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

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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′-TCGTCGGCAGCGTCAGATGTGTATAACCTACGGGNGGCWGCAG-3′ (forward) and 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-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

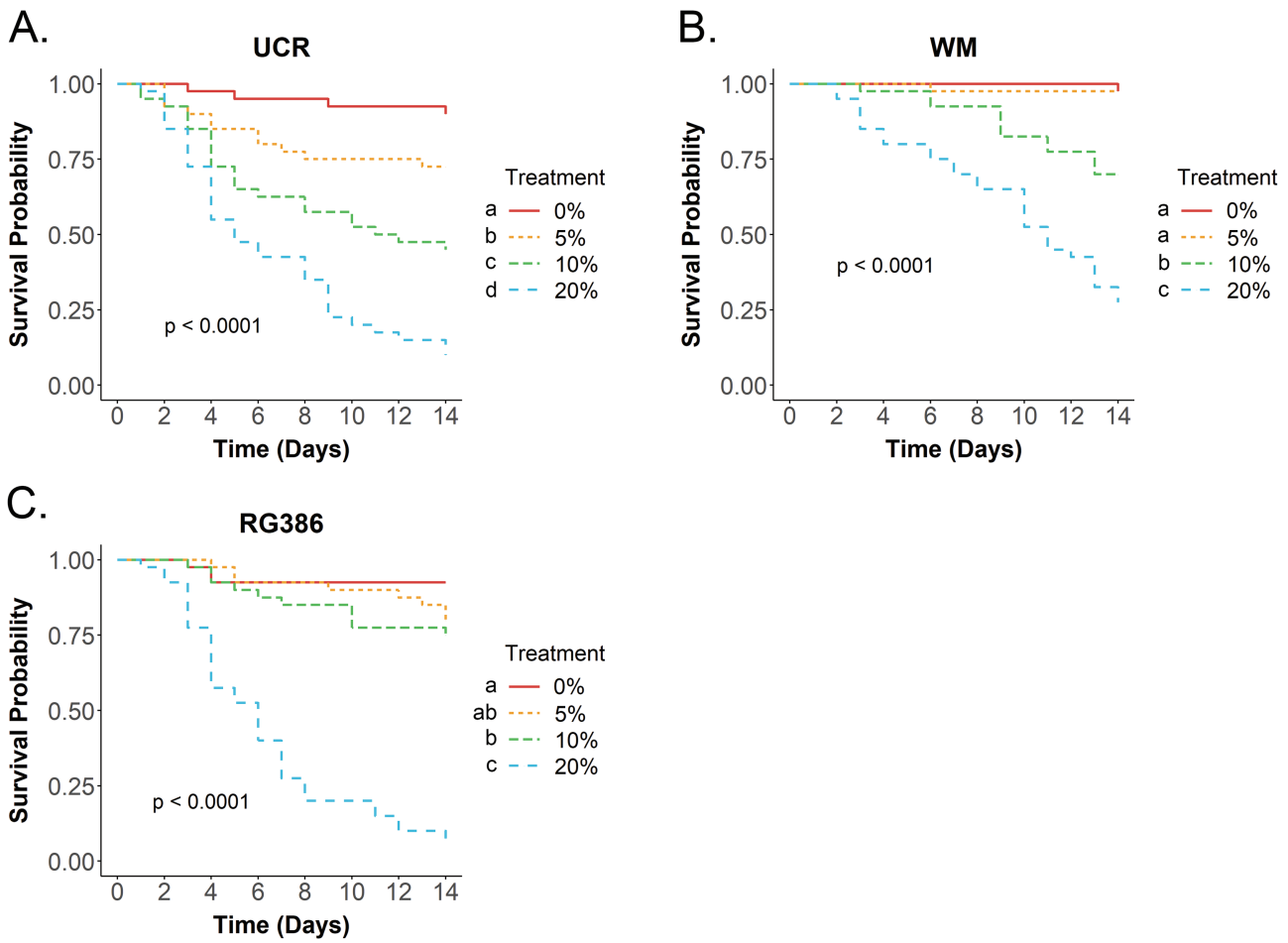

Fig. 1. Survivorship of A) UCR, B) WM, and C) RG386 strains exposed to 20%, 10%, 5%, or 0% (distilled water) sucralose solutions. Different letters by the figure legend denotes significant differences between treatments (Log-rank test; $\alpha = 0.05$).

