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# Glucose- and disaccharide-containing baits impede secondary mortality in glucose-averse German cockroaches

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Glucose aversion in the German cockroach, *Blattella germanica* (L.), results in behavioral resistance to insecticidal baits. Glucose-averse (GA) cockroaches reject foods containing glucose, even in relatively low concentrations, which protects the cockroaches from ingesting lethal amounts of toxic baits. Horizontal transfer of baits and the resulting secondary mortality have been documented in German cockroaches, including in insecticide resistant strains. However, the effects of the GA trait on secondary mortality have not been investigated. We hypothesized that ingestion of insecticide baits that contain glucose or glucose-containing disaccharides would result in behaviorally relevant glucose levels in the feces, possibly deterring coprophagy by GA nymphs. We fed adult female cockroaches hydramethylnon baits rich in either glucose, fructose, sucrose, or maltose and compared secondary mortality of GA and wild-type (WT) nymphs via coprophagy. When adult females were fed baits containing glucose, sucrose, or maltose and their feces offered to nymphs, secondary mortality was significantly lower in GA nymphs than in WT nymphs. However, survival of GA and WT nymphs was similar on feces generated by adult females fed fructose bait. Analysis of feces indicated that disaccharides in baits were hydrolyzed into glucose, some of which was excreted in the feces of females that ingested the bait. Based on these results, we caution that baits containing glucose or glucose-containing oligosaccharides may impede cockroach interventions; while GA adults and large nymphs avoid ingesting such baits, first instars reject the glucose-containing feces of any WT cockroaches that consumed the bait.

*Key words:* cockroach, insecticide resistance, behavioral resistance, secondary mortality, bait

## Introduction

Resistance to insecticides occurs widely across many insect taxa. Pest populations may evolve mechanisms to detoxify insecticides, reduce their cuticular penetration, sequester them away from target tissues, reduce their affinity to receptors with target-site mutations, or behaviorally avoid formulations that contain certain insecticides (Georghiou 1972, Hemingway et al. 2002, Zhu et al. 2016, Balabanidou et al. 2018). When a particular active ingredient or mode of action is utilized frequently and the insect has a short generation time and high fecundity, the risk of resistance evolution is heightened (Georghiou et al. 1983, Sparks and Nauen 2015).

Populations of the German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae), have evolved multiple resistance mechanisms to a wide array of insecticides with multiple modes of action (Scott et al. 1990, Siegfried and Scott 1992, Scott and Dong 1994, Scharf and Gondhalekar 2021). Several unique features of the German cockroach favor resistance evolution. Populations are limited to indoor environments, and so are relatively isolated with limited gene flow between populations. Being flightless also contributes to limiting gene flow while favoring inbreeding. Thus, as resistance mechanisms appear in a population, there is little opportunity for their dilution by susceptible alleles from nearby populations.

German cockroach populations have developed a unique behavioral resistance that reduces the effectiveness of insecticides across multiple classes delivered in bait formulations. German cockroach infestations are often treated with palatable baits (Appel and Rust 2021), which are ideal for crack-and-crevice applications that limit exposure of nontarget organisms, namely people and pets, to pesticides (DeVries et al. 2019). Baits rely on ingestion, which requires that they contain various phagostimulants, often sugars. Research

on cockroach food preferences indicated that glucose, a ubiquitous sugar in residential and food-processing settings, is a highly effective phagostimulant (Tsuji 1965, Gore and Schal 2004). Therefore, glucose frequently features in bait matrices, either as the pure monosaccharide, as a component of more complex oligosaccharides, or as a component of plant extracts such as corn syrup or molasses. However, heavy use of glucose-containing baits led to some cockroach populations evolving a heritable distaste of glucose (Silverman and Bieman 1993). Normally, aversion to such a common nutrient in the environment would be maladaptive. However, when glucose is coupled with an insecticide the inverse becomes true – glucose-averse (GA) cockroaches are uniquely protected from insecticidal baits.

Since its discovery, glucose aversion has been found in cockroach populations worldwide (Silverman and Bieman 1993, Wada-Katsumata et al. 2013). It is a partially dominant autosomal trait (Silverman and Bieman 1993); once it appears in a population it readily spreads with continued selection pressure, with GA cockroaches displacing the wild-type (WT) cockroaches. Physiologically, glucose aversion is a taste polymorphism based on changes in gustatory neurons within sensilla in the antennae and mouthparts, especially the paraglossae (Wada-Katsumata et al. 2011). In WT cockroaches, glucose stimulates appetitive (sweet) gustatory receptor neurons within these sensilla, driving appetitive behavior (Wada-Katsumata et al. 2013). In GA cockroaches, however, glucose also stimulates gustatory receptor neurons that normally respond to bitter compounds, driving aversive behavior (Wada-Katsumata et al. 2013). An important recent finding was the extension of glucose-aversion behavior to more complex di- and trisaccharides that contain glucose monomers; saliva hydrolyzes these sugars, releasing glucose, and thus stimulating glucose-aversion (Wada-Katsumata and Schal 2021, Wada-Katsumata et al. 2022).

