Evaluation of an artificial sweetener as a potential bait toxicant and an insecticide synergist against German cockroaches, an important indoor pest of public health

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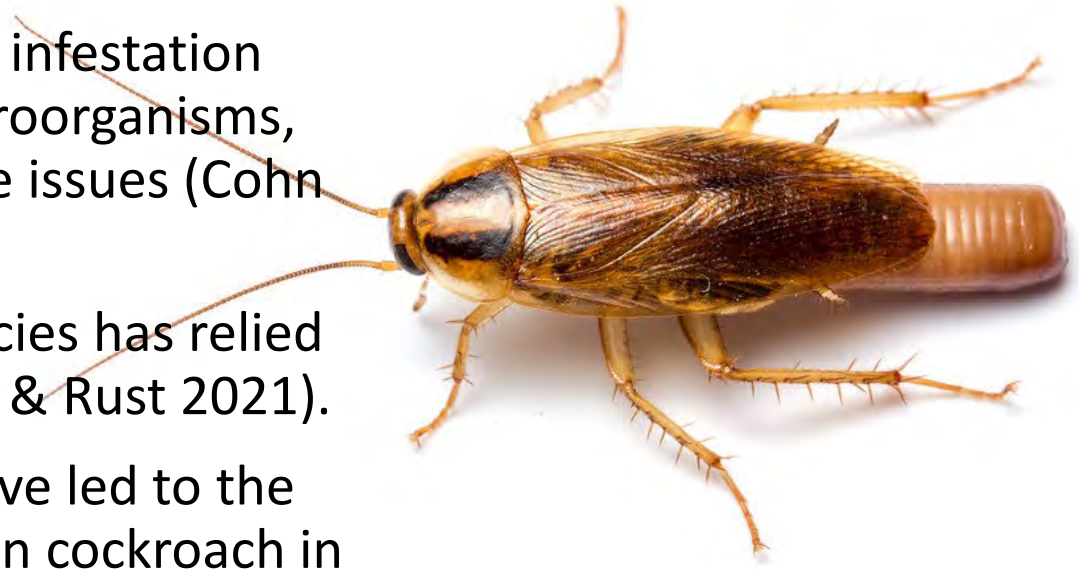
Background

- The German cockroach, *Blattella germanica*, is a major indoor public health insect pest in the United States, especially in residential premises and food preparative establishments such as restaurants, food courts, food packaging factories, etc. (Lee & Wang 2021).
- The negative consequences of a German cockroach infestation include mechanical transmission of pathogenic microorganisms, respiratory illness (allergy and asthma), and hygiene issues (Cohn et al. 2006; Schal & DeVries 2021).
- Pest management professionals' control of this species has relied heavily on residual insecticide sprays and baits (Lee & Rust 2021).
- Over-reliance and frequent usage of insecticides have led to the development of insecticide resistance in the German cockroach in the US, especially towards pyrethroids, phenyl pyrazole, and neonicotinoids (Scharf & Gondalekar 2021), and accumulation of pesticides in the indoor environment (Gunier et al. 2016; DeVries et al. 2019) that could seriously impact human health (Bao et al. 2019).

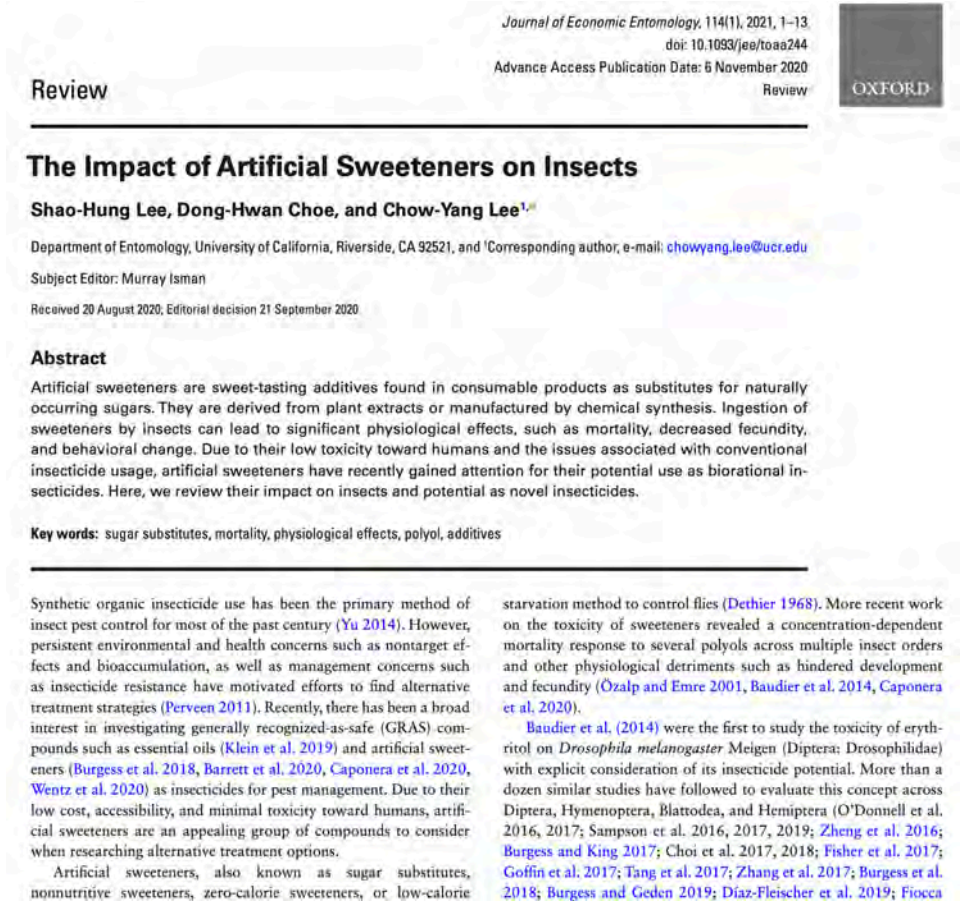


Background (2)

- Persistent environmental and health concerns such as effects on non-target organisms, bioaccumulation, and management challenges such as insecticide resistance have motivated the efforts to find alternative control strategies.
- Recently, there has been broad interest in investigating 'generally-recognized-as-safe' (GRAS) compounds such as essential oils and artificial sweeteners as insecticides for pest management.
- Artificial sweeteners are sweet-tasting additives found in beverages, food, drug, and other products.
- Due to their low cost, accessibility, and minimal toxicity towards humans, artificial sweeteners are an appealing group of compounds when considering alternative treatment options.
- Ingestion of sweeteners by insects can lead to significant physiological effects such as mortality, decreased fecundity, and behavioral change (Burgress et al. 2018; Barrett et al. 2020).

Background (3)

- Recent work on sweetener toxicity revealed concentration-dependent mortality response to several polyols across multiple insect orders and other physiological effects (Lee et al. 2021).
- The potential of using artificial sweeteners as toxicant against German cockroaches has never been reported.
- Our preliminary tests have shown that 10–20% sucralose, an artificial sweetener under trade name Splenda®, killed German cockroaches in choice and non-choice tests within 48 hours.
- This project's primary goal is to evaluate the potential of an artificial sweetener (sucralose) as a bait toxicant and as an insecticide synergist for boric acid for the effective management of insecticide-resistant German cockroaches.
- The second goal is to determine the possible mode of action(s) of sucralose in affecting the test insects' susceptibility to insecticides.



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Review

Review

The Impact of Artificial Sweeteners on Insects

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Abstract

Artificial sweeteners are sweet-tasting additives found in consumable products as substitutes for naturally occurring sugars. They are derived from plant extracts or manufactured by chemical synthesis. Ingestion of sweeteners by insects can lead to significant physiological effects, such as mortality, decreased fecundity, and behavioral change. Due to their low toxicity toward humans and the issues associated with conventional insecticide usage, artificial sweeteners have recently gained attention for their potential use as biorational insecticides. Here, we review their impact on insects and potential as novel insecticides.

Key words: sugar substitutes, mortality, physiological effects, polyol, additives

Synthetic organic insecticide use has been the primary method of insect pest control for most of the past century (Yu 2014). However, persistent environmental and health concerns such as nontarget effects and bioaccumulation, as well as management concerns such as insecticide resistance have motivated efforts to find alternative treatment strategies (Perveen 2011). Recently, there has been a broad interest in investigating generally recognized-as-safe (GRAS) compounds such as essential oils (Klein et al. 2019) and artificial sweeteners (Burgess et al. 2018, Barrett et al. 2020, Caponera et al. 2020, Wentz et al. 2020) as insecticides for pest management. Due to their low cost, accessibility, and minimal toxicity toward humans, artificial sweeteners are an appealing group of compounds to consider when researching alternative treatment options.

