Research Article

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Diet quality affects bait performance in German cockroaches (Dictyoptera: Blattellidae)

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Abstract

BACKGROUND: Bait formulations are widely used to control German cockroach (*Blattella germanica*) populations. To perform optimally, these formulations must compete favorably with non-toxic alternative foods present within the insect's habitat. We hypothesized that the nutritional history of cockroaches and their acceptance or avoidance of glucose would affect their food preference and thus bait efficacy. To test this hypothesis, we conducted a controlled laboratory experiment, first providing glucose-accepting and glucose-averse cockroaches nutritionally defined diets and then offering them identical diets containing the insecticide hydramethylnon as a bait proxy to evaluate the effect of diets of differing macronutrient composition on bait performance.

RESULTS: The interaction between diet composition and bait composition affected the survival of adult males as well as first-instar nymphs exposed to excretions produced by these males. Survival analyses indicated different responses of glucose-averse and glucose-accepting insects, but generally any combination of diet and bait that resulted in high diet intake and low bait intake reduced secondary kill.

CONCLUSIONS: This study represents a comprehensive examination of the effect of alternative foods on bait efficacy. We suggest that disparities between the nutritional quality of baits and the foods that are naturally available could profoundly impact the management of German cockroach infestations.

Keywords: *Blattella germanica*; hydramethylnon; coprophagy; secondary kill; diet; bait

1 INTRODUCTION

The German cockroach, *Blattella germanica*, is a widespread pest of human-built structures. Several proteins produced by this cockroach can trigger allergic and asthmatic episodes,[1–3] and *B. germanica* is a potential vector of pathogenic and antibiotic-resistant microorganisms.[4–7]

Bait formulations are currently the most effective method of controlling German cockroach populations.[8,9] These are toxic diets that contain an active ingredient (AI) incorporated within a food matrix, which generally contains a sugar as a phagostimulant. In contrast to other methods of control, such as broadcast sprays, baits offer a more targeted approach resulting in less AI required for control and less AI exposure to non-target organisms such as children and pets.[8–10] A number of effective, non-repellent active ingredients have been incorporated within baits.[8,9,11–15] Modern bait formulations can kill cockroaches through direct ingestion (primary kill), as well as through the uptake of translocated AI (secondary kill), whereby cockroaches, primarily first-instar cockroach nymphs, are adversely affected following ingestion of (coprophagy) or contact with excretions containing the toxicant.[12,15–19] Secondary kill through coprophagy is especially effective with slow-acting AIs, such as hydramethylnon.[12,13]

While bait formulations are effective, multiple cockroach populations have evolved a chemosensory-based behavioral resistance to baits; in response to selection with glucose-containing bait formulations, some cockroaches have evolved a taste aversion to glucose.[20] Although glucose is a common sugar and virtually universal phagostimulant, glucose-averse *B. germanica* reject bait formulations with glucose, surviving pest control efforts.[21,22]

In addition, for bait formulations to control German cockroach populations effectively, these toxic nutritive formulations must be preferred over non-toxic alternative foods, and previous work has emphasized the importance of sanitation and the removal of alternative food sources.[8–10,23–25] The nutrient composition of diets can affect acceptance in this omnivore,[26–30] as prolonged exposure to suboptimal diets can result in specific nutrient deficiencies and physiological stress,[31–34] increasing the efficacy of palatable baits that satisfy these deficiencies.[10] *B. germanica* self-select optimal diets based upon their nutritional needs, reaching diet mixtures of approximately 1:3 protein–carbohydrate ratio.[27–30] They also compensate for low dietary protein levels by elevating consumption, but extremely high dietary proteins can suppress food intake.[35]

We hypothesized that the efficacy of baits would be influenced by the composition of the food eaten, and we proposed

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that the mechanisms underlying these differences would be reflected in disparate bait consumption and lipid accumulation under different diet–bait combinations. We also hypothesized that glucose-averse cockroaches would reject diets containing glucose and thus suffer high mortality when subsequently exposed to palatable fructose-containing baits. To test these hypotheses, we exposed glucose-accepting and glucose-averse cockroaches to diets of varying protein–carbohydrate ratios, then introduced hydramethylnon-amended diets (hereafter termed 'baits') of similar or different macronutrient composition and recorded the mortality of adults that ingested baits and of nymphs secondarily exposed to the adult excretions.

2 EXPERIMENTAL METHODS

2.1 Stock insect colonies and rearing conditions

The strains of *B. germanica* used in these studies were Orlando normal and T164. Orlando normal is a glucose-accepting and insecticide-susceptible strain with no known prior exposure to insecticides; T164 glucose-averse cockroaches were collected from a Florida apartment in 1991.[21] Glucose aversion has been maintained in T164 by periodic laboratory selection with bait containing 11.8% glucose and 2% hydramethylnon. Neither strain has been reported to be resistant to hydramethylnon, the active ingredient used in this study. Colonies were maintained on Purina 5001 Rodent Diet (PMI Nutrition International, St Louis, MO) prior to the start of the study in laboratory rearing conditions of 25 ± 1 °C, 37 ± 5% RH and LD 12:12.

2.2 Characterization of susceptibility of strains to hydramethylnon

Hydramethylnon was serially diluted with acetone and 0.5 µL of the solution was applied to the ventral surface of an adult male cockroach, between the coxae of the fore and middle legs, with a repeating micropipette (Hamilton Company, Reno, NV). Thirty males were treated individually with each dose. Following treatment, cockroaches were maintained in three groups of ten in 10 cm diameter petri dishes (Fisher Scientific, Pittsburgh, PA) with rat chow and water. Cockroaches topically treated with hydramethylnon were monitored for mortality daily for 5 days. Insects that could not right themselves within 30 s when flipped were considered to be dead and were removed from the petri dish. Values for LD_{50} and LD_{90}, and their respective fiducial limits, were estimated by probit analysis in Polo Plus (LeOra Software Company, Petaluma, CA).[36]

2.3 Composition and preparation of diets and baits

Diets were modified from Raubenheimer and Jones,[28] and ingredients are listed in Table 1. The digestible carbohydrate was either glucose or fructose. All diets and baits contained a fixed amount of diluent (alpha-cellulose) and agar. Because *B. germanica* has the capacity to digest cellulose as a secondary mechanism of nutritional regulation on nutritionally dilute foods,[27] the amount of cellulose remained the same in all treatments, as in related studies (e.g. Jones and Raubenheimer[27]), to avoid confounding the results. To prepare the diets, all dry ingredients were blended with 150 mL of dH_2O. Separately, agar was heated in 150 mL dH_2O until boiling. The agar mixture was allowed to cool after boiling to avoid denaturing the protein while being continuously agitated to prevent congealing, before adding the other ingredients.