Secondary mortality results from the horizontal transfer of an insecticide from foraging individuals to more sedentary individuals within aggregations. The German cockroach is a gregarious insect that rests in aggregations within harborage spaces, where insects remain when not foraging for food. The small size of early instars of the German cockroach limits the extent and speed of foraging and increases susceptibility to desiccation; they therefore remain close to the harborage while more robust adults and older nymphs travel farther for food (Cloarec and Rivault 1991). Thus, coprophagy is more prominent in first instars than in all other stages; feces are teeming with both the nutrients and microbial inocula they need to thrive (Kopanic et al. 2001, Carrasco et al. 2014). Normally, the sedentary nature of first instars would minimize their exposure to toxic baits. However, sufficiently slow-acting insecticides allow foraging adults and large nymphs to return to the harborage and defecate; these feces may contain partially digested bait and toxicant, which will then be consumed by first instars (Kopanic and Schal 1997, Durier and Rivault 2000). Although the contribution of secondary mortality to overall cockroach population control has not been fully quantified, it is thought that secondary mortality keeps early nymphs from evading pest control efforts. Even if nymphs are exposed directly to baits, mortality can be hastened under laboratory conditions by the presence of feces from intoxicated adults (Buczkowski et al. 2001).

Previous studies have shown the potential for insecticide resistance in adult cockroaches to affect secondary mortality (Ko et al. 2016). However, secondary mortality has not been investigated in the context of behavioral resistance, specifically glucose aversion. We hypothesized that some undigested or partially digested dietary sugar might pass into the feces, and thus we predicted that glucose-containing baits would result in lower secondary mortality in GA nymphs than fructose-containing baits. Moreover, we posited that more complex sugars (e.g., disaccharides) might be hydrolyzed by salivary and digestive enzymes and pass into feces as sugar monomers. If so, maltose- and sucrose-containing baits would release glucose, and if glucose appears in feces in behaviorally relevant concentrations, then we would expect differential secondary mortality in WT and GA cockroaches. We used four otherwise identical baits containing different mono- or disaccharides; we incorporated into each bait the same amount of hydramethylnon, a slow-acting insecticide. After feeding these baits to adult WT females, which defecated and then died, we removed all the bait, and concurrently exposed WT and GA first instars to the feces in the same cages.

## Materials and Methods

### Insects

Two strains of cockroaches were used in this study. The Orlando Normal strain, a glucose-accepting strain with no known prior exposure to insecticides and no insecticide resistance, was collected in 1947 in Florida and maintained in the laboratory. This strain was considered the WT *B. germanica* in this study. For secondary mortality experiments, we used first instars of a naturally-occurring orange-body variant (Ross and Cochran 1962) of the Orlando Normal strain. T164 is a GA strain collected in a Florida apartment in 1989 and maintained in the laboratory with regular selection using a bait containing 11.8% glucose and 2% hydramethylnon. Both strains are fully susceptible to hydramethylnon, which we used in this study. All insects were reared at 27 ± 1°C (range), 35–60% RH (range), and a 12:12 h L:D cycle. Colony insects had ad libitum access to water and rodent diet (Purina 5001 Rodent Diet, PMI Nutrition International, St. Louis, MO).

### Baits

Baits were modified from the diets reported in McPherson et al. (2021). Ingredients for 25 g of bait are listed in Table 1. The protein-to-carbohydrate ratio for the baits was 1:3, known to be appropriate and phagostimulatory to all life stages of *B. germanica* (Jones and Raubenheimer 2001, McPherson et al. 2021), with the carbohydrate portion being provided by one of four sugars: fructose, glucose, maltose (two glucose monomers), or sucrose (fructose and glucose monomers).

The Vanderzant vitamins that were included in previous diets were replaced with an equal volume of cellulose due to the presence of glucose in the vitamins. Technical grade hydramethylnon (Bayer Corporation, Research Triangle Park, NC), 2% by dry mass, was dissolved along with the cholesterol and linoleic acid in 40 ml of chloroform before combining with the cellulose and casein. After evaporating the chloroform, all other ingredients except agar and water were mixed in. Agar was dissolved in 100 ml water, brought to a short boil in a microwave, cooled to 60°C, and then combined with the other ingredients. The mixture was poured in a thin layer into 90 × 15 mm Petri dishes, lyophilized, and stored at –20°C.