Artificial sweeteners, also known as sugar substitutes, nonnutritive sweeteners, zero-calorie sweeteners, or low-calorie

starvation method to control flies (Dethier 1968). More recent work on the toxicity of sweeteners revealed a concentration-dependent mortality response to several polyols across multiple insect orders and other physiological detriments such as hindered development and fecundity (Özalp and Emre 2001, Baudier et al. 2014, Caponera et al. 2020).

Baudier et al. (2014) were the first to study the toxicity of erythritol on *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) with explicit consideration of its insecticide potential. More than a dozen similar studies have followed to evaluate this concept across Diptera, Hymenoptera, Blattodea, and Hemiptera (O'Donnell et al. 2016, 2017; Sampson et al. 2016, 2017, 2019; Zheng et al. 2016; Burgess and King 2017; Choi et al. 2017, 2018; Fisher et al. 2017; Goffin et al. 2017; Tang et al. 2017; Zhang et al. 2017; Burgess et al. 2018; Burgess and Geden 2019; Díaz-Fleischer et al. 2019; Fiocca

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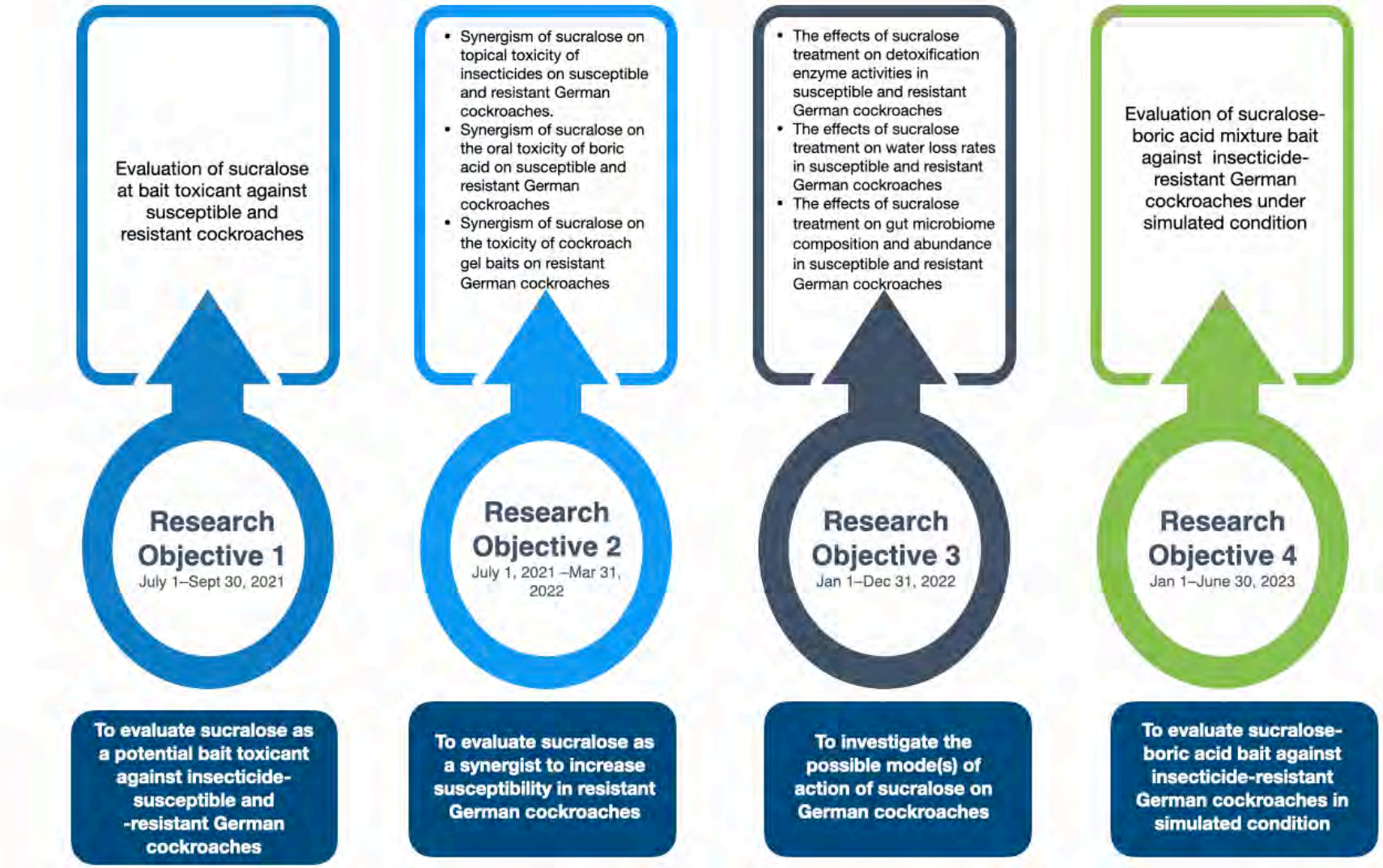


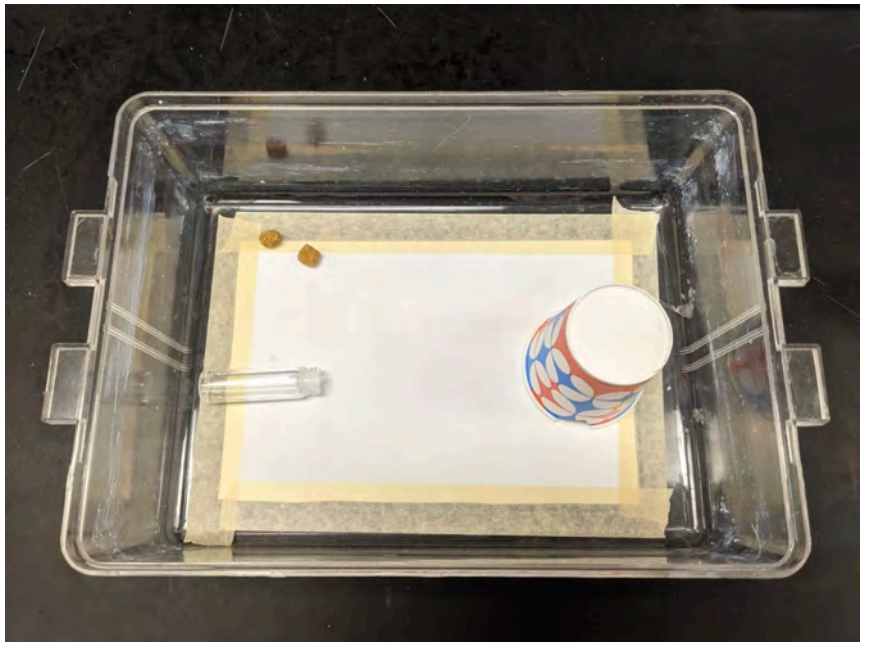
Insects

- We used 1 susceptible strain (UCR) and 2 insecticide-resistant strains (WM and RG386) of the German cockroach in all experiments.
- The **UCR strain** has been reared for >40 years without insecticide exposure and was originally a subculture of Orlando normal.
- **WM** and **RG386** were collected from low-income housing in Los Angeles, CA in 2018 and 2019, respectively.
- All insects were mass-reared inside 121-liter garbage bins equipped with electrical barriers, under indoor conditions of 24 $\pm$ 2^{o}C, ambient RH of 30–50%, and 12-hour photoperiod.
- Dog food, water, and cardboard harborages were provided *ad libitum*.

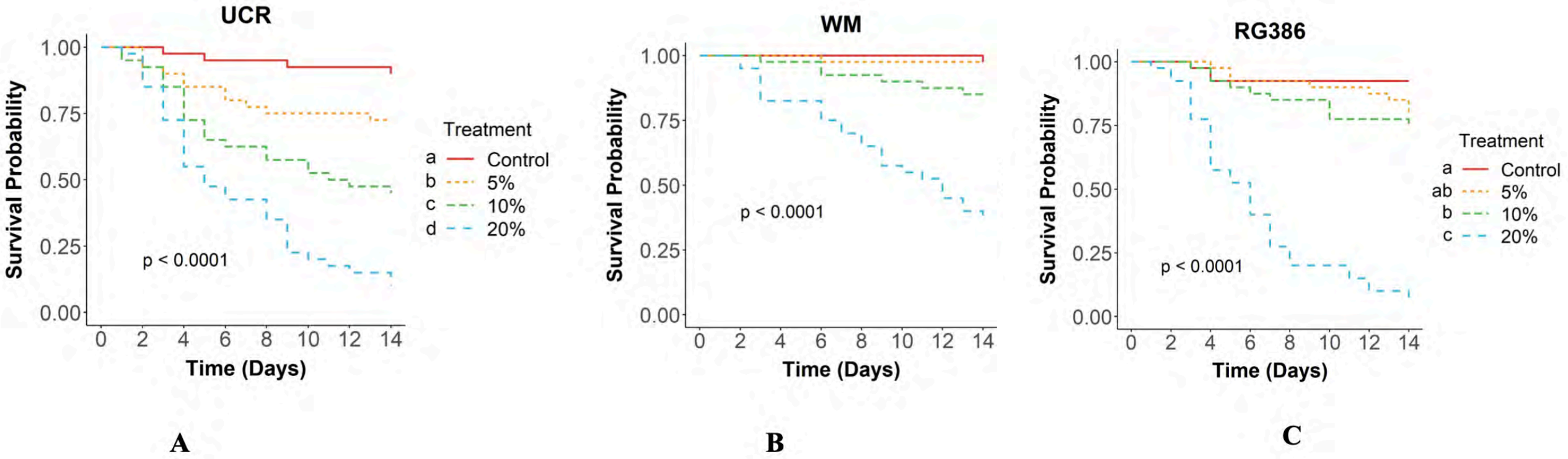
Objective #2

To evaluate the potential of sucralose as a bait toxicant against insecticide-susceptible and –resistant German cockroaches

