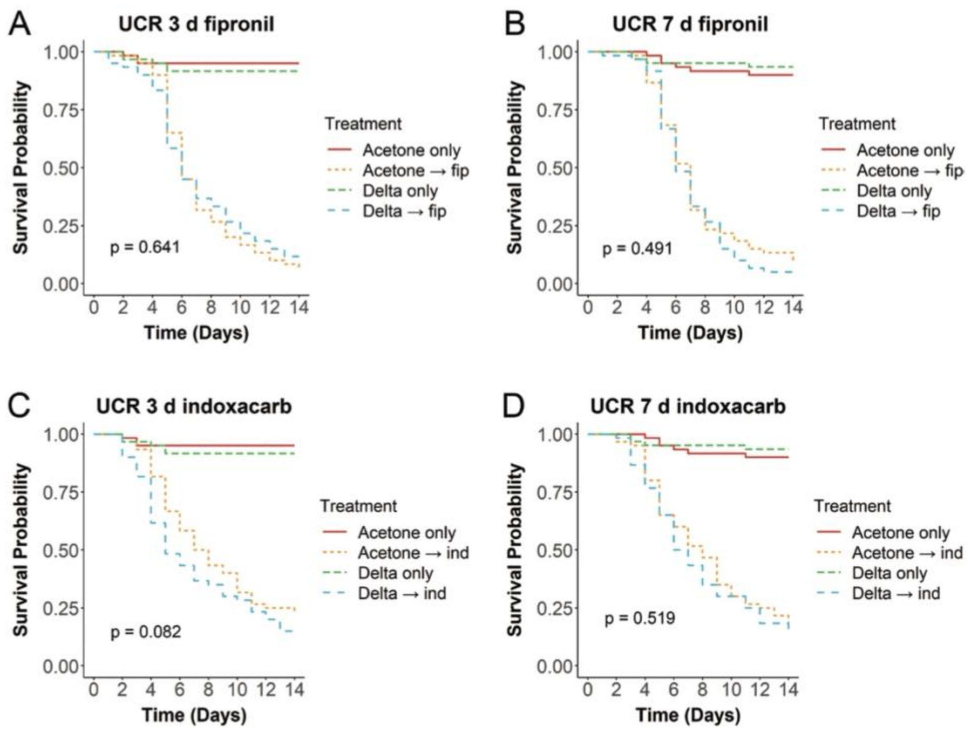

Fig. 2. Survivorship of UCR adult males exposed to fipronil- or indoxacarb-treated dog food (5 d LC_{50}) 3 d or 7 d after a first sublethal exposure to deltamethrin (topical 3 d LD_{20}). Treatment groups are as follows: (A) fipronil treatment started 3 d after deltamethrin treatment, (B) fipronil treatment 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; $\alpha = 0.05$).

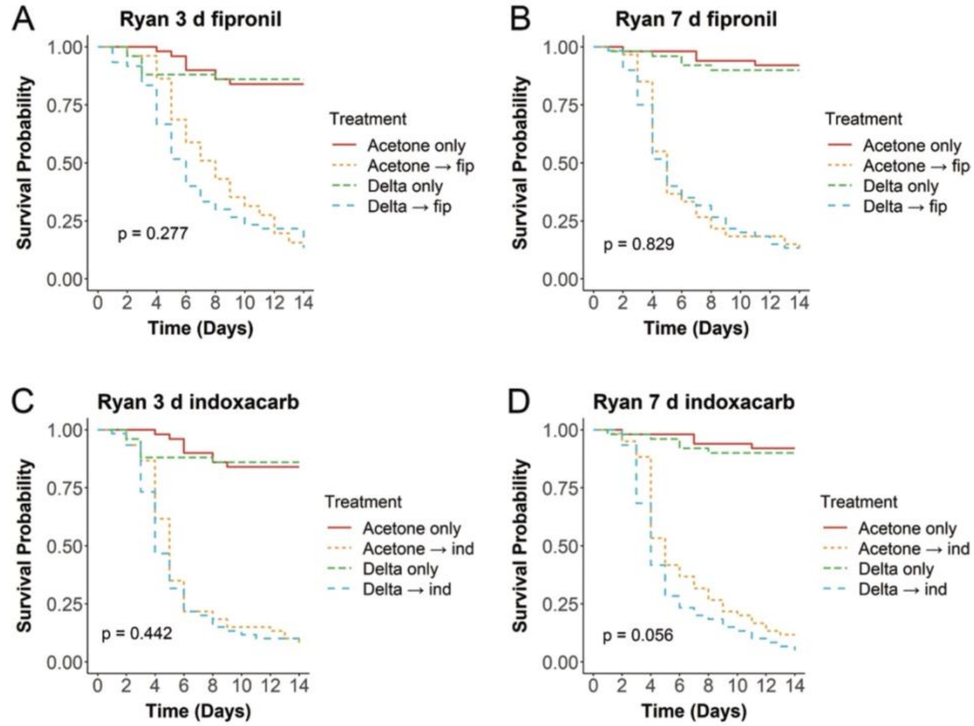

Fig. 3. Survivorship of Ryan adult males exposed to fipronil- or indoxacarb-treated dog food (5 d LC_{50}) 3 d or 7 d after a first sublethal exposure to deltamethrin (topical 3 d LD_{20}). Treatment groups are as follows: (A) fipronil treatment started 3 d after deltamethrin treatment, (B) fipronil 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 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.

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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.

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