### Primary Mortality of Donor Wild-type Adult Females: Generating the Feces

Adult Orlando Normal females, 2–4 days post eclosion, were starved for 24 h, cold-anesthetized, and placed into cylindrical jars (10 cm ID × 10 cm high, walls greased with petroleum jelly to prevent escape), 20 females per jar. We provisioned each jar with water in a 1.5 ml cotton-stoppered centrifuge tube and bait placed into a vial lid. The lid was anchored in the center of the jar with a small square of parafilm to minimize bait dispersal. Females were assessed daily for mortality;

**Table 1.** Ingredients in 25 g of bait

| Ingredient (unit) | Source,[a] Catalog # | CAS #[b] | Purity[b] | Amount | % |
|---|---|---|---|---|---|
| Protein | | | | 3.76 | 15.02 |
| Casein (g) | C7078 | 9000-71-9 | 13.5–15.0% nitrogen | 1.88 | 7.51 |
| Peptone (g) | 83059 | 100209-45-8 | ≥9.5% nitrogen | 0.94 | 3.76 |
| Albumin (g) | A5503 | 9006-59-1 | ≥98% | 0.94 | 3.76 |
| Sugar (g)[c] | below | | | 11.25 | 44.95 |
| Fructose | F9048 | 57-48-7 | ≥98.5% | | |
| Glucose | G7021 | 50-99-7 | ≥99.5% | | |
| Maltose | M5885 | 6363-53-7 | ≥99% | | |
| Sucrose | S9378 | 57-50-1 | ≥99.5% | | |
| α-Cellulose (g) | C8002 | 9004-34-6 | NA | 6.38 | 25.49 |
| Agar (g) | A1296 | 9002-18-0 | NA | 2.25 | 8.99 |
| Cholesterol (g) | C8667 | 57-88-5 | ≥99% | 0.13 | 0.52 |
| Linoleic acid (ml) | L1012 | 60-33-3 | ≥99% | 0.13 | 0.52 |
| Hydramethylnon (g) | Bayer | 67485-29-4 | 95% | 0.5 | 2.00 |
| Wesson salt mix (g) | W1374 | NA | NA | 0.63 | 2.52 |

[a]Sigma-Aldrich, unless otherwise specified.
[b]NA, Not applicable or Not available.
[c]Each bait contained one of four sugars: fructose, glucose, maltose, or sucrose.

we also removed dead insects and changed the water simultaneously. We considered insects that exhibited movement but could not right themselves within 30 s as moribund but left them in the jar until they were dead (no movement) to maximize fecal collection. However, for analysis of mortality in adult females, we considered moribund insects as dead. After 3–4 days, when ≥90% of the females were dead or moribund, all females and bait were removed from the jar. Each jar was carefully examined on a white background to ensure that there were no bait fragments in the jar. Bait fragments were readily apparent due to the bright yellow color of hydramethylnon. Jars with fecal material were used for secondary mortality within 1 day of removing the primary mortality (donor) females. We used 5 replicate jars (100 total females) for each of the four baits.

## Secondary Mortality of Recipient Wild-type and Glucose-averse Nymphs

Secondary mortality via coprophagy was assessed with first instars within 1 day of hatching. Nymphs hatched in cages provisioned with the same rodent diet used for rearing. Twenty orange-body nymphs from the WT Orlando normal strain and 20 nymphs from the GA T164 strain were gently transferred to the jars previously used for primary mortality. As with the adult females, they received a cotton-stoppered 1.5 ml water tube that was changed daily. For the first 24 h, the nymphs had access to only water and fecal excretions from the primary mortality females. After this initial 24 h period, the jars were provisioned with rodent diet and their mortality was assessed daily. We used 5 replicate jars (100 of each strain) for each of the four baits.

## Feces Collection for Glucose Analysis

We collected feces from separate groups of adult females from the experimental females to assess its glucose content. For feces collection, 20 females (2–4 days old) were starved for 24 h and placed in a jar with one type of bait, as in the primary mortality experiments, but with an added harborage made from a single egg crate cell. After 2 h feeding on the bait, the harborage and females were transferred to an identically sized feces collection jar, which contained only a water tube.

After 20–24 h, females were transferred as before, using the harborage, back to the feeding jar for another 2 h round of feeding. Water was provided ad libitum in both feeding and feces collection jars. The cycle of feeding and feces collection was repeated; females that became moribund were left in the collection jar but discarded after they died. On day 3 or 4 (for disaccharides and monosaccharides, respectively) all females in the feces collection jar were removed. Feces was removed from the jar, fully dried in a 50°C oven (2–5 days), weighed (1712 MP8, Sartorius, Goettingen, Germany), and stored at −30°C until analysis. For each sugar we used nine replicate jars to generate feces.