- Sucralose (Supplement Partners LLC, Phoenix, AZ) was diluted in distilled water to create 20%, 10%, and 5% (w/v%) solutions.
- 10 adult male cockroaches were introduced and allowed to acclimatize in arenas (27.5 x 20 x 9 cm) with dog food, water, paper cup harborage, and a sheet of filter paper lining the basin (Figure 1).
- The sides of the arena were coated with fluon (BioQuip Products, Rancho Dominguez, CA) to prevent insects from escaping.
- At the start of the trial, the water source was replaced with a 5, 10, or 20% sucralose solution in a glass vial.
- Control groups were provided with distilled water only.
- Mortality was determined by an insect's inability to walk or right itself when probed with forceps and was recorded daily for 14 days.
- Each concentration was replicated 4 times.



Impact of sucralose solutions on (A) UCR, (B) WM, and (C) RG386 survivorship



P-values represent overall differences in survivorship, and different letters by treatment names indicate a significant difference in impact (log-rank test; $\alpha = 0.05$).

Mean survival time and total mortality of susceptible and field strains of German cockroaches after sucralose treatment

Strain	Treatment	Mean Survival Time (95% CI) (hour)	% Mortality at 14 days
UCR	20%	6.675 (5.378 - 7.972)	90.0%
	10%	9.475 (7.929 - 11.021)	55.0%
	5%	11.575 (10.249 - 12.901)	27.5%
	Control	13.375 (12.552 - 14.198)	10.0%
WM	20%	10.025 (8.667 - 11.383)	62.5%
	10%	13.1 (12.323 - 13.877	15.0%
	5%	13.8 (13.413 - 14.187)	2.5%
	Control	14 (14 - 14)	2.5%
RG386	20%	6.4 (5.252 - 7.548)	92.5%
	10%	12.325 (11.228 - 13.422)	25.0%
	5%	13.1 (12.256 - 13.944)	20.0%
	Control	13.225 (12.381 - 14.069)	7.5%

Comments on the results

- Sucralose had a concentration-dependent effect on survivorship of all strains.
- The 20% treatment had a significantly greater impact on survivorship than all other treatments.
- 20% treatment resulted in the greatest overall mortality and shortest mean survival times: 90% and 6.675 days, 62.5% and 10.025 days, and 92.5% and 6.4 days for UCR, WM, and RG386, respectively.
- **Summary and conclusion:** Sucralose alone showed oral toxicity against both susceptible and field strain (insecticide-resistant) German cockroaches.

Objective 3

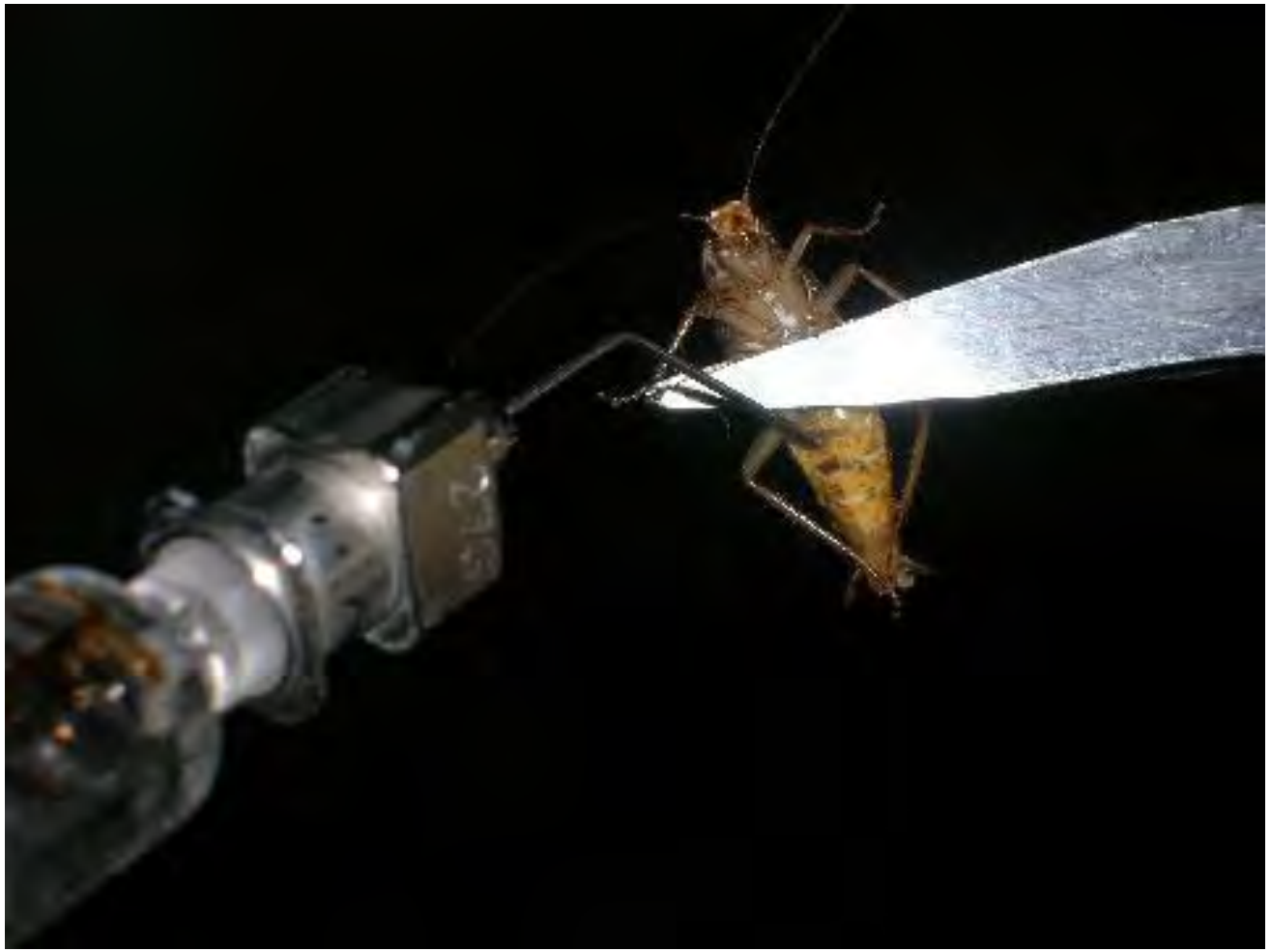
To evaluate sucralose as a synergist to increase susceptibility in resistant German cockroaches.

- Objective 3.1 - assess the synergism effect of sucralose on topical toxicity of insecticides on susceptible and resistant German cockroaches
- Objective 3.2 - assess the synergism effect of sucralose on the oral toxicity of boric acid on susceptible and resistant German cockroaches
- Objective 3.3 - assess the synergism effect of sucralose on the toxicity of cockroach gel baits on resistant German cockroaches

3.1: Assess the synergism effect of sucralose on topical toxicity of insecticides on susceptible and resistant German cockroaches

- 3 strains of German cockroaches were used: UCR susceptible strain, WM and RG386 strains.
- **Diagnostic dose determination**: A range of doses that caused 10–90% mortality was made by diluting technical grade hydramethylnon or fipronil in acetone.
- The UCR adult males were anesthetized with CO_2 and 0.5 µl of insecticide solution was applied to the abdominal sternites with a microapplicator.
- The treated cockroaches were provided with food & water, and mortality was scored at 72 h (fipronil) and 120 h (hydramethylnon).

Apply 0.5 µl of insecticide on the abdominal sternite of an adult male German cockroach



3.1: Assess the synergism effect of sucralose on topical toxicity of insecticides on susceptible and resistant German cockroaches

- Mortality was pooled and analysed using probit analysis.
- The LD_{95} value obtained was multipled by 10 or 3 to determine the diagnostic dose.