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

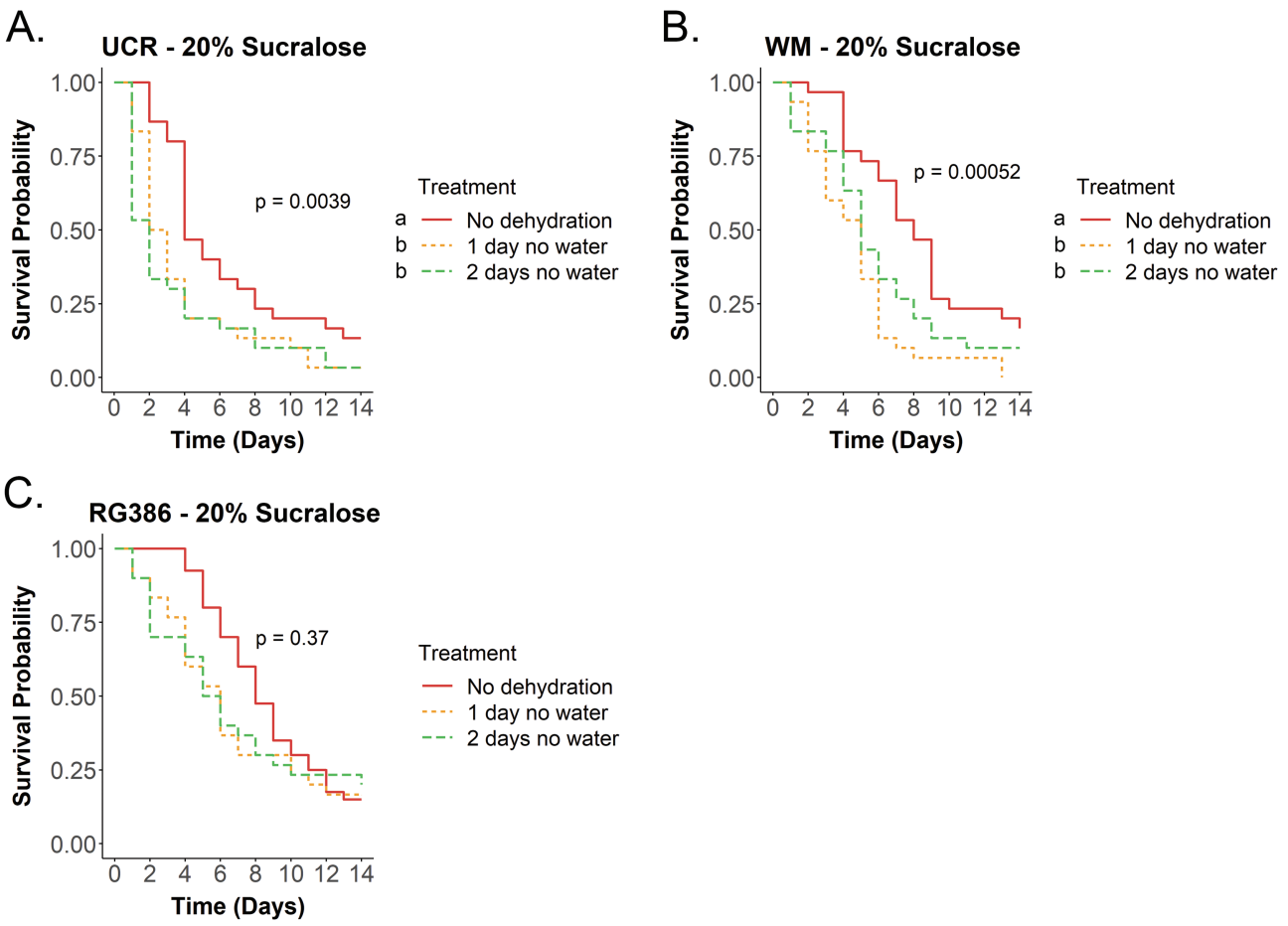

Fig. 2. Survivorship of A) UCR, B) WM, and C) RG386 strains treated with 20% sucralose solutions after 2, 1, or 0 days without water. Different letters by the figure legend denote significant difference, and the *P*-value represents an overall difference between all treatments (Log-rank test; $\alpha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