## Analysis of Feces and Baits for Glucose

Prior to analysis, the mass of individual fecal pellets was determined for each sugar by weighing 3 groups of 10 fecal pellets, averaging the masses, and dividing by 10. We divided fecal materials into 10 mg aliquots to prepare for analysis. Feces remained separated by bait sugar and replicate. Simultaneously, 10 mg of the corresponding bait was prepared for every replicate. About 300 µl of water (HPLC-grade, as is water used in other steps) and 300 µl of chloroform were added to the feces to remove lipids. We then homogenized the samples with glass beads in a bead beater (FastPrep24 5G, MP Biomedicals, Irvine, CA). After centrifugation for 3 min at 12,000 rpm, the top aqueous layer was transferred into a clean vial. The chloroform was removed and discarded from the precipitated solids. The addition of 300 µl chloroform and 300 µl water, along with the subsequent steps, was repeated twice more. Each time the aqueous layer was removed, it was placed into the same tube, for a total of approximately 900 µl of aqueous fecal extract.

The aqueous extract was filtered in increments of 200 µl through a 10 kDa filter (10 KDa NMWCO, UFC5010, Amicon Ultra-0.5 Centrifugal Filter Unit, Millipore-Sigma, St. Louis, MO) by centrifugation at 1,200 rpm to remove proteins and large particles. After all the extract was filtered, the empty vial was rinsed with 400 µl of clean water, which was then used to elute the filter. The filtered extract and final elution were then dried completely in a Jouan Evaporator (Jouan RCT 60, Thermo Scientific, Waltham, MA).

All samples were then analyzed with a colorimetric kit (K606 from BioVision/Abcam, Cambridge, UK). Dried samples were first resuspended in 40 µl of assay buffer from the kit. Due to the known high glucose concentration of the glucose bait, we took 2 µl out of the initial 40 µl from the sample and added it to 414 µl of buffer, for an estimated concentration of 2–3 nmol/µl. All other

samples were analyzed directly from the initial 40 µl dilution. We analyzed all samples ($N$ = 9 bait samples per bait type and 9 fecal samples per bait type) according to kit instructions in amounts ranging from 1 to 10 µl per well. Fecal samples were run with corresponding background wells due to pigment that remained despite our initial processing. Samples were read in a PowerWave 200 scanning microplate spectrophotometer (Bio-Tek, Winooski, VT) at 570 nm.

### Statistics

All survival data were analyzed using R (R Core Team 2020). The packages 'survminer' (Kassambara et al. 2021) and 'survival' (Therneau and Grambsch 2000, Therneau 2021) were used to analyze both primary and secondary mortality data. Adult female survival on baits containing four different sugars was compared with a log-rank test ($\alpha$ = 0.05) with a Benjamini–Hochberg correction for multiple comparisons (Benjamini and Hochberg 1995, Benjamini et al. 2009) applied to the $P$-values with a false discovery rate of 0.05. Survival of WT versus GA nymphs (secondary mortality) was analyzed for each sugar using a log-rank test; no multiple comparison correction was necessary as only two strains were compared. Glucose content in baits and feces was analyzed in Prism (GraphPad Software, San Diego, CA) with an ANOVA followed by Tukey's HSD test ($\alpha$ = 0.05).

## Results

### Primary Mortality of Adult Females

Survival of adult females was significantly different in all pairwise comparisons of baits containing different sugars (Table 2). Different sugars resulted in different speed of mortality of adult females, presumably representing their palatability, and thus the amount of bait that females ingested. From most to least rapid mortality, the sugars were: maltose, sucrose, glucose, and fructose (Fig. 1). Thus, the baits containing monosaccharides acted more slowly than baits containing disaccharides, and the mono- and disaccharides were more similar to their own group than the other group (i.e., baits containing sucrose or maltose were more similar to each other than to baits containing either glucose or fructose, and vice-versa).

### Secondary Mortality of Nymphs

Secondary mortality was significantly different between the two strains on feces generated from females that fed on glucose, sucrose, and maltose, but not fructose (Fig. 2). The difference between the two strains was most pronounced when they had access to feces produced from maltose-containing bait, followed by sucrose and glucose baits.

Within each strain, the effect of each feces type roughly followed the pattern set by the respective sugar in primary mortality of adult females. However, whereas survivorship of females fed each sugar differed significantly from females fed every other sugar, with secondary mortality this was not the case (Table 3). The WT nymphs showed no significant difference in survival on feces from maltose and sucrose baits. The GA nymphs showed no significant difference in survival on adult feces generated from eating glucose and maltose baits.