Table 1: Toxicity of hydramethylnon and fipronil on UCR strain

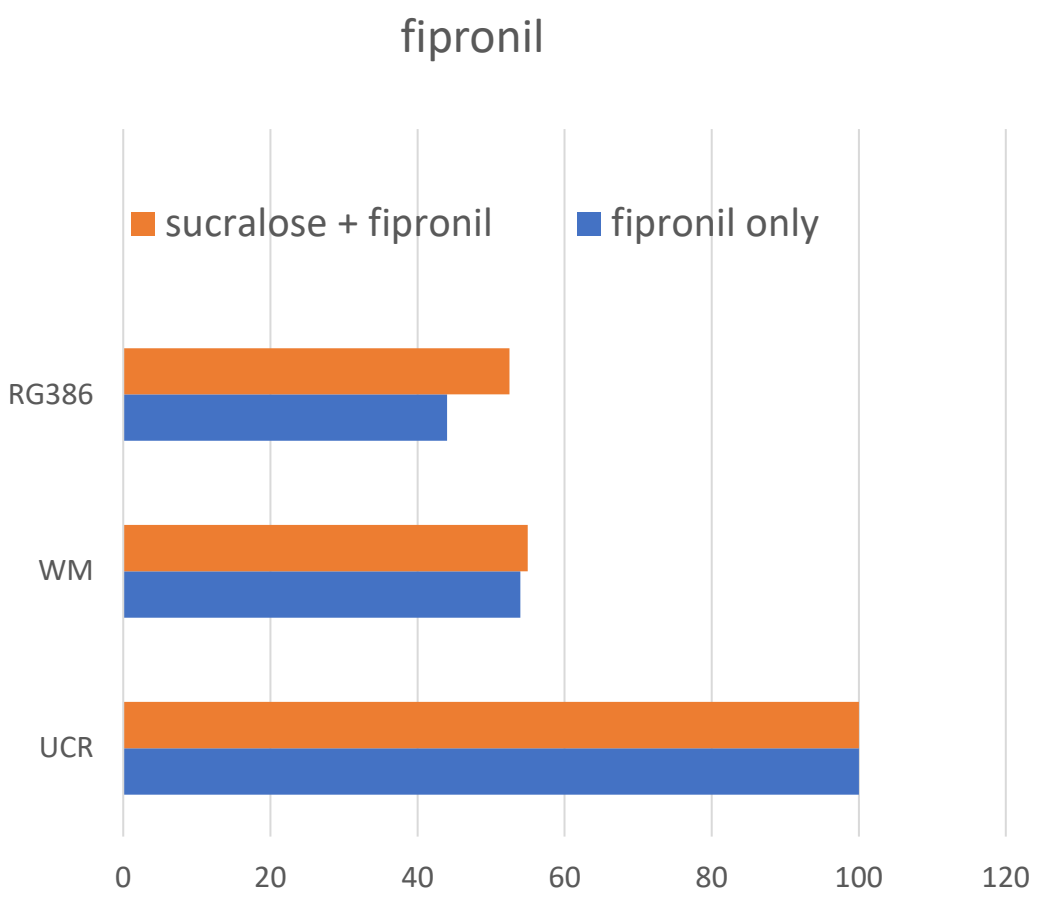
Insecticide	N	LD_{50} (95% FL) (μg/insect)	LD_{95} (95% FL) (μg/insect)	Slope ± SEM	χ^2 (df)
hydramethylnon	600	3.33 (2.28–4.26)	14.19 (10.79–22.18)	2.611 ± 0.246	5.576 (4)
fipronil	200	0.0013 (0.0011–0.0014)	0.0036 (0.0031–0.0048)	3.595 ± 0.424	4.806 (6)

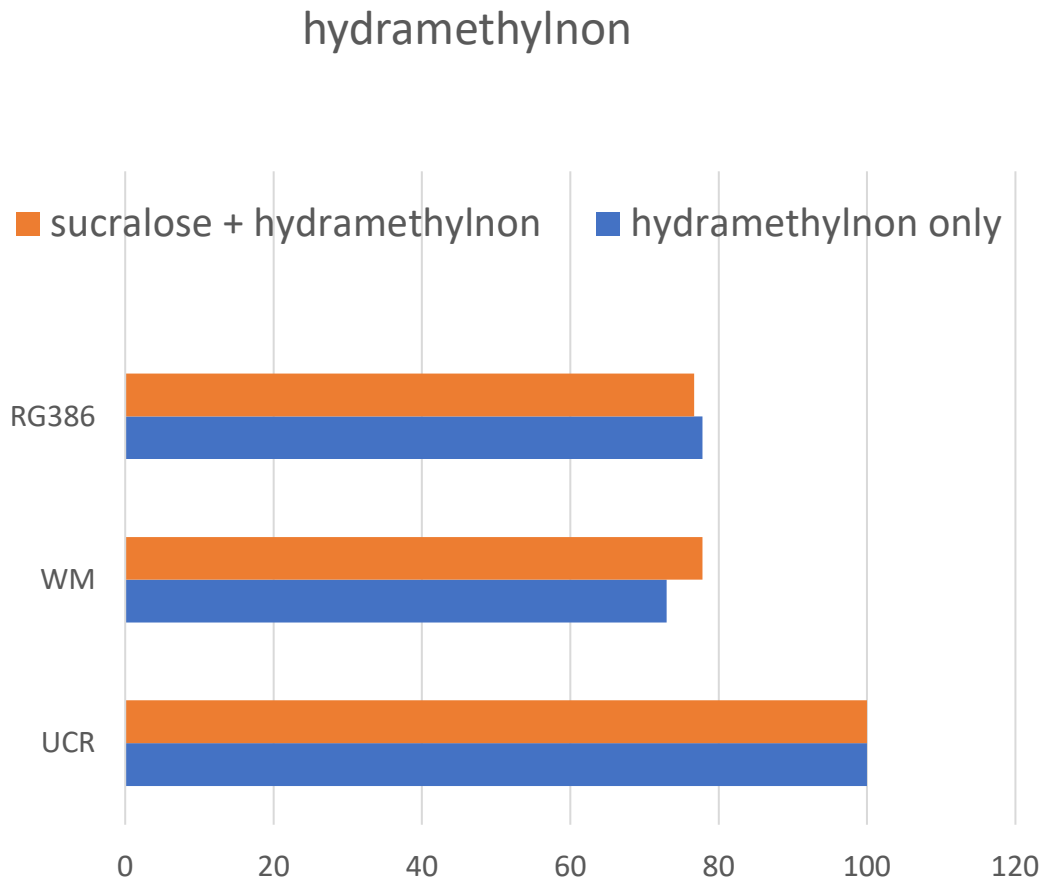
10 x LD_{95} diagnostic dose (fipronil) = 0.036 μg/insect
3 x LD_{95} diagnostic dose (hydramethylnon) = 42.57 μg/insect

3.1: Assess the synergism effect of sucralose on topical toxicity of insecticides on susceptible and resistant German cockroaches

- Two groups of adult males of WM and RG386 were prepared.
- The first group was treated with diagnostic doses of hydramethylnon or fipronil.
- The second group was first fed with 20% sucralose solution for 48 hours. After that, they were treated with diagnostic doses of hydramethylnon or fipronil.

Mortality of German cockroaches after treatment with 20% sucralose and diagnostic dose of fipronil and hydramethylnon





Pre-exposure to sucralose did not significantly (P > 0.05) impact mortality against the diagnostic dose of hydramethylnon or fipronil