Table 1. Compositions of *B. germanica* diets and baits

Ingredients[a]	P:C 1:3[b]	P:C 3:1[b]
Casein	7.50	22.50
Peptone	3.75	11.25
Albumin	3.75	11.25
Carbohydrate	45.00	15.00
Cellulose	26.90	26.90
Agar	4.00	4.00
Vitamin mixture	0.81	0.81
Hydramethylnon	1.00	1.00
2-propanol	6 mL	6 mL
Oleic acid	1 mL	1 mL

[a] Ingredients comprising diets (no hydramethylnon) and baits (with hydramethylnon). The carbohydrate source was either glucose or fructose. Ingredients were obtained from the following suppliers: casein – Sigma Aldrich, St Louis, MO (C5890); peptone – BDH (440754 K); albumin – BDH, Poole, UK (Cat. No. 830083G; alpha-cellulose – Sigma (C8002); agar – Oxoid, Basingstoke, UK (L11); hydramethylnon (Bayer Corporation); Vanderzant vitamin mix – Sigma (V1007).
[b] P:C 1:3 and 3:1 indicate protein:carbohydrate ratios. Unless noted otherwise, compositions of ingredients are in grams.

Oleic acid dissolved in isopropanol was mixed into the agar solution, which was then mixed with the dry ingredient solution, and blended until homogeneous. To create the baits, hydramethylnon (CAS 67485-29-4, obtained from Bayer Corporation, Research Triangle Park, NC) was dissolved in oleic acid and then in isopropanol and added to the nutrient solution, as described above. Diets and baits were poured into petri plates (100 mm × 15 mm), allowed to cool at room temperature for several hours, stored at −20 °C for 24 h and then freeze dried for 5 days, which also removed the isopropanol.

2.4 Effect of protein:carbohydrate (P:C) ratio and sugar type on diet consumption and lipid content of strains

We evaluated the effect of P:C ratio and sugar type on diet consumption and body lipid content to determine acceptability and carbohydrate assimilation of the treatment diets in both strains. We starved newly eclosed *B. germanica* males of the Orlando normal and T164 strains for 24 h, with water provided, and then added one of four diets (P:C 1:3 with fructose, 1:3 with glucose, 3:1 with fructose, 3:1 with glucose) for 3 days. We used 15 replicates for each treatment. Each male only had access to a single treatment after eclosion, so its body lipid content was a direct consequence of the diet it consumed as an adult. To measure diet consumption, we cut the freeze-dried diets into cubes (approximately 0.125 cm^3), dried the diets for 1 week at 50 °C, cooled them in a desiccator and weighed them to the nearest 10 µg (Sartorius Model 1712 MP8). After diets had been exposed to the insects for the 3 day consumption period, they were dried for an additional week at 50 °C, cooled in a desiccator and weighed again. Diet consumption was calculated as the difference in dry mass of the diets before and after insect feeding.

After we offered one of four diets for 3 days, each insect was dried for 1 week at 50 °C, weighed and then immersed for 1 week in 10 mL of anhydrous diethyl ether in a 20 mL glass scintillation vial, with periodic agitation to extract lipids. After lipid extraction, diethyl ether was removed from the vials, and insects were dried at 50 °C for an additional week and then weighed. Lipid content

was calculated as the difference between dry mass of insects before and after extraction. To control for differences in starting dry mass between the two strains, 20 control insects of each strain were dried prior to the start of the experiment (mean ± SEM, Orlando normal = 14.32 ± 0.295, T164 = 12.67 ± 0.342), and percentage lipid content was normalized to dry body mass.

2.5 Effect of diet and bait composition on male *B. germanica* mortality

To determine the effect of diet composition on bait efficacy, we exposed Orlando normal and T164 adult males to P:C 1:3 and 3:1 diets, with the carbohydrate being either glucose or fructose. Males were deprived of food but provided with water for 24 h before introducing the diets. Diets were provided for 3 days, and then baits were added, allowing cockroaches access to both. There were no untreated controls in this experiment. We performed five replicates with ten males per replicate. Mortality was recorded, and dead insects were removed daily for 17 days. At the end of the experiment, the remaining insects were removed from the jars so that only adult excreta remained. Fifty adults were used per treatment (1600 total).

2.6 Effect of diet–bait interactions on the toxicity of cockroach excretions

We predicted that excretions produced by male cockroaches exposed to different diet–bait combinations would vary in their availability, palatability and nutritional content, and thus would have different effects on secondary mortality of nymphs. We exposed newly emerged glucose-accepting orange-body first-instar nymphs (Orlando normal) and glucose-averse (T164) first-instar nymphs to the residues produced by adult male *B. germanica* exposed to various diet and bait combinations, and recorded nymphal mortality. Ten first-instar nymphs of each strain were confined to the same jar with the residues. The Orlando normal nymphs were distinguished from T164 nymphs by body color using orange variants within the Orlando normal colony.[37] Nymphs were first exposed to adult male excretions for 24 h, and then rat chow was added to the same jar. Five replicates per treatment were performed. Nymphal mortality was recorded and dead nymphs were removed daily for 10 days. Approximately 50 nymphs were used per treatment, but several nymphs had escaped during the course of the experiment and were not included in the analysis, resulting in 1167 in total.

2.7 Statistical analysis

Susceptibility of the two strains to hydramethylnon was statistically compared using the lethal dose ratio test, whereby tested strains are significantly different if the upper and lower 95% confidence intervals do not encompass 1.[36] The effect of diet on percentage lipid content per dry body mass was analysed with ANOVA and *post hoc* Tukey HSD for multiple comparisons. Diet consumption and lipid content analysis was implemented in R (v.3.1.2). One T164 adult escaped, and thus only 59 adults were used for T164, as opposed to 60 Orlando normal adults. For both primary and secondary kill experiments, treatments were compared using Kaplan–Meier survival analysis and log-rank tests. All survival analyses were implemented in SAS 9.3 (SAS Institute, Cary, NC). A Sidak adjustment was used for the log-rank test multiple comparisons.