### Glucose in Baits and Adult Feces

As expected, within our baits only the glucose bait tested positive for the presence of glucose (Fig. 3A). This confirmed that there was no glucose contamination in our baits, and that the disaccharide sugars had not degraded during storage or during our analysis procedure. The ANOVA for comparing fecal sugars was significant ($F_{(3, 32)} = 28.86$, $P < 0.01$). Post-hoc analysis with Tukey's test revealed that the fructose bait yielded significantly less glucose in feces than the other bait (Fig. 3B). The presence of glucose in the fructose-only feces was an unexpected result. German cockroaches are capable of some, though low, cellulose digestion. However, more cellulose is digested by cockroaches fed dilute carbohydrate foods (Jones and Raubenheimer 2001), whereas our diets contained high amounts of digestible carbohydrates. Some glucose in feces could also be a normal state of the cockroach representing background metabolic waste. However, it is also possible that dietary fructose was converted to glucose. In mammals, dietary fructose is cleared by the intestine, with extensive fructose-derived glucose found in the blood (Jang et al. 2018), and likely some in feces. Unfortunately, isotope

**Table 2.** Statistical comparisons of survival of wild-type adult *B. germanica* females (primary mortality)

| Sugars[a] | $\chi^2$ | $P$-value[b] |
|---|---|---|
| Glucose > Fructose | 4.1 | 0.0425 |
| Glucose < Maltose | 24.0 | <0.0010 |
| Glucose < Sucrose | 9.8 | 0.0027 |
| Fructose < Maltose | 41.4 | <0.0010 |
| Fructose < Sucrose | 23.8 | <0.0010 |
| Maltose > Sucrose | 4.2 | 0.0425 |

[a]Paired comparisons of sugars are denoted by which sugar resulted in significantly faster mortality.
[b]Comparisons are pairwise (df = 1). $P$-values underwent a Benjamini–Hochberg correction for multiple comparisons with a false discovery rate of 0.05. $N$ = 100 for each type of sugar.

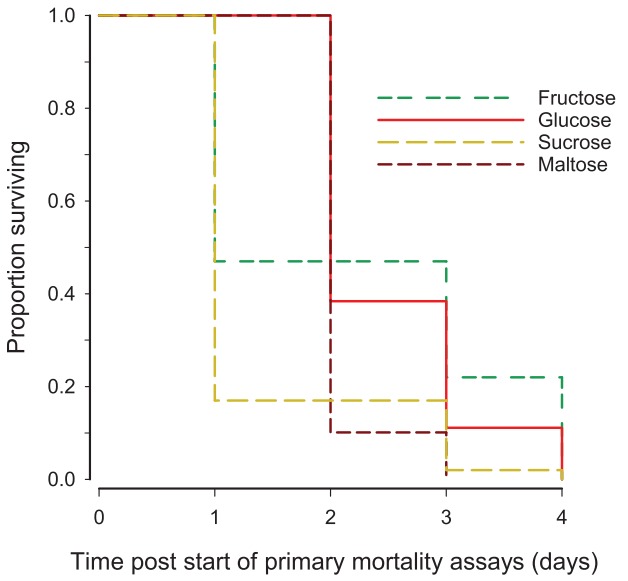


**Fig. 1.** Survival of wild-type *B. germanica* females fed 2% hydramethylnon bait with different types of sugars (primary-mortality assays). Different sugars are denoted by different colors and line types. $N$ = 100 total females in five replicates for each type of sugar used. These data were analyzed with a log-rank test ($\alpha$ = 0.05) with a Benjamini–Hochberg correction for multiple comparisons applied to the $P$-values with a false discovery rate of 0.05. The results of the statistical analysis are shown in Table 2.

tracing with mass spectrometry to track the fate of dietary fructose has not been conducted in insects.

Nevertheless, due to the low concentration in the diet, significantly lower fecal concentration than in the other treatments, and apparent behavioral unimportance of this trace amount of glucose, we proceeded with our analyses. Although the mean glucose contents in feces from glucose, maltose, and sucrose baits were not significantly different from each other, baits containing glucose and maltose yielded more glucose in feces than baits containing sucrose, consistent with the molecular structure of each sugar type. Individual fecal pellets from all four baits had an average mass of 0.2 mg, translating to an approximate mass of 12.0, 71.0, 86.7, and 87.8 ng of glucose per fecal pellet for fructose, sucrose, maltose, and glucose, respectively.