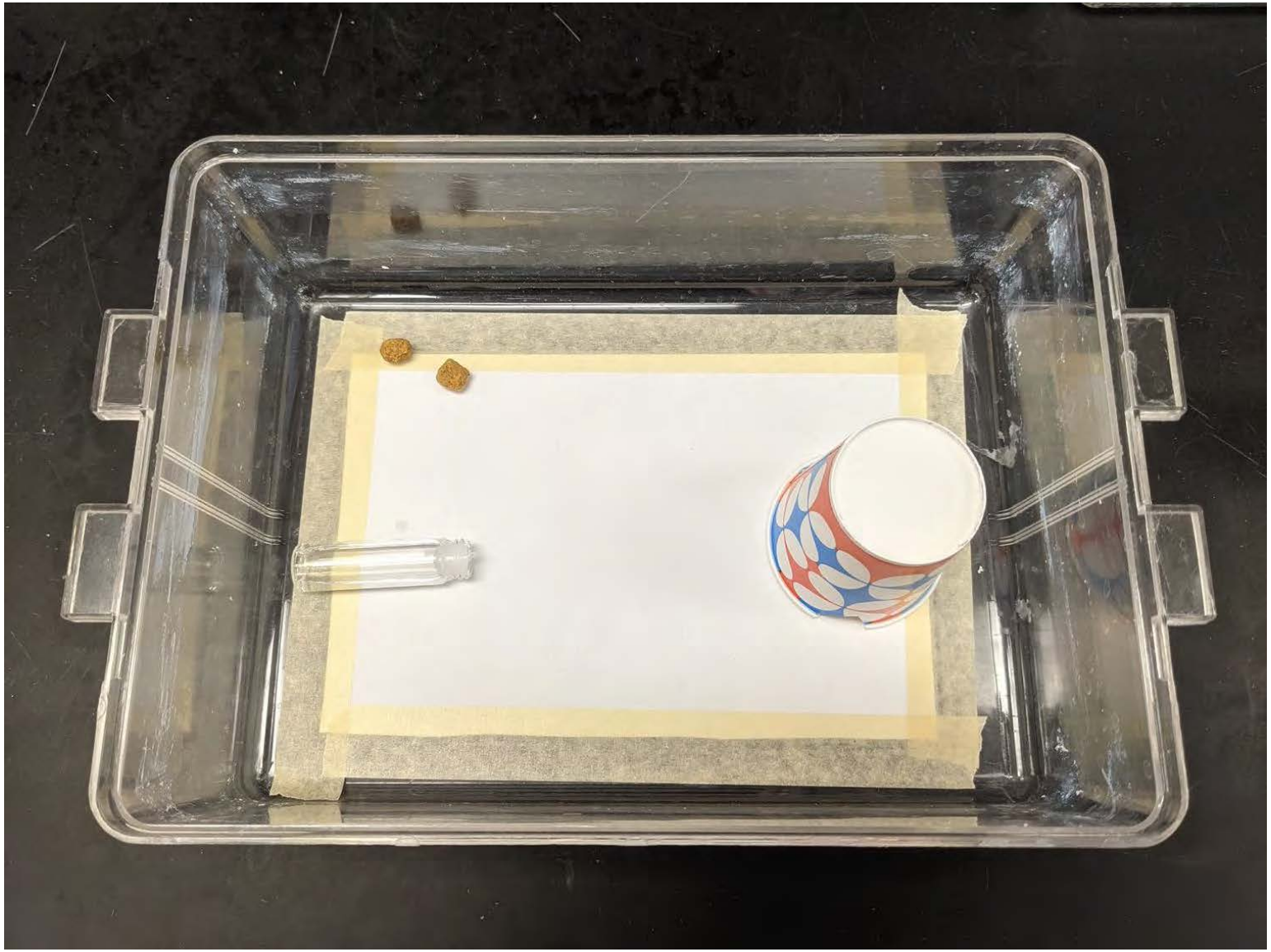
3.2: Assess the synergism effect of sucralose on oral toxicity of boric acid on susceptible and resistant German cockroaches

- **Cockroaches**: UCR susceptible strain, WM, and RG386 field strains.
- **Methodology**: The following liquid baits were prepared by diluting materials in distilled water (w/v%):

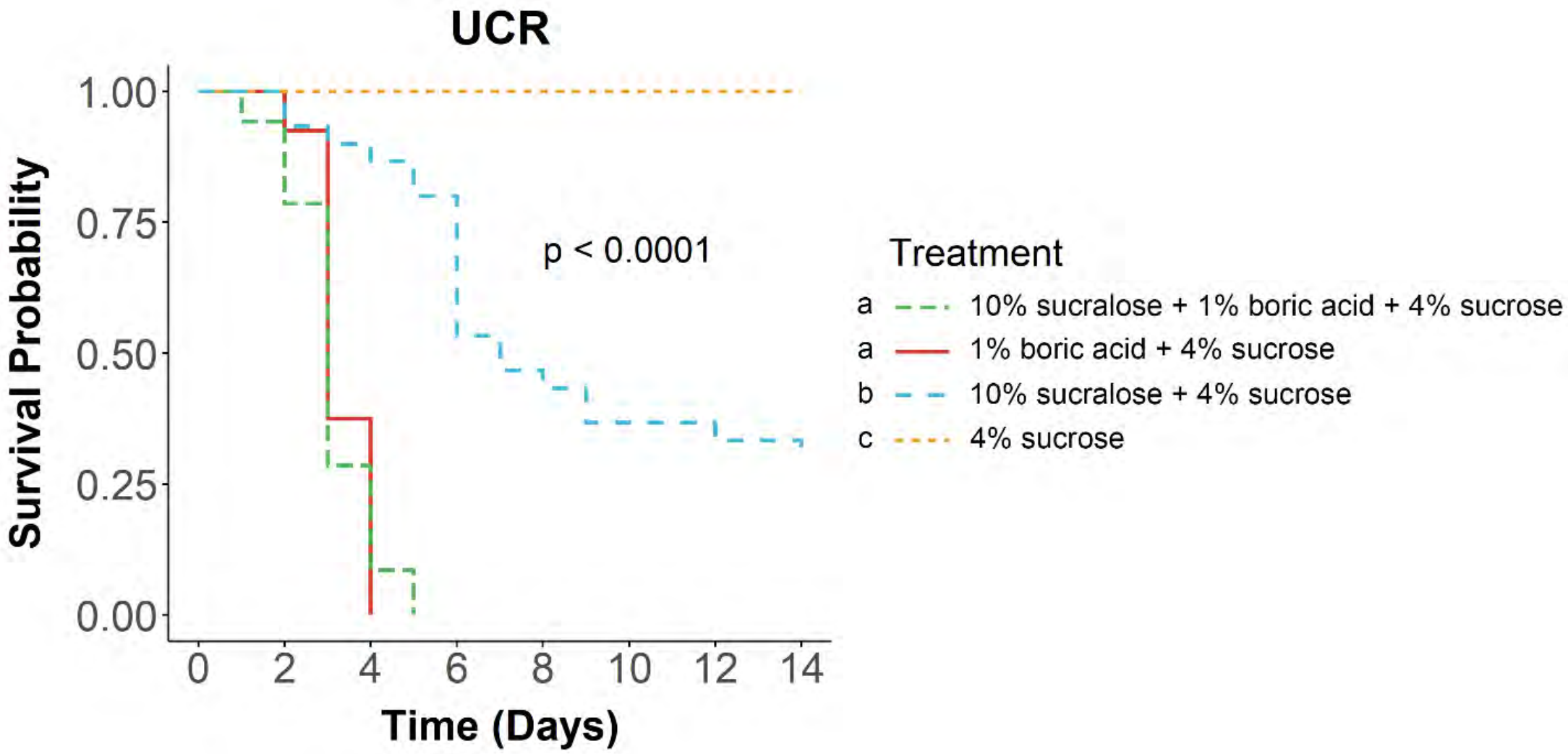
  10% sucralose + 4% sucrose + 1% boric acid (toxicant)
  10% sucralose + 4% sucrose
  4% sucrose + 1% boric acid (toxicant)
  4% sucrose (control)

- Ten adult male cockroaches of the UCR, WM, or RG386 strains were introduced into arenas (27.5 x 20 x 9 cm) with dog food, cardboard harborage, and a sheet of filter paper lining the base.
- One solution was introduced into the arena at the start of the trial, and mortality was recorded daily until complete mortality or 14 days.
- Data were analyzed with Kaplan-Meier survival analysis and compared amongst all treatments. Each treatment was replicated a minimum of 3 times.

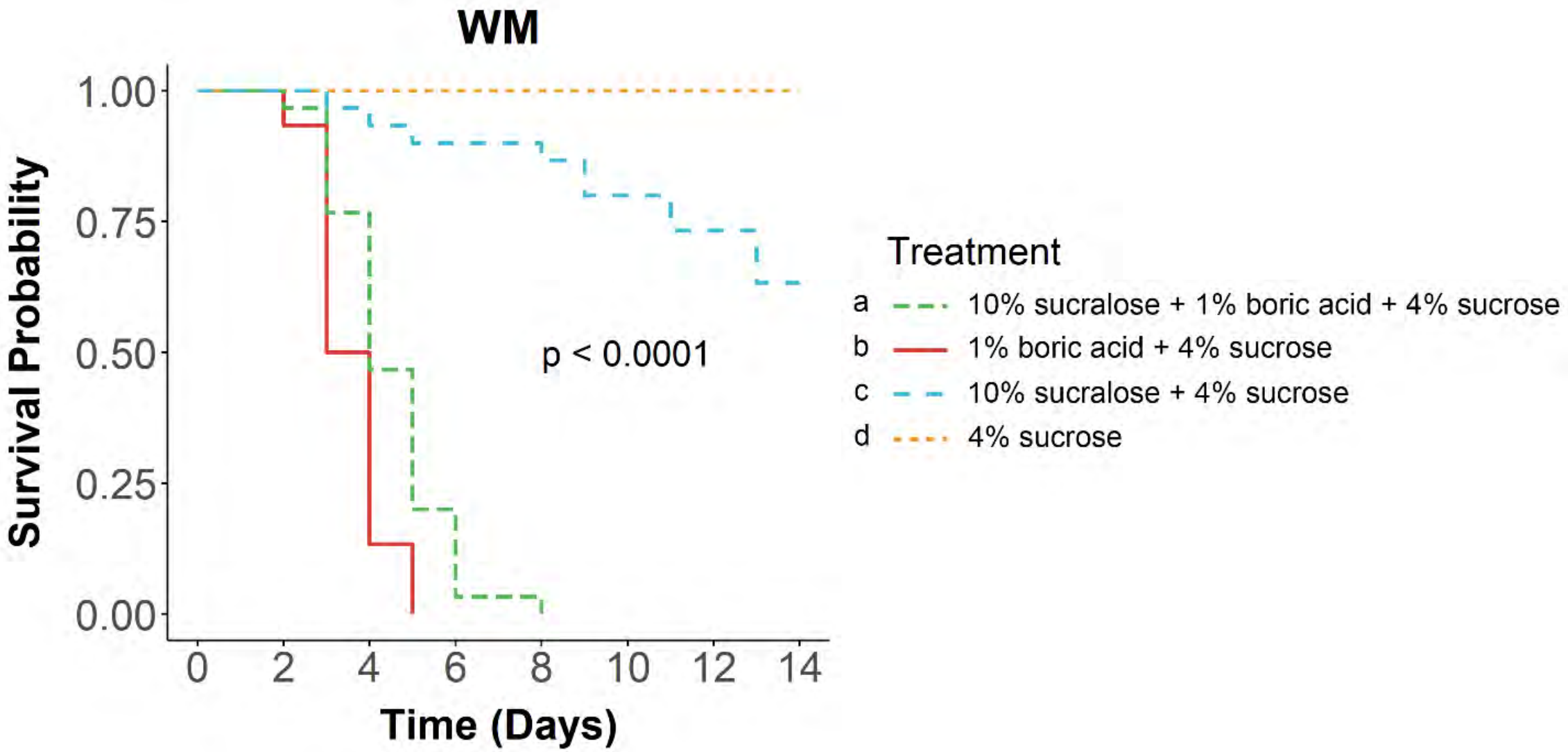
The experimental set up for testing bait