3 RESULTS AND DISCUSSION

3.1 Susceptibility of Orlando normal and T164 to hydramethylnon

Topical application of 0.5 µL of acetone did not cause any mortality. The LD_{50} values (95% CI) of Orlando normal and T164 were 21.33 (15.93–27.81, $\chi^2 = 6.8094$) and 37.90 (34.68–41.90, $\chi^2 = 3.9430$) µg g^{-1} respectively, and were significantly different (lethal dose ratio test; 95% CI, 0.449–0.705). The LD_{90} values (95% CI) of Orlando normal and T164 were 60.75 (44.62–95.92) and 61.97 (53.99–76.12) µg g^{-1} respectively, and were not significantly different (0.710–1.355). The LD_{50} and LD_{90} resistance ratios (RRs) of T164 relative to Orlando normal were 1.78 and 1.02 respectively. Newly eclosed Orlando normal males weighed significantly more [47.33 ± 0.871 mg dry mass (± SEM)] than T164 males (42.84 ± 0.654; *t*-test: $t = 4.114_{35.3}$, $P = 0.00022$).

3.2 Effect of protein:carbohydrate ratio and sugar type on diet consumption and lipid content

Whole-body lipids generally increase as sugar and fat intake increases.[27,29] Varying the dietary P:C ratio and the incorporated sugar significantly affected the amount of diet consumed by the two strains of cockroaches (Fig. 1). Orlando normal males fed high-carbohydrate diets containing fructose or glucose consumed more food than on high-protein diets, and as expected, their lipid contents were highest (Fig. 1). Generally, consumption of P:C 3:1 diets was lower than consumption of 1:3 diets, as expected, and Orlando normal males on these diet treatments significantly differed in the conversion of food to body lipids. Orlando normal males consuming high-carbohydrate diets of either sugar contained more lipids than males given high-protein diets (ANOVA: $F_7 = 11.372$, $P < 0.0001$) (Fig. 1), resulting in a positive correlation between diet intake and body lipids (Spearman correlation: $S = 23394$, $n = 60$, $r_s = 0.3450$, $P = 0.0061$).

T164 glucose-averse males largely rejected glucose-containing diets and consumed significantly greater amounts of fructose-containing diets, regardless of their P:C ratio. T164 males also consumed less carbohydrate-rich diet than Orlando normal males, even when the sugar offered was fructose (Fig. 1). The pattern of diet consumption and consequent lipid accumulation differed in T164 males. Males provided with P:C 1:3 or 3:1 fructose-containing diets accumulated lipids in a similar pattern to Orlando normal males, with more body lipids on sugar-rich diets. However, T164 males offered glucose-containing diets exhibited an unusual reversed relationship between intake and lipid accumulation. Males fed high-protein (3:1) glucose diet ate more but accumulated fewer lipids than males fed 1:3 glucose diet. Thus, males were able to extract more lipid precursors from the unpalatable 1:3 glucose diet than from the more palatable but non-preferred high-protein diet. In contrast to the positive correlation between diet intake and body lipid content in Orlando normal males, no overall correlation was found in T164 males when all four diets were considered (Spearman correlation: $S = 31728$, $n = 59$, $r_s = 0.0728$, $P = 0.5837$).

These results, suggesting that T164 insects may have different metabolic/nutritional requirements from Orlando normal, are consistent with those of Shik *et al.*[29] and Jensen *et al.*,[30] who also reported lower fructose diet intake in T164 than in Orlando normal insects. However, whereas Shik *et al.*[29] found similar lipid content in T164 and Orlando normal nymphs fed 1:3 fructose diets, we found less lipid in T164 males (Fig. 1). It is likely that differences

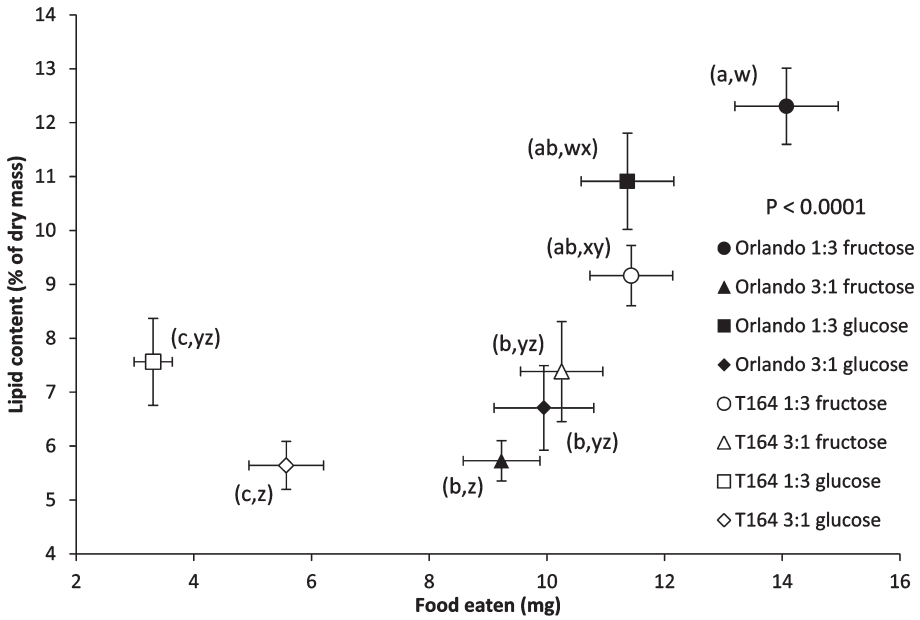

Figure 1. Relationship between dry mass of food eaten (mg) and lipid content (percentage of dry body mass) for Orlando normal (closed symbols) and T164 (open symbols) adult males. Ratios (1:3 and 3:1) represent protein:carbohydrate ratios. Points with different letters in parentheses are significantly different from each other with respect to their axes [food eaten (a, b, c, d), lipid content (w, x, y, z)] (one-way ANOVA, *post hoc* Tukey test for multiple comparisons). Each mean (± SEM) represents 15 replicates for diet intake and 15 replicates for lipid content.

in experimental insects (nymphs versus adults), their physiological condition (pre-molt versus post-molt) and experimental conditions (number of days in the experiment) contributed to these divergent results.