## Discussion

### Bait Palatability

German cockroach females fed otherwise identical insecticide baits that contained different sugars experienced significantly different survival. Maltose resulted in the fastest time-course of mortality, followed by sucrose, glucose, and fructose. Assuming that the rate of mortality depends mainly on the amount of bait consumed, and hence the amount of active ingredient ingested, these results suggest that, of the sugars tested, maltose was the most preferred by *B. germanica*, and fructose was least preferred. These results are consistent with earlier studies that examined the palatability of various sugars dissolved in water (Tsuji 1965, Gore and Schal 2004), although we used different insecticides, bait formulations, and sugar concentrations. Whereas we included the same mass percentage (45%) of all sugars, Gore and Schal (2004) evaluated each sugar at a concentration of 0.1 M. Thus, we used much higher sugar concentrations in our solid baits, with monosaccharides represented at 2.5 M and disaccharides at 1.25 M. We also suspect that sugars dissolved in water or formulated in gels may be more phagostimulatory than in lyophilized formulations. Nevertheless, despite these differences, the disaccharide baits were still significantly more effective in our assays than those containing monosaccharides, presumably due to their superior palatability. It is worth noting that these differences appear to be robust despite other differences in methodology, including no-choice assays in our assays versus choice assays in Gore and Schal (2004) and our use of adult females rather than the adult males utilized in the earlier work, or the mixed cohort of adults and large nymphs in Tsuji (1965).

### Factors Influencing Secondary Mortality

First instars of the GA strain with access to the feces of females that were fed insecticide baits had significantly higher survival than the