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

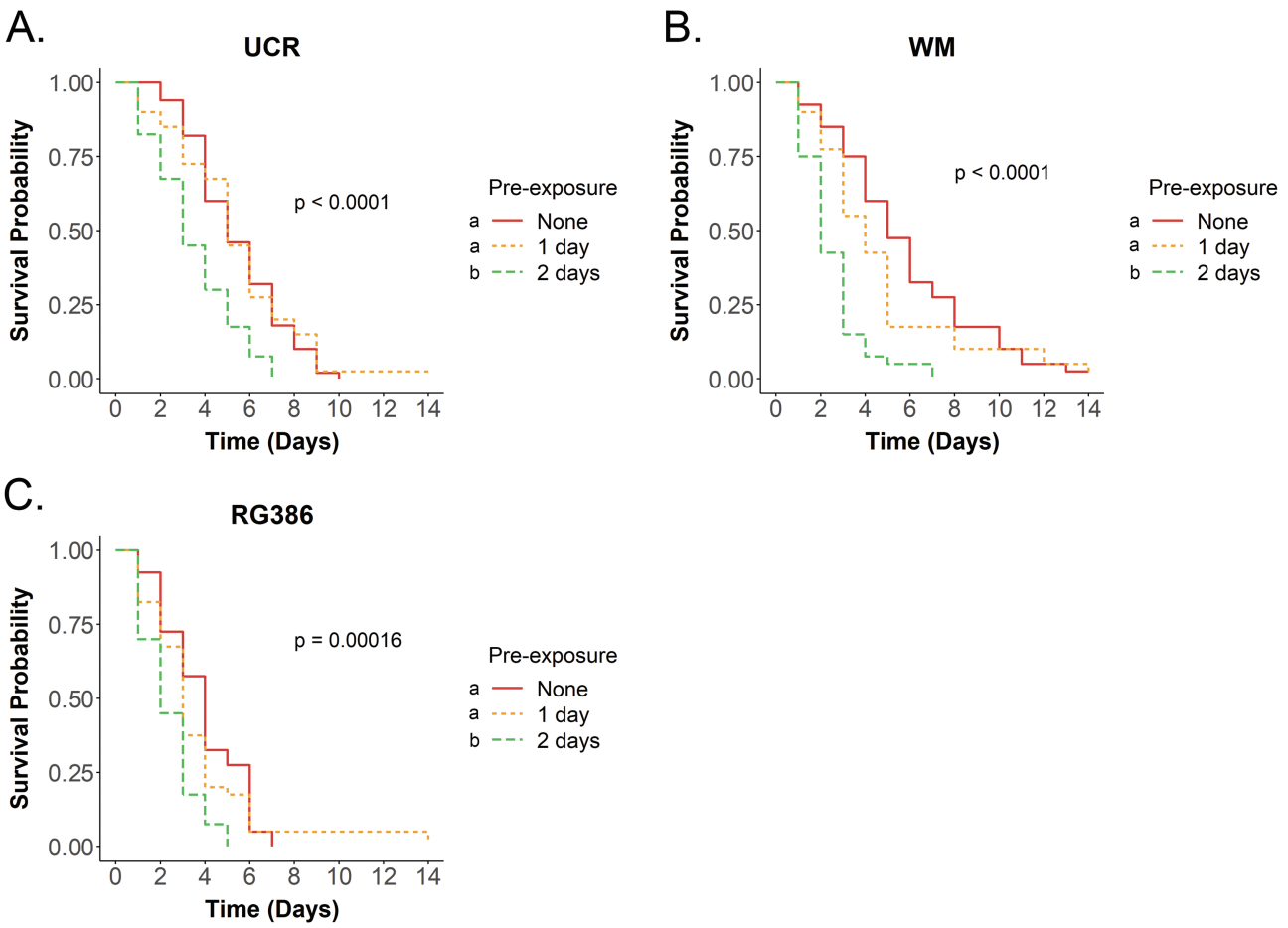

Fig. 3. Survivorship of A) UCR, B) WM, and C) RG386 strains without water after 2, 1, or 0 days exposure to 20% sucralose solution. Different letters by the figure legend denote significant differences, and the *P*-value represents an overall difference between all treatments (Log-rank test; $\alpha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