These results add to a growing body of evidence that evolutionary shifts in diet breadth of cockroaches[20,29,30,38,39] may have downstream effects on life history characteristics such as sexual maturation[40] and courtship.[41] We suggest that glucose-averse cockroaches have evolved lower carbohydrate needs, an adaptation that may facilitate the persistence of glucose aversion in German cockroach populations in the absence of selection with glucose-containing baits.

3.3 Effect of diet and bait composition on male *B. germanica* mortality

Mortality of Orlando normal males was affected by the P:C ratios of both their diet and the hydramethylnon bait. Three days of feeding on a suboptimal P:C 3:1 diet improved the efficacy of all bait combinations tested (Figs 2B and D), while feeding on optimal 1:3 diets reduced bait performance, regardless of the type of sugar used (Figs 2A and C). Greater separation of survival curves was observed on the 1:3 diets, revealing preferences of Orlando normal insects for high-carbohydrate baits, regardless of sugar type, and for high-protein (3:1) glucose baits over high-protein fructose baits (Figs 2A and C). Nevertheless, optimal 1:3 baits of either sugar performed well, regardless of the composition of the pretreatment diets. We suggest that even slight differences in sugar preferences may have significant effects on bait performance.

With T164 males, fructose-containing baits always performed the best (Figs 2E to H; Table 2). While many T164 glucose-averse males survived treatments with glucose-containing baits, nearly all survived when first provided with 1:3 fructose diets (Fig. 2G). Baits containing fructose were most effective against T164 when insects were first exposed to non-preferred diets (1:3 glucose, 3:1 glucose or 3:1 fructose) (Figs 2E, F and H). Thus, satiating cockroaches with optimal diets that match their intake targets (1:3 fructose) rendered both glucose baits and high-protein fructose baits ineffective, and reduced the effectiveness of 1:3 fructose baits by 60% (Fig. 2G). This result further illustrates that the macronutrient composition of food and satiety state of insects can dramatically affect bait choice in glucose-averse insects.

Despite aversion to glucose by T164 males, glucose-containing baits still killed insects that had fed on a low-quality or non-preferred diet for 3 days (Figs 2E, F and H). These findings suggest that males that were essentially deprived of food for 3 days (high-glucose diets dramatically suppress feeding in T164 males) (Fig. 1) accept some glucose bait. It is also possible that persistent exposure to glucose in petri dishes caused some sensory adaptation to glucose-containing foods.[42] Regardless, aversion to glucose by T164 insects trumps macronutrient composition.[29]

3.4 Effect of diet–bait interactions on the toxicity of cockroach excretions

First-instar *B. germanica* nymphs ingest the feces of conspecifics, an adaptive behavior whereby these relatively sedentary nymphs procure critical nutrients for development.[12,16–18] The macronutrient composition of feces produced by adult *B. germanica* largely mirrors that of their diet.[18] We demonstrated that nymphs were profoundly affected by the macronutrient compositions of the diet and bait offered to adult males. As with male mortality, nymphal secondary mortality was also a function of sugar type, P:C ratio and strain. Specifically, any diet (no toxicant) and bait (diet + hydramethylnon) combination that lowered adult diet intake and promoted bait intake (and hence hydramethylnon excretion) also elevated secondary kill of nymphs.

For example, nymphs of both strains suffered high mortality (~80%) when exposed to feces produced by males fed a high-protein diet (i.e. lower intake) followed by high-fructose hydramethylnon bait (i.e. higher intake) (Fig. 3C). Feces of glucose-averse T164 males fed a high-glucose diet (i.e. lower