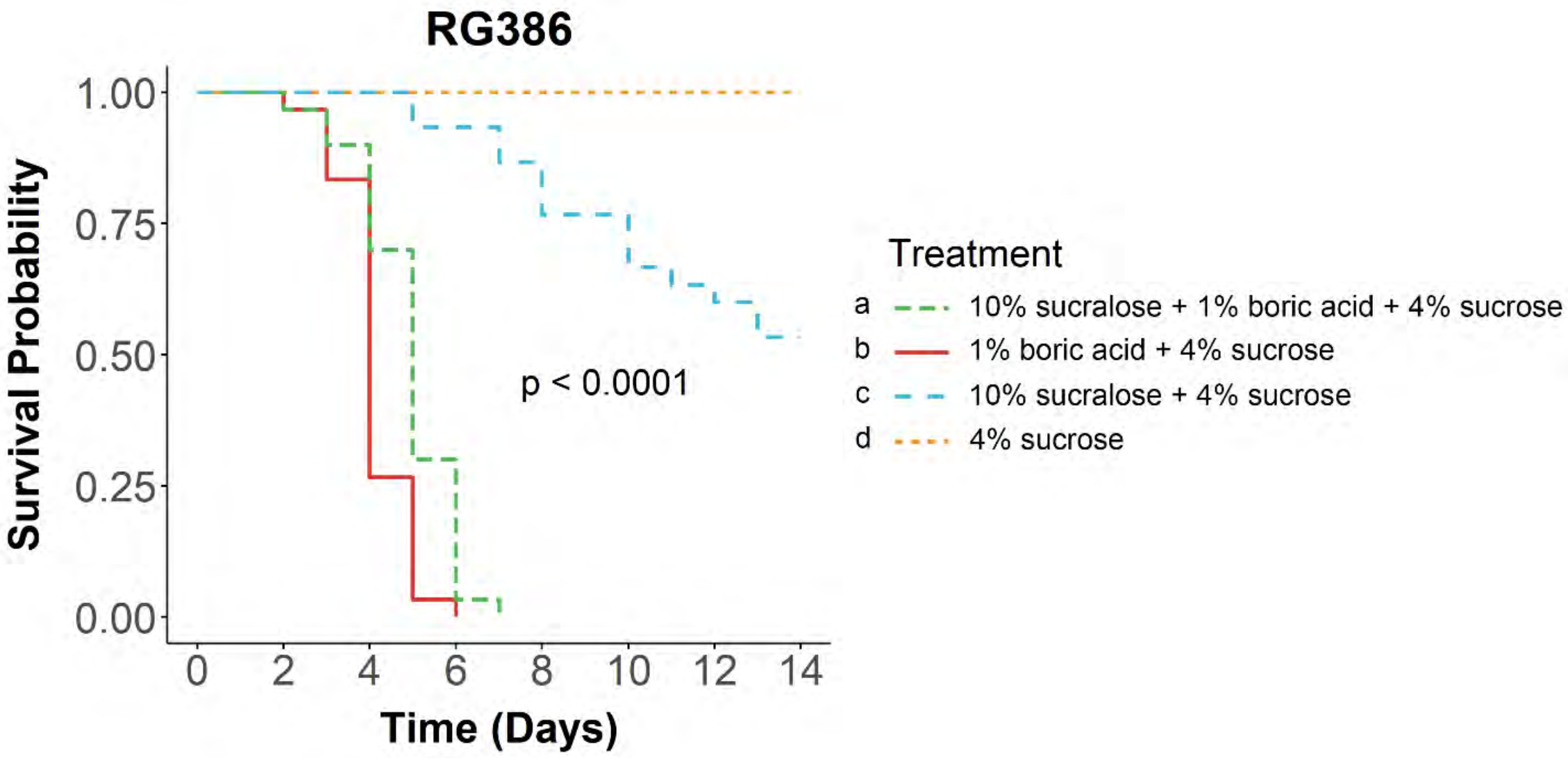
UCR susceptible strain



WM strain (resistant)



RG386 strain (resistant)



Mean survival times of different treatments against insecticide-susceptible and resistant German cockroaches

Strain	Treatment	Mean Survival Time (days)	95% CI	Std. Error	% Mortality at 14 Days
UCR	10% sucralose + 1% boric acid + 4% sucrose	3.1	2.9–3.3	0.115	100.0
	1% boric acid + 4% sucrose	3.3	3.1–3.5	0.96	100.0
	10% sucralose + 4% sucrose	8.7	7.2–10.2	0.785	70.0
	4% sucrose	14	-	-	0.0
WM	10% sucralose + 1% boric acid + 4% sucrose	4.5	4.0–4.9	0.234	100.0
	1% boric acid + 4% sucrose	3.6	3.3–3.9	0.149	100.0
	10% sucralose + 4% sucrose	12.2	11.9–13.3	0.585	36.7
	4% sucrose	14	-	-	0.0
RG386	10% sucralose + 1% boric acid + 4% sucrose	4.9	4.5–5.3	0.2	100.0
	1% boric acid + 4% sucrose	4.1	3.8–4.4	0.147	100.0
	10% sucralose + 4% sucrose	11.7	10.6–12.8	0.55	46.7
	4% sucrose	14	-	-	0.0

Results

- The addition of 10% sucralose to the 1% boric acid + 4% sucrose bait caused a negligible effect on survivorship in the UCR strain.
- Both treatments caused an average survival time of 3.1–3.3 days.
- For the WM and RG386 strains, sucralose **decreased** the effectiveness of 1% boric acid + 4% sucrose.
- Bait with 10% sucralose extended survival time by ~1 day.
- Bait with sucralose + sucrose (without boric acid) caused 37–70% mortality at 14 days post-treatment.
- All treatments with boric acid caused 100% mortality by 14 days and no mortality was observed from the 4% sucrose treatments across all strains.

Conclusion

- **We did not find significant synergistic effect with the addition of sucralose to the boric acid-sucrose bait formulation.**

3.3 Assess the synergism effect of sucralose on the toxicity of cockroach gel baits on resistant German cockroaches

- Strains used: UCR susceptible strain, WM and RG386 strains.
- Sucralose solution containing 20% sucralose and 0.025% Nile Blue A were prepared.
- Nile Blue A is a lipid-soluble dye to verify the consumption of sucralose solution.
- Control were 0.025% Nile Blue A only, or water only.
- 10 adult males were pre-fed with sucralose solution for 48 h. After the exposure, the insects were provided with 1 g of Siege gel bait (containing 2% hydramethylnon).
- Mortality was recorded every 12 h for the first 72 h, and then daily up to 14 d.