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

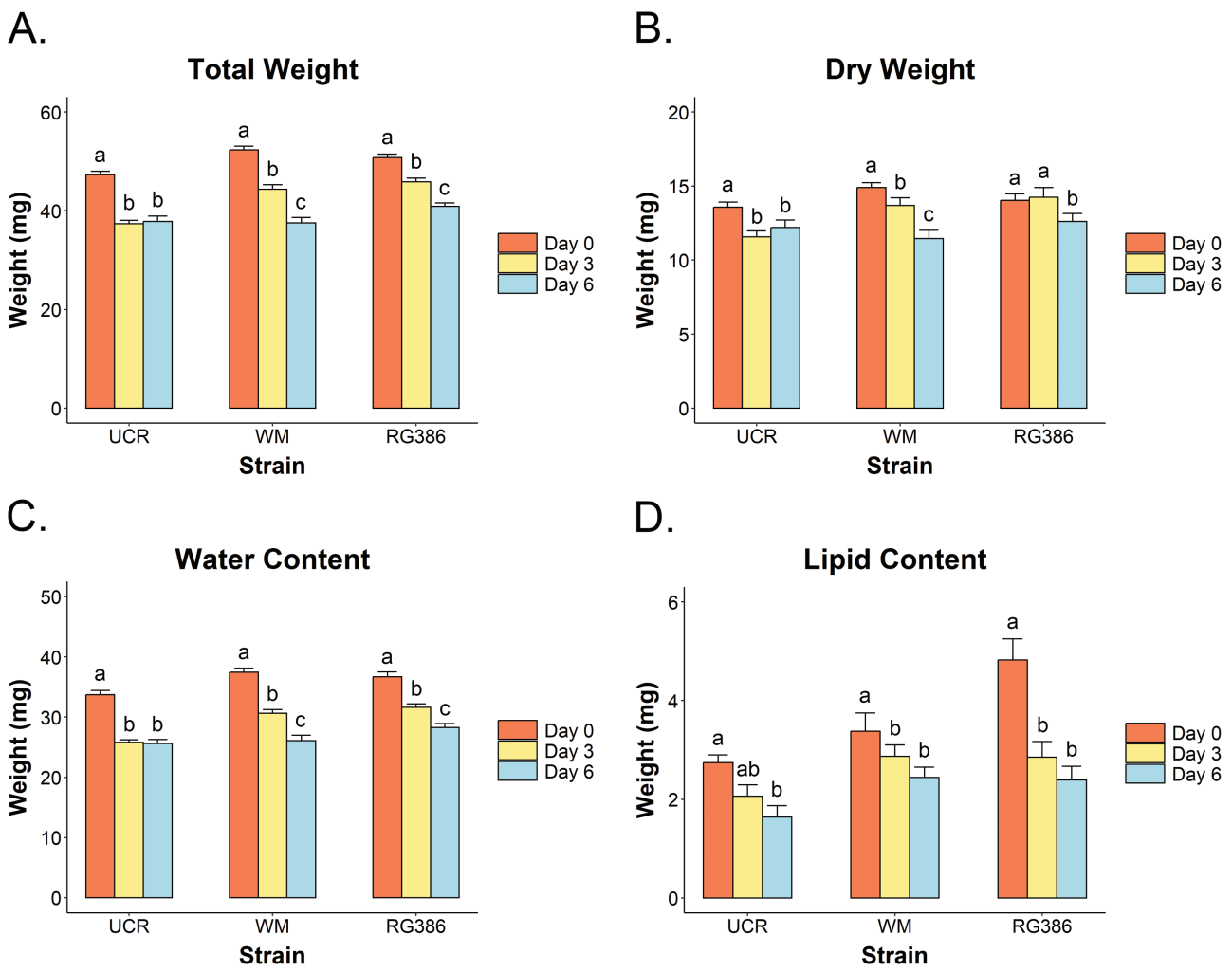

Fig. 4. A) Total weight, B) dry weight, C) water content, and D) lipid content of UCR, WM, and RG386 strains after 0, 3, or 6 days exposure to 20% sucralose solution. Different letters indicate significant difference between days (Wilcoxon rank sum test; $\alpha = 0.05$).