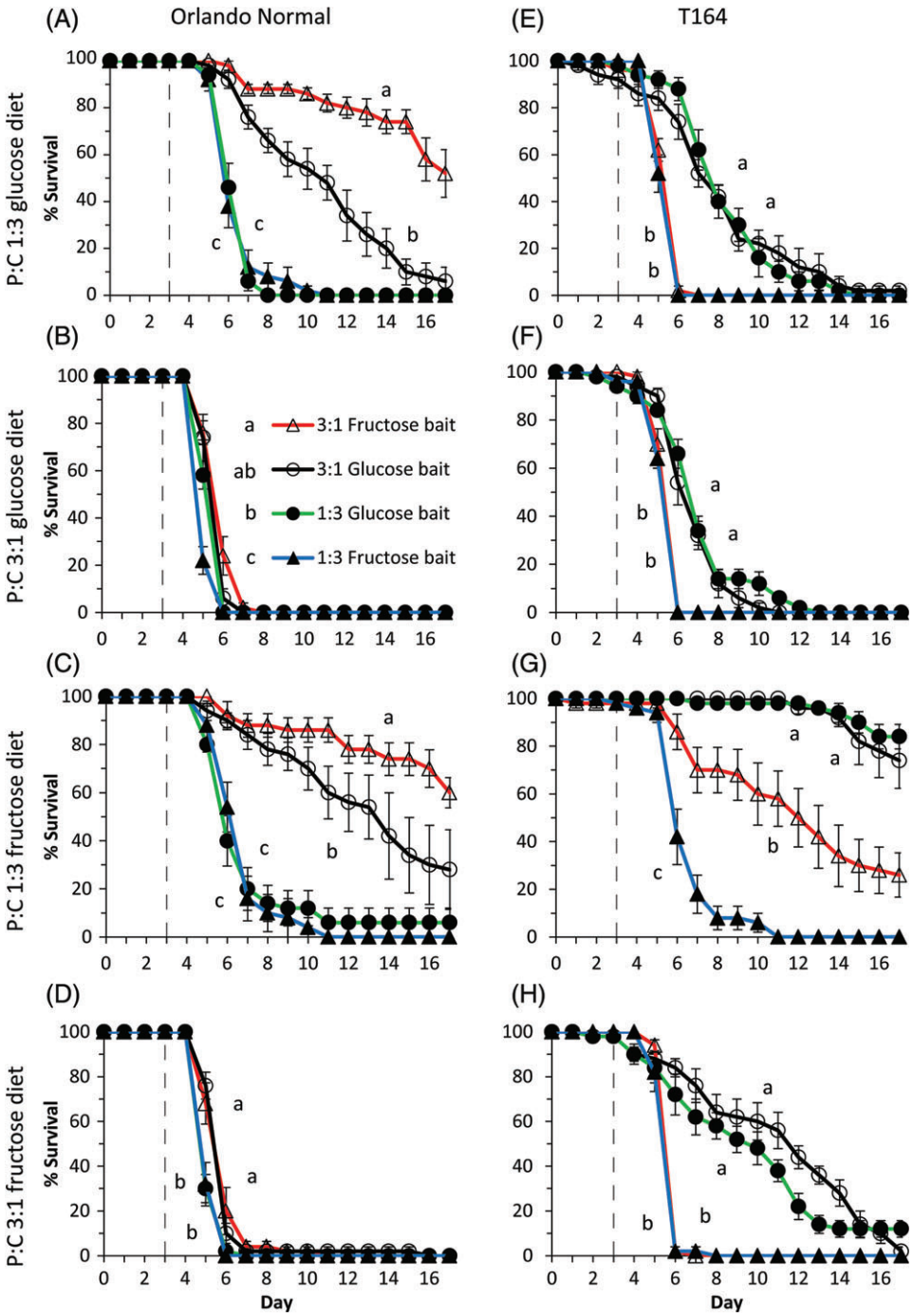

Figure 2. Effects of two bait monosaccharides and protein:carbohydrate (P:C) ratios on adult male survival in relation to their feeding history on various diets. Orlando normal and T164 males were fed one of four different diets (noted on left) for 3 days, followed by one of four baits on day 3, represented by the dashed vertical line. SEM shown for each mean, and statistical analyses are shown in Table 2. Fifty insects were used per treatment (1600 total).

intake) and then exposed to a more palatable fructose-containing high-protein hydramethylnon bait also caused higher secondary kill of nymphs (Fig. 3B). T164 males presumably avoided the unpalatable diet in favor of the fructose-containing bait, even though its P:C ratio was suboptimal, and thus they excreted more hydramethylnon-laden feces.

Conversely, any diet and hydramethylnon bait combination that increased adult diet intake and reduced bait intake also reduced secondary kill of nymphs. For example, offering Orlando normal males a combination of a preferred diet (high-glucose or high-fructose diet) and a non-preferred bait (high-protein bait) produced low secondary nymphal mortality (Figs 3A, B and E). Feeding either Orlando normal or T164 males high-fructose diet (P:C 1:3 fructose) resulted in little secondary mortality of nymphs with 3:1 bait (Fig. 3E), presumably because little bait was consumed and excreted. Similarly, few Orlando normal and T164 nymphs died on the excretions of adult Orlando normal males that had been fed 1:3 glucose diet followed by 3:1 fructose bait (Fig. 3B), presumably because Orlando normal adults preferred the optimal 1:3 diet over

Table 2. Statistical comparisons of primary (male) mortality for all diet–bait combinations shown in Fig. 2

Strain	Diet type	Comparison of bait types[a]		df	χ^2	Sidak adjusted *P*-value
		Global		3	171.13	<0.0001
Orlando normal		PC 3:1 fructose	PC 3:1 glucose		25.12	<0.0001
		PC 3:1 fructose	PC 1:3 fructose		119.00	<0.0001
	P:C 1:3 glucose	PC 3:1 fructose	PC 1:3 glucose		116.90	<0.0001
		PC 3:1 glucose	PC 1:3 fructose		18.60	<0.0001
		PC 3:1 glucose	PC 1:3 glucose		17.65	0.0002
		PC 1:3 fructose	PC 1:3 glucose		0.02	1.0000
		Global		3	47.76	<0.0001
		PC 3:1 fructose	PC 3:1 glucose		3.12	0.3826
		PC 3:1 fructose	PC 1:3 glucose		12.41	0.0026
	P:C 3:1 glucose	PC 3:1 fructose	PC 1:3 fructose		43.00	<0.0001
		PC 3:1 glucose	PC 1:3 glucose		2.96	0.4154
		PC 3:1 glucose	PC 1:3 fructose		21.86	<0.0001
		PC 1:3 glucose	PC 1:3 fructose		8.50	0.0211
		Global		3	123.89	<0.0001
		PC 3:1 fructose	PC 3:1 glucose		7.96	0.0284
		PC 3:1 fructose	PC 1:3 glucose		71.69	<0.0001
	P:C 1:3 fructose	PC 3:1 fructose	PC 1:3 fructose		79.89	<0.0001
		PC 3:1 glucose	PC 1:3 glucose		24.90	<0.0001
		PC 3:1 glucose	PC 1:3 fructose		28.93	<0.0001
		PC 1:3 glucose	PC 1:3 fructose		0.17	0.9989
		Global		3	38.55	<0.0001
		PC 3:1 fructose	PC 3:1 glucose		0.00	1.0000
		PC 3:1 fructose	PC 1:3 glucose		18.47	0.0001
	P:C 3:1 fructose	PC 3:1 fructose	PC 1:3 fructose		18.52	0.0001
		PC 3:1 glucose	PC 1:3 glucose		18.36	0.0001
		PC 3:1 glucose	PC 1:3 fructose		18.34	0.0001
		PC 1:3 glucose	PC 1:3 fructose		0.00	1.0000
		Global		3	107.96	<0.0001
T164		PC 1:3 glucose	PC 3:1 glucose		0.02	1.0000
		PC 1:3 glucose	PC 1:3 fructose		42.82	<0.0001
	P:C 1:3 glucose	PC 1:3 glucose	PC 3:1 fructose		38.27	<0.0001
		PC 3:1 glucose	PC 1:3 fructose		41.61	<0.0001
		PC 3:1 glucose	PC 3:1 fructose		37.12	<0.0001
		PC 1:3 fructose	PC 3:1 fructose		0.10	0.9997
		Global		3	68.44	<0.0001
		PC 1:3 glucose	PC 3:1 glucose		1.01	0.8958
		PC 1:3 glucose	PC 1:3 fructose		37.71	<0.0001
	P:C 3:1 glucose	PC 1:3 glucose	PC 3:1 fructose		33.24	<0.0001
		PC 3:1 glucose	PC 1:3 fructose		23.35	<0.0001
		PC 3:1 glucose	PC 3:1 fructose		20.02	<0.0001
		PC 1:3 fructose	PC 3:1 fructose		0.12	0.9996
		Global		3	213.47	<0.0001
		PC 1:3 glucose	PC 3:1 glucose		0.43	0.9867
		PC 1:3 glucose	PC 3:1 fructose		30.97	<0.0001
	P:C 1:3 fructose	PC 1:3 glucose	PC 1:3 fructose		123.40	<0.0001
		PC 3:1 glucose	PC 3:1 fructose		23.43	<0.0001
		PC 3:1 glucose	PC 1:3 fructose		104.40	<0.0001
		PC 3:1 fructose	PC 1:3 fructose		21.43	<0.0001
		Global		3	87.64	<0.0001
		PC 3:1 glucose	PC 1:3 glucose		0.93	0.9144
		PC 3:1 glucose	PC 3:1 fructose		35.10	<0.0001
	P:C 3:1 fructose	PC 3:1 glucose	PC 1:3 fructose		41.62	<0.0001
		PC 1:3 glucose	PC 3:1 fructose		21.67	<0.0001
		PC 1:3 glucose	PC 1:3 fructose		26.70	<0.0001
		PC 3:1 fructose	PC 1:3 fructose		0.28	0.9958