Experimental set up for testing bait

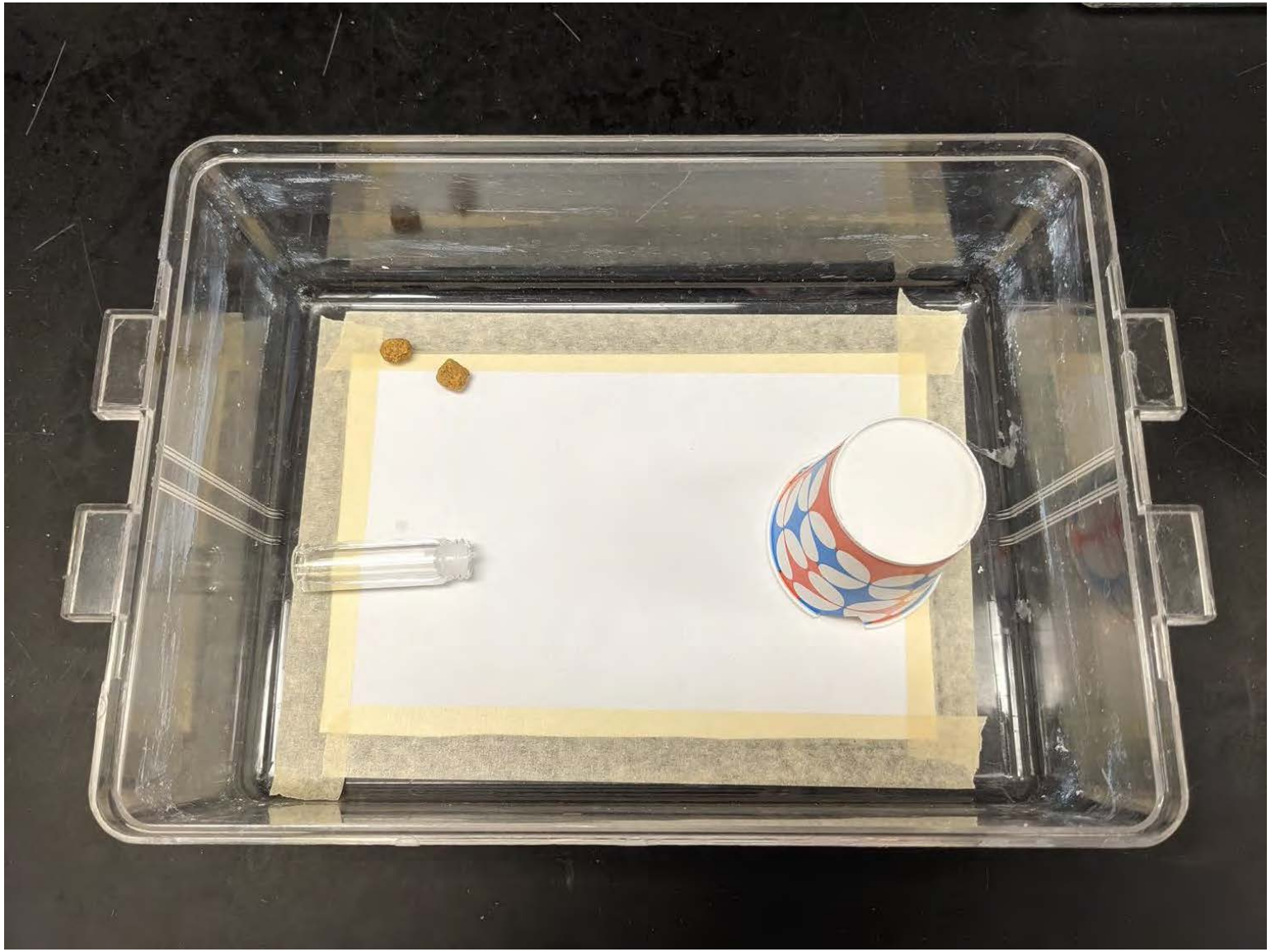
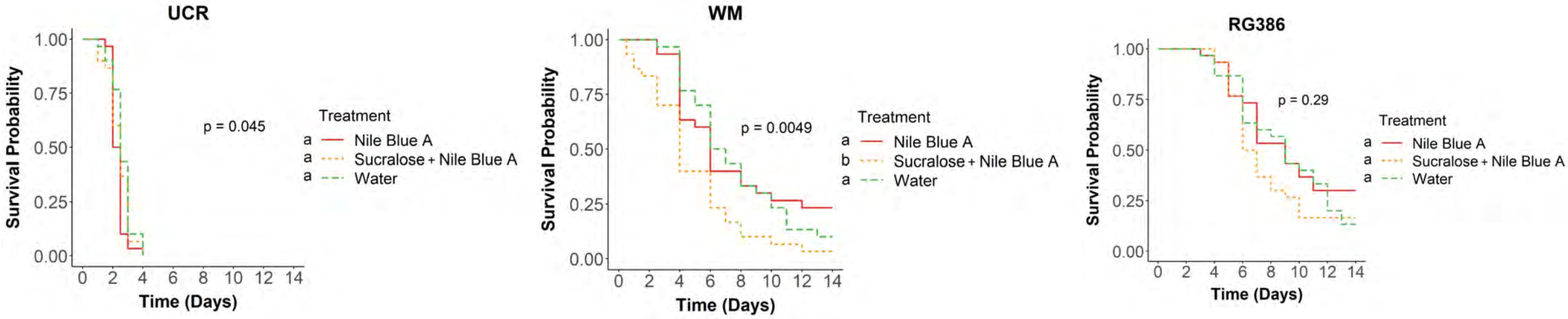


Table 5. Mean survival time of cockroaches pre-exposed to sucralose solutions and tested against Siege bait.

Strain	Pre-exposure	Treatment	Mean Survival Time (Days)	95% CI	Std. Error	% Mortality at 14 Days
UCR	Sucralose + Nile Blue A	Siege	2.433	2.165 - 2.702	0.137	100.0%
	Nile Blue A	Siege	2.317	2.151 - 2.483	0.085	100.0%
	Water	Siege	2.633	2.385 - 2.882	0.127	100.0%
	Sucralose + Nile Blue A	Control	-	-	-	6.7%
	Nile Blue A	Control	-	-	-	3.3%
	Water	Control	-	-	-	6.7%
WM	Sucralose + Nile Blue A	Siege	4.883	3.744 - 6.023	0.582	96.7%
	Nile Blue A	Siege	7.567	6.099 - 9.035	0.749	76.7%
	Water	Siege	7.583	6.389 - 8.777	0.609	90.0%
	Sucralose + Nile Blue A	Control	-	-	-	13.3%
	Nile Blue A	Control	-	-	-	13.3%
	Water	Control	-	-	-	10.0%
RG386	Sucralose + Nile Blue A	Siege	7.8	6.659 - 8.941	0.582	83.3%
	Nile Blue A	Siege	9.167	7.847 - 10.487	0.673	70.0%
	Water	Siege	9	7.767 - 10.233	0.629	86.7%
	Sucralose + Nile Blue A	Control	-	-	-	3.3%
	Nile Blue A	Control	-	-	-	3.3%
	Water	Control	-	-	-	3.3%

Survivorship of UCR (A), WM (B), and RG386 (C) pre-exposed to sucralose solutions and treated with Siege bait.



There was no statistical difference in survivorship between sucralose pre-exposed and unexposed UCR groups and between RG386 groups.

Sucralose pre-exposed WM had significantly decreased survivorship compared to Nile Blue A or water exposed groups

Objective 4: To investigate the possible mode of action of sucralose on German cockroaches

- 4.1: Effects of sucralose treatment on detoxification enzyme activities in susceptible and resistant German cockroaches.
- 4.2: Effects of sucralose treatment on water loss rates in susceptible and resistant German cockroaches.
- 4.3: Effects of sucralose treatment on gut microbiome composition and abundance in susceptible and resistant German cockroaches.

Effects of sucralose treatment on detoxification enzyme activities in susceptible and resistant German cockroaches

- 5 enzyme assays performed using procedures described in Lee et al. (2022)
  - Monooxygenase titration assay
  - Esterase assay (1-naphthyl acetate)
  - Esterase assay (2-naphthyl acetate)
  - Esterase assay (p-nitrophenyl acetate)
  - Glutathione S-transferase assay
- Partially completed.