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; $\alpha = 0.05$).

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.

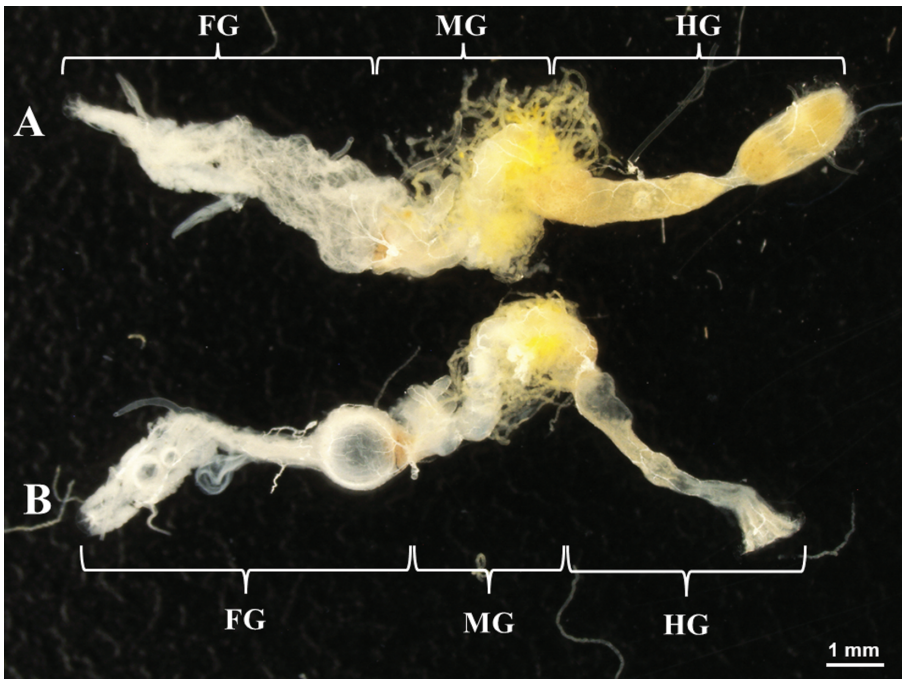

Fig. 5. Alimentary tracts of German cockroaches provided A) sterile water for 3 days and B) 20% sucralose solution for 3 days. FG—foregut; MG—midgut; HG—hindgut.