[a] Pairwise comparisons (six) of the four survival curves within a given graph represented in Fig. 2. Global indicates overall differences among all four survival curves within a graph.

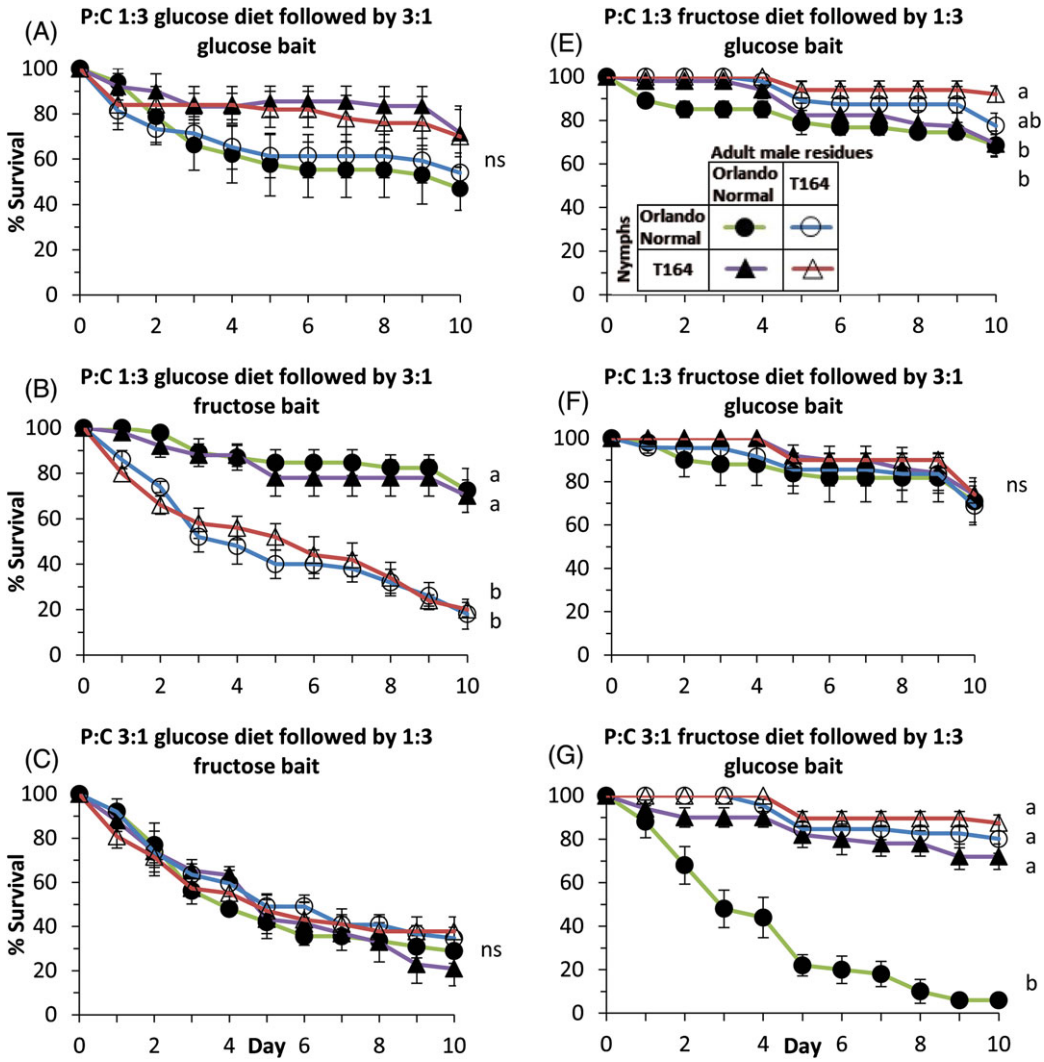

Figure 3. Mortality of first-instar nymphs on residues left by adult male *B. germanica* fed various diets and exposed to various baits. Ratios (P:C 1:3 and 3:1) represent protein:carbohydrate ratios. SEM shown for each mean, and statistical analyses are shown in Table 3. Survival lines with different letters indicate significant differences, with 'ns' representing non-significant differences among lines. Approximately 50 nymphs of each strain were used per treatment (1167 used in total).

the 3:1 suboptimal bait, resulting in limited excretion of hydramethylnon in feces. Low adult mortality in the Orlando normal strain (Fig. 2A, red line) given this diet and bait combination reflects these results. Finally, low nymphal mortality occurred when male cockroaches of either strain were fed a P:C 1:3 glucose diet followed by a 3:1 glucose bait (Fig. 3A), presumably because of two different mechanisms. Orlando normal males were likely satiated and thus consumed little of the 3:1 bait, whereas T164 males likely avoided both the diet and bait because they contained glucose; both strains would produce little feces after bait exposure and cause little secondary mortality.