Metabolic detoxification enzyme activities in susceptible and resistant German cockroaches after treatment with sucralose

Strain	1-NA esterase[a]				2-NA esterase[b]				PNPA esterase[c]				GST[d]				P450[e]			
	No treatment		Sucralose		No treatment		Sucralose		No treatment		Sucralose		No treatment		Sucralose		No treatment		Sucralose	
	n	Reading	n	Reading	n	Reading	n	Reading	n	Reading	n	Reading	n	Reading	n	Reading	n	Reading	n	Reading
UCR	95	90.92	24	174.29*	95	107.68	24	172.01*	96	519.10	24	540.25	96	0.38	24	0.68*	-	-	-	-
WM	96	118.60	64	131.00	96	142.61	64	168.48*	95	631.48	64	847.12*	96	1.18	64	1.54*	49	54.62	56	143.45*
RG386	-	-	-	-	-	-	-	-	24	734.66	24	872.90	24	0.90	22	1.00	-	-	-	-

[a]1-naphthol (ng/min/mg protein)

[b]2-naphthol (ng/min/mg protein)

[c]*p*-nitrophenyl (nmol/min/mg protein)

[d]GST conjugate (mmol/min/mg protein)

[e]P450 estimation (µg/mg protein)

*Indicates significant difference from no treatment (t-test; $\alpha = 0.05$)

Water loss from sucralose exposure

20%
sucralose

Time

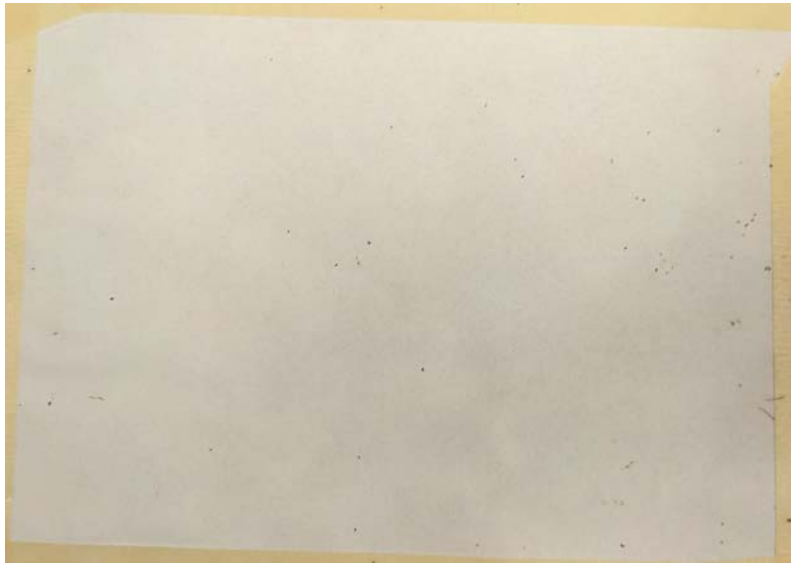
0 days 3 days 6 days

Total weight, dry weight, % body water

Strain	Time	Total Weight (mg)	Dry Weight (mg)	% Body Water*
UCR	Day 0	47.28 a	13.57 a	71.18% a
	Day 3	37.37 b	11.56 b	69.17% ab
	Day 6	37.82 b	12.2 b	67.94% b
WM	Day 0	52.34 a	14.9 a	71.48% a
	Day 3	44.33 b	13.69 b	69.22% b
	Day 6	37.55 c	11.45 c	69.45% ab
RG386	Day 0	50.74 a	14.04 a	72.12% a
	Day 3	45.87 b	14.24 a	69.11% b
	Day 6	40.87 c	12.61 b	69.19% b

* (Total Weight - Dry Weight)/Total Weight x 100

Sucralose causes “splattering”



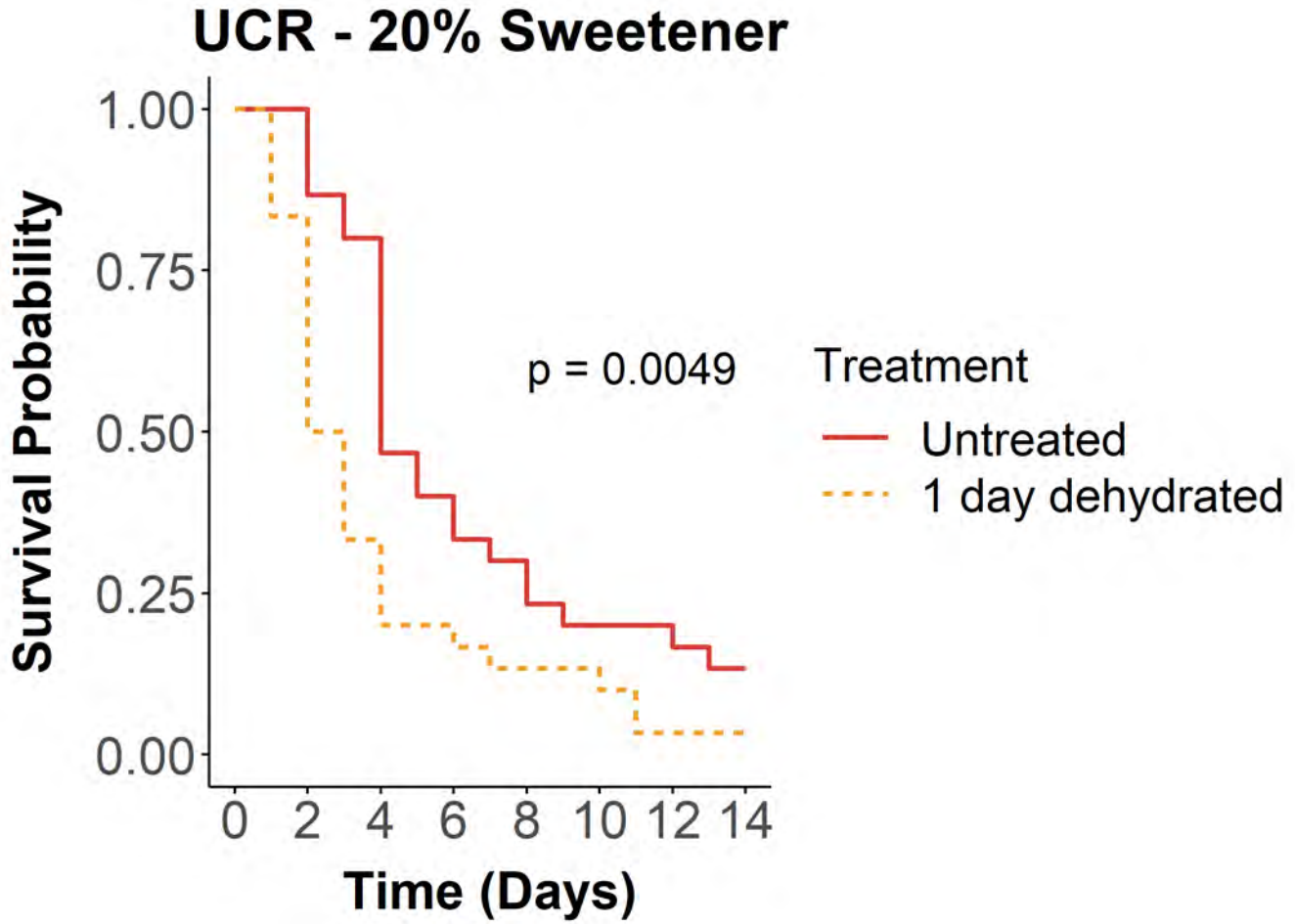
No sucralose exposure

20% sucralose

Forced vomiting or excretion: Death by dehydration?

Additional experiment:
Effect of water stress on sucralose toxicity

- Ten cockroaches acclimate with or w/o water for 1 day
- 20% sucralose for 14 days



Summary

- **Objective 2:** 5, 10, 20% sucralose solutions
  - Findings: Concentration dependent mortality
- **Objective 3**: Pre-exposure to 20% sucralose solution
  - Findings: No impact on topical toxicity (deltamethrin and fipronil DD) or bait toxicity (hydramethylnon)
- **Objective 3:** Combination with boric acid in liquid bait
  - Findings: No apparent synergistic or additive effect; possible antagonism
- **Objective 3**: Combination with commercial gel baits
  - Findings: Pre-exposure to sucralose had marginal impact on mortality against hydramethylnon bait.
- **Objective 4**: Effects on detoxification enzyme activities
  - Findings: Did not reduce the activities of detoxification enzymes
- **Objective 4**: Effects on water loss rates
  - Findings: Sucralose possibly disrupted homeostasis in treated cockroaches due to water loss.

Objective	Task	Deadline	Status
2 – Assess the lethal effects of sucralose at different concentrations on susceptible and resistant German cockroaches	2.1 (same)	September 30, 2021	Completed
3 – Evaluate sucralose as a synergist to increase susceptibility in insecticide-resistant German cockroaches	3.1 – Assess the synergistic toxicity effects of sucralose in combination with other insecticides	December 31, 2021	Completed
	3.2 – Assess the synergistic oral toxicity effects of sucralose and boric acid on susceptible and resistant German cockroaches	December 31, 2021	Completed
	3.3 – Assess the synergistic effect of sucralose on cockroach gel bait toxicity for resistant cockroaches	March 31, 2022	Completed
4 – Investigate the possible mode of action of sucralose on German cockroaches	4.1 – Investigate the effects of sucralose treatment on detoxification enzyme activities in susceptible and resistant German cockroaches	September 30, 2022	On-going
	4.2 – Investigate the effects of sucralose treatment on water loss rates in susceptible and resistant German cockroaches	September 30, 2022	Completed
	4.3 – Investigate the effects of sucralose treatment on gut microbiome composition and abundance in susceptible and resistant German cockroaches	December 31, 2022	Started
5 – Evaluate sucralose-boric acid bait against insecticide resistant German cockroaches	5.1 – Evaluate the performance of sucralose-boric acid mixture in bait formulation against insecticide-resistant strains of German cockroaches under simulated conditions	May 31, 2023	

Acknowledgement