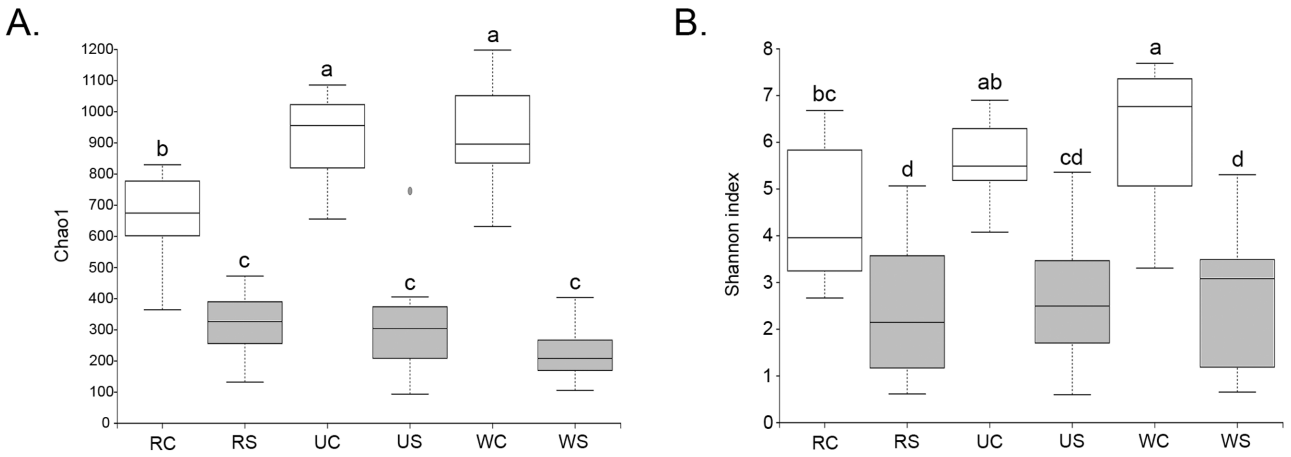

Fig. 6. Boxplots of richness, A) Chao1 and alpha diversity, (B) Shannon, indices. RC and RS are RG386 untreated and treated, respectively. UC and US are UCR untreated and treated, respectively. WC and WS are WM untreated and treated, respectively. Treated groups are shaded and untreated groups are unshaded. 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

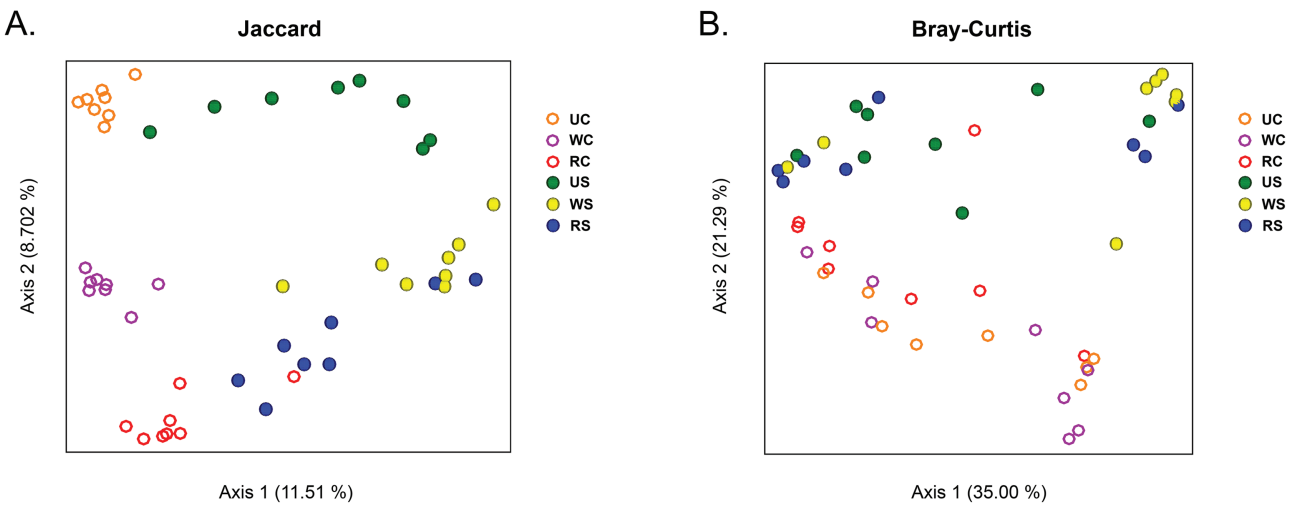

Fig. 7. Principal coordinate analysis plots of beta-diversity metrics A) Jaccard and B) Bray–Curtis. UC and US are UCR untreated and treated, respectively. WC and WS are WM untreated and treated, respectively. RC and RS are RG386 untreated and treated, respectively. Untreated groups are represented by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