Comparisons of Figs 2D and H illustrate how differences in sensory mechanisms can affect both primary and secondary kill. Moderate mortality was evident in T164 adults provided with P:C 3:1 fructose diet followed by 1:3 glucose bait (Fig. 2H, green line), likely owing to low intake of the 1:3 glucose bait. Little nymphal mortality was observed in both strains on the adult T164 residues (Fig. 3F, red and blue lines), a probable consequence of limited hydramethylnon in the adult excretions. However, most Orlando normal males fed this same diet and bait combination died, likely owing to a preference for the 1:3 glucose bait over 3:1 fructose diet (Fig. 2D, green line). When exposed to the excretions produced by these males, most Orlando normal nymphs died while most T164 nymphs survived (Fig. 3F). Because there is no substantial difference in the susceptibility of the two strains to hydramethylnon, we propose that T164 nymphs survived because they avoided glucose in the excreted feces. While previous studies have demonstrated positive correlation between ingested and excreted carbohydrates in the feces of German cockroaches[18] and locusts,[43] the presence of specific monosaccharides that reflect dietary intake will need to await confirmation through direct analysis.

Comparisons of adult male Orlando normal mortality when offered P:C 1:3 fructose or 3:1 fructose diet followed by 1:3 glucose bait (Figs 2C and D) with Orlando normal nymphal mortality (Figs 3D and F) gave an interesting insight into the role of nutrition in secondary kill. When Orlando normal adults were given 3:1 fructose diet followed by 1:3 glucose bait, mortality occurred very quickly, and all insects died by day 6, i.e. 3 days after bait introduction (Fig. 2D, green line). However, when Orlando normal

Table 3. Statistical comparisons of the nymphal secondary mortality curves shown in Fig. 3

Diet[a]	Bait[a]	Adult strain[b]	Nymphs exposed[b]	Compared with:	Adult strain[c]	Nymphs exposed[c]	df	χ^2	Sidak adjusted *P*-value[d]
P:C 1:3 glucose	P:C 3:1 glucose			Global			3	7.79	0.0506
		Orlando	T164		T164	T164		0.01	1.0000
		Orlando	T164		T164	Orlando		2.36	0.5497
		Orlando	T164		Orlando	Orlando		4.82	0.1574
		T164	T164		T164	Orlando		2.62	0.4882
		T164	T164		Orlando	Orlando		5.17	0.1300
		T164	Orlando		Orlando	Orlando		0.43	0.9862
P:C 1:3 glucose	P:C 3:1 fructose			Global			3	62.81	<0.0001
		Orlando	Orlando		Orlando	T164		0.04	1.0000
		Orlando	Orlando		T164	T164		31.08	<0.0001
		Orlando	Orlando		T164	Orlando		32.66	<0.0001
		Orlando	T164		T164	T164		27.93	<0.0001
		Orlando	T164		T164	Orlando		29.41	<0.0001
		T164	T164		T164	Orlando		0.02	1.0000
P:C 3:1 glucose	P:C 1:3 fructose			Global			3	1.61	0.6567
		T164	Orlando		T164	T164		0.01	1.0000
		T164	Orlando		Orlando	Orlando		0.47	0.9828
		T164	Orlando		Orlando	T164		1.15	0.8648
		T164	T164		Orlando	Orlando		0.40	0.9890
		T164	T164		Orlando	T164		1.06	0.8854
		Orlando	Orlando		Orlando	T164		0.16	0.9990
P:C 1:3 fructose	P:C 1:3 glucose			Global			3	10.30	0.0162
		T164	T164		T164	Orlando		2.40	0.5406
		T164	T164		Orlando	T164		7.88	0.0296
		T164	T164		Orlando	Orlando		7.70	0.0327
		T164	Orlando		Orlando	T164		1.54	0.7648
		T164	Orlando		Orlando	Orlando		1.33	0.8196
		Orlando	T164		Orlando	Orlando		0.02	1.0000
P:C 1:3 fructose	P:C 3:1 glucose			Global			3	1.06	0.7871
		T164	T164		Orlando	T164		0.01	1.0000
		T164	T164		T164	Orlando		0.78	0.9411
		T164	T164		Orlando	Orlando		0.34	0.9929
		Orlando	T164		T164	Orlando		0.64	0.9629
		Orlando	T164		Orlando	Orlando		0.25	0.9970
		T164	Orlando		Orlando	Orlando		0.10	0.9998
P:C 3:1 fructose	P:C 1:3 glucose			Global			3	133.00	<0.0001
		T164	T164		T164	Orlando		0.64	0.9639
		T164	T164		Orlando	T164		2.31	0.5630
		T164	T164		Orlando	Orlando		86.56	<0.0001
		T164	Orlando		Orlando	T164		0.55	0.9747
		T164	Orlando		Orlando	Orlando		73.51	<0.0001
		Orlando	T164		Orlando	Orlando		58.88	<0.0001

[a] Diet and bait combinations given to the adult males.
[b,c] Survival of nymphs[b] exposed to residues from adults[b] of a given strain compared with another survival curve of nymphs[c] given residues from adults.[c]
[d] All pairwise comparisons (six) are shown within each group, with the corresponding significance values. Significance of the global comparisons indicate differences among all four survival curves within a given group represented in Fig. 3.

adults were given 1:3 fructose diet rather than 3:1 fructose diet, and then given 1:3 glucose bait, mortality occurred more slowly, resulting in only 60% mortality of adults by day 6 (Fig. 2C, green line). In the first scenario, with Orlando normal adults fed 3:1 fructose diet and then 1:3 glucose bait, their excretions killed 90% of the Orlando normal nymphs (Fig. 3F, green line). However, in the second scenario, when adults were fed a 1:3 fructose diet and then a 1:3 glucose bait, only 33% of Orlando normal nymphs died on the adult excretions (Fig. 3D, green line). Thus, the secondary kill properties of bait formulations are considerably affected by available alternative foods, the palatability of food and bait and insecticide resistance,[44] which affect the ingestion of bait by foraging insects, their survival and the amount of feces they deliver to aggregation sites.