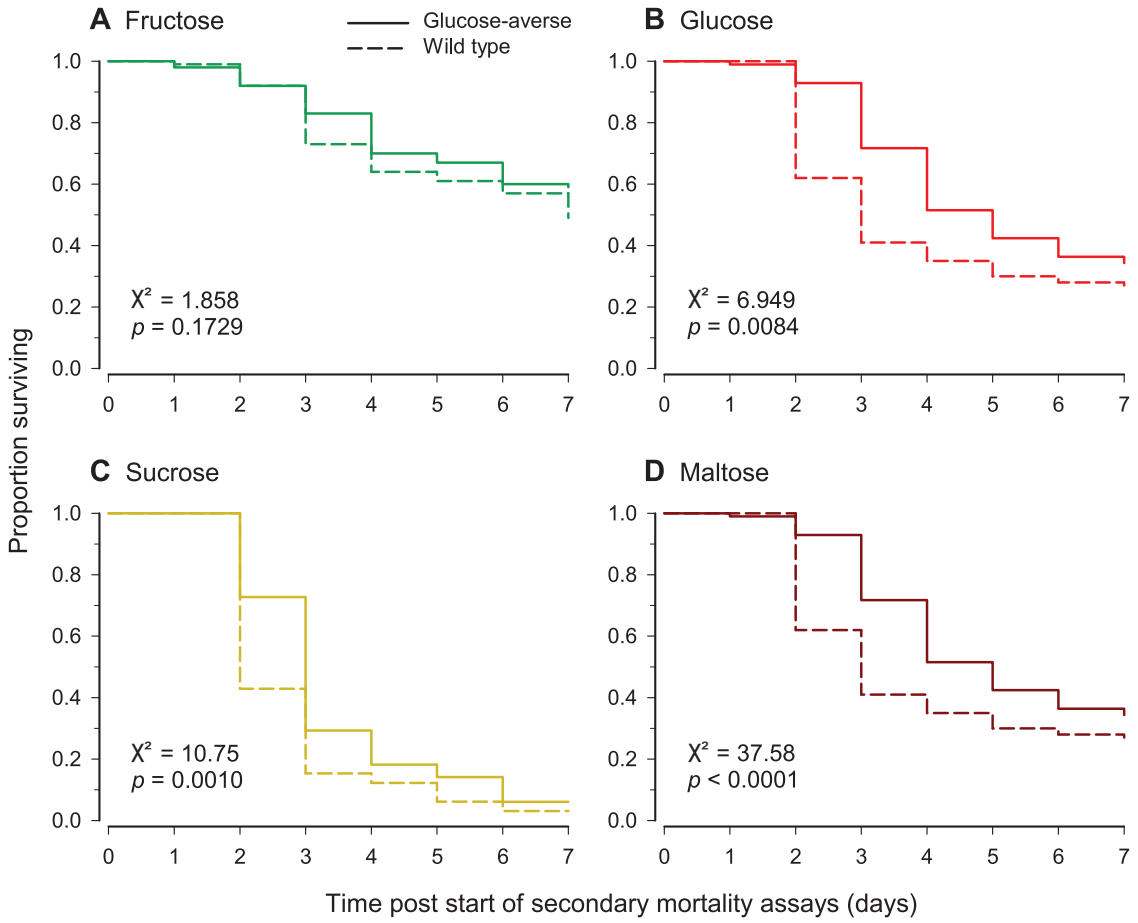


**Fig. 2.** Secondary mortality of wild-type and glucose-averse *B. germanica* nymphs that were offered the feces of wild-type *B. germanica* females fed 2% hydramethylnon bait with different types of sugars, including fructose (A), glucose (B), sucrose (C), or maltose (D). Log-rank tests ($\alpha = 0.05$) were used to compare the two strains, and $\chi^2$ and *P*-values are displayed for each pairwise comparison (df = 1). The two strains are denoted by different line types. These data were also analyzed with a log-rank test ($\alpha = 0.05$) with a Benjamini–Hochberg correction for multiple comparisons applied to the *P*-values with a false discovery rate of 0.05; these results are shown in Table 3.

**Table 3.** Statistical comparisons of survival of *B. germanica* nymphs (secondary mortality)

| Sugars[a] | Strain | $\chi^2$ | *P*-value[b] |
|---|---|---|---|
| Glucose > Fructose | Wild-type | 17.8 | <0.001 |
| Glucose < Maltose | Wild-type | 17.8 | <0.001 |
| Glucose < Sucrose | Wild-type | 23.4 | <0.001 |
| Fructose < Maltose | Wild-type | 85.0 | <0.001 |
| Fructose < Sucrose | Wild-type | 93.4 | <0.001 |
| Maltose = Sucrose | Wild-type | 1.1 | 0.3000 |
| Glucose > Fructose | Glucose-averse | 11.5 | <0.001 |
| Glucose = Maltose | Glucose-averse | 2.7 | 0.1032 |
| Glucose < Sucrose | Glucose-averse | 39.2 | <0.001 |
| Fructose < Maltose | Glucose-averse | 22.6 | <0.001 |
| Fructose < Sucrose | Glucose-averse | 78.5 | <0.001 |
| Maltose < Sucrose | Glucose-averse | 19.2 | <0.001 |

[a]Paired comparisons of sugars are denoted by which sugar resulted in significantly faster mortality. If there was no significant difference, they are marked as equal.
[b]Comparisons are pairwise (df = 1). *P*-values underwent a Benjamini–Hochberg correction for multiple comparisons with a false discovery rate of 0.05. *N* = 100–102 for each type of sugar.

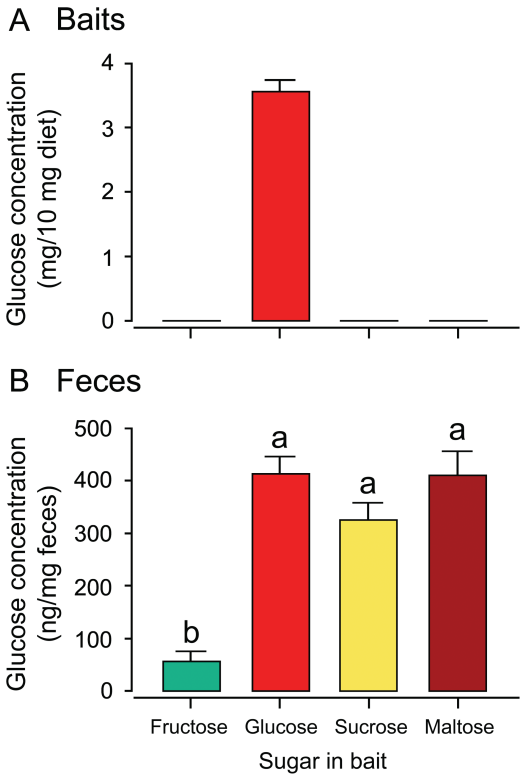


**Fig. 3.** Glucose concentrations in 2% hydramethylnon baits offered to *B. germanica* adult females (A) and in the feces they produced (B). *N* = 9 bait samples per bait type and 9 fecal samples per bait type.

WT strain when the bait contained glucose, sucrose, or maltose. On the other hand, both strains had similar survivorship when fed feces that originated from fructose bait. There are several inferences and potential implications of these results. First, dietary sugars remain partially undigested as they pass through the digestive system and thus intact sugars are found in the feces of cockroaches fed sugar baits. Our analytical results confirm this prediction, with glucose appearing not only in feces from the glucose-containing bait but in feces generated from disaccharide-containing baits as well. It is especially interesting that when primary mortality females consumed maltose, the most phagostimulatory of the four sugars, their feces resulted in the most dramatic difference in secondary mortality between GA and WT nymphs. The reason for this is likely that the hydrolysis of maltose releases two glucose monomers, whereas sucrose would release one glucose and one fructose molecule. Thus, feces produced from ingesting glucose baits and maltose baits resulted in the highest survival of GA nymphs relative to WT nymphs, feces from sucrose baits less so, and feces from fructose-containing baits resulted in no differences between GA and WT nymphs.