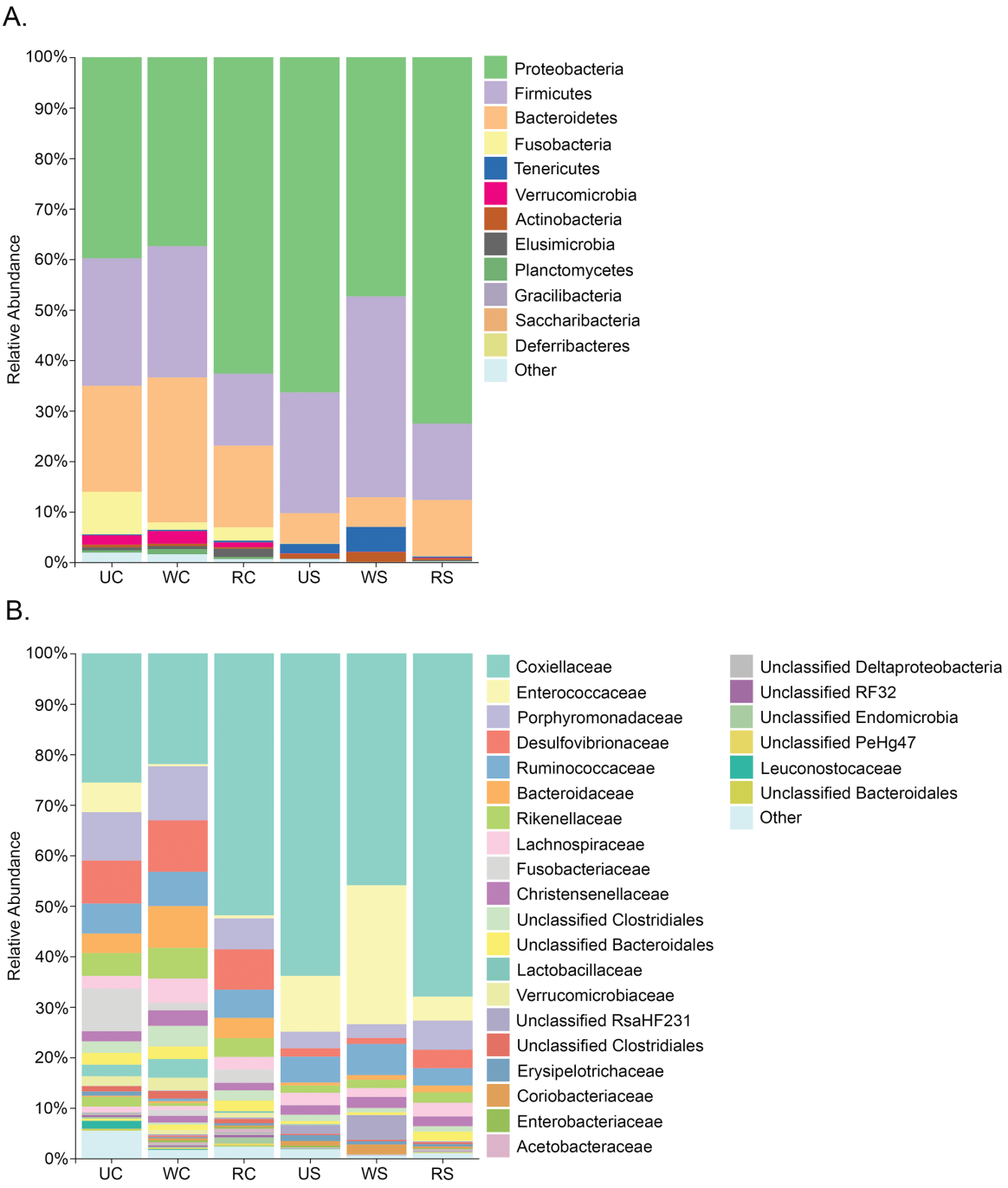

Fig. 8. Relative abundance of bacterial taxa at the A) phylum and B) family level. Taxa detected at <1% relative abundance are grouped in Other. UC and US are UCR untreated and treated, respectively. WC and WS are WM untreated and treated, respectively.

(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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