These results highlight that both primary and secondary kill performance of highly preferred baits can be compromised by

the availability of equally preferred alternative foods through three mechanisms: (1) adults eat less of the bait because they have become satiated on the alternative preferred diet; (2) adults produce less toxicant-laden feces; (3) the toxicant becomes diluted in feces produced from the optimal non-toxic diet. The differential prominence of these mechanisms will depend upon the array of foods available, the physiological stage of adults and nymphs and their life stages. For example, Kopanic *et al.*[18] demonstrated that the development of first instars was better supported by adult female feces than by equal amounts of male feces, suggesting that the nutritional quality of male and female feces may differ.

3.5 The role of alternative foods in bait performance and insecticide resistance

Alternative food sources are thought to interfere with cockroach control, and their removal (i.e. improved sanitation) has been promoted as a key component of effective cockroach control, especially with baits.[8–10,23–25] However, no studies have examined how the relative qualities and acceptability of these foods and baits affect pest control. In this study we examined the effect of various foods on bait performance in a controlled laboratory environment where food and bait consumption, lipid content and mortality (primary and secondary kill) could be quantified. It is important to note, however, that under normal operational conditions in the field, and with commercial baits, the consumption of baits will be substantially guided by olfactory cues (attraction) and gustatory cues (palatability and phagostimulation). Olfactory cues can improve baiting success by luring German cockroaches over long distances,[45,46] and phagostimulants can bias the insects' consumption, independent of nutritional needs;[45] indeed, commercial baits rank differently on attractiveness[46] and palatability scales.[45] Moreover, in many insects, responses to olfactory[47] and gustatory[48] cues are heightened when either starved or deprived of key nutrients. Field *B. germanica* appear unable to meet their macronutrient intake target,[33] suggesting that nutritionally balanced baits with effective attractants and phagostimulants should be highly effective in these nutritionally austere environments. Lastly, consumption of diets may also be influenced by neophilia, as nutritionally deficient American cockroaches,[49] domestic rats[50] and grasshoppers[51,52] are known to become more neophilic compared with their nourished counterparts. Because baits introduced into the cockroach environment may be considered to be a novel food source, bait consumption in the field may be initially more driven by its novelty than by its inherent nutrient composition, especially if field *B. germanica* are lacking sufficient nutrient resources.

The effects of palatable baits can be magnified through secondary kill, whereby the bait is ingested and translocated by foraging cockroaches and excreted within or near cockroach aggregations, and the AI is ingested again by non-foraging members of the population. The magnitude of secondary kill can be modulated by many factors, but primary among these is the type of AI and the amount of bait ingested.[13,45] Our results show that both nutritional and sensory mechanisms can reduce the interaction of nymphal cockroaches with adult feces. The effects of sensory mechanisms on the interaction of cockroach nymphs with adult feces are most readily seen in T164 nymphs; these nymphs ingested less feces produced from glucose-rich baits, thus not only reducing the efficacy of baits but also exposing cockroaches to sublethal doses of AI. Insecticide resistance has also been shown to result in sublethal doses of AI delivered to coprophagous nymphs,[44] suggesting that the presence of alternative foods may influence the development of resistance in a similar manner.[53]

Finally, numerous studies have linked cockroach nutritional status[54,55] and fat body accumulation[56] with insecticide resistance through greater capacity to metabolize xenobiotics,[57] suggesting that alternative foods might play a role in insecticide resistance beyond simply influencing the palatability of commercial baits.

4 SUMMARY AND CONCLUSIONS

Bait efficacy is influenced by many factors, including its attractiveness and palatability,[45,46] the toxicity of different active ingredients,[14,15,45] the presence and frequency of resistance alleles within the target insect population,[44,58–63] its ability to affect secondary mortality[12–14] and practical considerations such as bait distribution.[64] Here we demonstrate that bait performance could also be affected by a predictable interaction between the nutritional condition of the German cockroach, which is a consequence of its nutritional history, and bait macronutrient composition. The relative quality and palatability of the bait, in relation to the previous food consumed, affect bait intake and primary mortality, as well as AI excretion and secondary mortality. Because baits are also used to control rodents[65] and other pests, this study has broad applicability. Indeed, alternative food sources can compete with rodent baits, as the presence of human (restaurants, bakeries, bars, food markets) and animal (domestic pet) food was found to be negatively correlated with bait consumption.[65]

The mortality profiles we observed reflected the *B. germanica* macronutrient intake target of 1:3 protein:carbohydrate reported by Jones and Raubenheimer,[27] Raubenheimer and Jones,[28] Shik *et al.*[29] and Jensen *et al.*,[30] lending further support to a close and predictable relationship between diet and bait intake levels and mortality. Baits were most effective when they matched the intake target and were preceded by food that departed from the intake target. Conversely, baits became ineffective when cockroaches fed on high-quality food before they were offered the bait. These principles readily extended to glucose-averse cockroaches, with the added constraint that glucose transformed nutritionally adequate diets and baits (i.e. P:C 1:3) into poor-quality analogs. Thus, baits that corresponded to the intake target performed poorly on T164 if they contained glucose.

Moreover, we demonstrated that the toxicity of male excretions to nymphs (secondary kill) varied with cockroach strain, diet and bait P:C ratio and sugar type. The combination of high diet intake and low bait intake resulted in less secondary kill, whereas the combination of low diet intake and high bait intake resulted in greater secondary kill. The complexity of this diet–bait macronutrient interaction was further extended when glucose-averse *B. germanica* adults were offered diets and baits with and without glucose. Here, intake of diet and/or bait was a function of the glucose aversion trait, and foods that would normally be preferred (1:3) were rejected. Thus, glucose aversion had the potential both to increase acceptance of diets (if baits contained glucose) and to increase bait efficacy (if diets contained glucose). Consequently, the survival of nymphs exposed to the excretions was the result of the macronutrient interactions of the diet and bait the adults were given.

Sensory mechanisms (olfaction and taste) and insecticide resistance are two factors that play prominent roles in the efficacy

of cockroach baits. This study, and a related investigation,[44] have shown that the efficacy of baits can be compromised, resulting in sublethal doses of AI being received by foraging cockroaches and coprophagous nymphs, potentially hastening the evolution of insecticide resistance.[53]

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