Primary mortality results seem to follow the same pattern as secondary mortality for different sugars. For example, the fructose-containing bait performed the worst with our primary mortality females, and feces from it proved the least effective on nymphs relative to feces generated from other dietary sugars. Less palatable baits result in less overall consumption, with the obvious consequence of less feces produced and therefore less insecticide deposited. However, all nymphs in the secondary mortality assays had access to an overabundance of fecal material, so secondary mortality would not have been improved by having more toxic feces. It may be that less palatable baits (e.g., containing fructose) generate feces that are themselves less palatable and thus consumed less by nymphs.

## Experimental Design and Potential Follow-up Questions

Our assessment of secondary mortality used an orange-body morph of WT cockroaches and GA nymphs with typical black-body coloration. This design controlled for potential differences between separate jars in the amount and quality of feces from primary-mortality (donor) females; the two strains were simultaneously exposed to identical conditions. However, a previous study found that WT black-body cockroaches exhibited delayed mortality compared to orange-body insects of the same WT strain when hydramethylnon was used as the active ingredient for secondary mortality (Ko et al. 2016). Nonetheless, differences between the color morphs of WT cockroaches were minor compared to the differences between the two strains found in our bioassays. Additionally, since both strains responded similarly to exposure to fructose-containing bait, it seems unlikely that the color morph played a significant role in bait susceptibility. If the orange-body trait conferred some disadvantage we would expect faster mortality of these insects than of black-body GA nymphs.

Our study focused on glucose aversion exclusively in the nymphs used for secondary mortality. Only WT females were used for primary mortality. What might occur if GA females were in the primary mortality position, serving as donors? The results with fructose bait would be expected to be largely the same as our results from WT females. In a no-choice setting, the GA females would of course consume much less glucose bait, and presumably less of the sucrose and maltose baits as well given the rapid hydrolysis of disaccharides via salivary digestion (Wada-Katsumata and Schal 2021). These GA females would then defecate much less active ingredient, seriously confounding not only primary mortality, but secondary mortality as well.

Although other routes for secondary mortality might operate in the field, our design assumed that coprophagy was the primary route of secondary mortality with hydramethylnon, and we removed dead adults from the jars before introducing nymphs. Thus, emetophagy, cannibalism, and necrophagy were prevented in these assays. Emetophagy, where nymphs consume

exudates from the mouths of dying primary-mortality insects, has been documented with the neuroactive insecticides fipronil and indoxacarb (Buczkowski and Schal 2001, Buczkowski et al. 2008). However, previous examination of exudates from the head and abdomen of insects fed hydramethylnon indicated that coprophagy was the primary mechanism of secondary mortality (Silverman et al. 1991). Mortality due to cannibalism of hydramethylnon-intoxicated insects has been documented with male German cockroaches consuming first instars (Gahlhoff Jr et al. 1999, Buczkowski et al. 2008). The inverse, where nymphs feed on live or dead adults, has not been examined. Both cannibalism and necrophagy are uncommon in German cockroaches with sufficient alternative foods (Appel et al. 2008), and size differences would likely preclude cannibalism by the smaller nymphs on adults. Nevertheless, we observed necrophagy of dead nymphs by live nymphs in our assays, even after rodent diet was added to the secondary mortality jar. As GA nymphs are deterred from eating feces that contains some glucose, perhaps other phagostimulants ingested during cannibalism or necrophagy might mask the aversive taste of glucose and thus contribute more to secondary mortality in GA insects than coprophagy.

Many factors must be considered in decisions regarding bait formulations, and bait performance of different strains is one of many considerations. If this were the sole metric considered then fructose, which performed most poorly, would be selected for a formulation because it performed the same in WT and GA insects. Yet maltose or sucrose is much more effective phagostimulants in WT cockroaches. Integrating both palatability and relatively high performance in the two strains suggests that sucrose might be the superior sugar; it performed similarly to maltose in WT nymphs but outperformed maltose in the GA strain. This is likely due to the presence of two different monosaccharides in the disaccharide – when sucrose is hydrolyzed into fructose and glucose, fructose might partially mask the bitter taste of glucose to GA nymphs, reminiscent of high-fructose corn syrup, which contains both glucose and fructose. Ultimately, bait formulations might need to include non-sugar phagostimulants, and prime candidates would be lipids that appear to function along with sugars as phagostimulants during courtship displays (Kugimiya et al. 2002, 2003; Wada-Katsumata et al. 2009).

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