Research Article

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Insecticide resistance and diminished secondary kill performance of bait formulations against German cockroaches (Dictyoptera: Blattellidae)

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Abstract

BACKGROUND: Bait formulations are considered to be the most effective method for reducing German cockroach (*Blattella germanica*) infestations. An important property of some bait formulations is secondary kill, whereby active ingredient (AI) is translocated in insect-produced residues throughout the cockroach population, especially affecting relatively sedentary early-instar nymphs.

RESULTS: *B. germanica* was collected from a location where baits containing hydramethylnon, fipronil or indoxacarb had become ineffective, and these AIs were topically applied to adult males. Results revealed the first evidence for hydramethylnon resistance, moderate resistance to fipronil and extremely high resistance to indoxacarb. Insecticide residues excreted by field-collected males that had ingested commercial baits effectively killed nymphs of an insecticide-susceptible laboratory strain of *B. germanica* but failed to kill most nymphs of the field-collected strain.

CONCLUSIONS: We report three novel findings: (1) the first evidence for hydramethylnon resistance in any insect; (2) extremely high levels of indoxacarb resistance in a field population; (3) reduced secondary mortality in an insecticide-resistant field-collected strain of *B. germanica*. We suggest that, while secondary mortality is considered to be advantageous in cockroach interventions, the ingestion of sublethal doses of AI by nymphs may select for high insecticide resistance by increasing the frequency of AI resistance alleles within the population.

Keywords: *Blattella germanica*; fipronil; indoxacarb; hydramethylnon; insecticide resistance; bait; secondary kill

1 INTRODUCTION

The German cockroach (*Blattella germanica*) is a widespread urban pest of significant health concern, mainly because it produces asthma-triggering allergens[1,2] and can vector pathogenic microorganisms.[3–5] It is generally recognized that the most effective way to control German cockroach populations is with bait formulations.[6] These products include nutrients that stimulate feeding and a toxic active ingredient (AI). The targeted nature of bait formulations reduces insecticide exposure to humans and their pets.[6,7] Several bait formulations have secondary[8,9] and even tertiary[10] kill properties through various mechanisms, including coprophagy,[8] cannibalism[11] and emetophagy.[12] Deposition of insecticide-containing feces within harborages and consumption of feces by early-instar nymphs via coprophagy can make the insecticide more accessible to the cockroach population.[8,13–15]

Although bait formulations are largely effective, several *B. germanica* field populations have been reported to be resistant to some bait AIs (sulfluramid,[16] fipronil,[17–20] indoxacarb,[21] abamectin,[18,19] imidacloprid[19]). Hydramethylnon[22] has been a very effective AI in bait products against the German cockroach. In spite of its longstanding use since the 1980s, no hydramethylnon-resistant populations of any insect have been found, and the highest resistance ratio (RR) reported in the German cockroach is 1.5.[16,22–25]

In August 2012, we collected *B. germanica* from an apartment in Puerto Rico where the performance of Advion® (AI – indoxacarb), Maxforce FC Magnum® (AI – fipronil) and Maxforce Pro Roach Killer® (AI – hydramethylnon) gel baits was poor. We conducted both laboratory bait efficacy studies and topical assays with fipronil, indoxacarb and hydramethylnon to determine whether insecticide resistance in this strain (PR-712) might explain, in part, the observed poor bait performance. Moreover, we investigated secondary kill in this strain. All studies to date on cockroach secondary kill have been performed with longstanding insecticide-susceptible laboratory colonies. Yet, the efficacy of baits may be further compromised in resistant populations by

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poor secondary kill performance. Our goals were (1) to characterize the collected field strain for resistance through topical application of AI and ingestion of formulated bait, and (2) to compare the toxicity of adult excreta to nymphs from the field-collected strain and from a laboratory-susceptible strain of *B. germanica*.

2 EXPERIMENTAL METHODS

2.1 Insect strains and rearing conditions

We compared two *B. germanica* strains: (1) Orlando Normal, an insecticide-susceptible strain maintained in the laboratory for over 70 years; (2) PR-712, collected in August 2012 from a single apartment in Monseratte Tower 1, Carolina, Puerto Rico. The cockroach population in this unit could not be controlled with a range of commercial bait products. We propagated newly collected PR-712 for 2–3 generations, then allocated these insects to four treatments: (1) unselected, (2) fipronil selected (Maxforce FC Magnum gel bait), (3) indoxacarb selected (Advion gel bait) and (4) hydramethylnon selected (Maxforce Pro Roach Killer). Insecticide selection was accomplished by placing approximately 2 g of bait in a rearing container for 3 days, then removing any remaining bait. After bait exposure, living insects were moved to a clean container. Food (Purina 5001 Rodent Diet; PMI Nutrition International, St Louis, MO) and water were provided *ad libitum*. This process was repeated every 2 months for 2 years, after which time the experiments detailed below were performed. Laboratory conditions for insect rearing and all experiments were 25 ± 1 °C, 37 ± 5% RH and 12:12 h L:D.

2.2 Topical application of insecticides

Technical insecticides were serially diluted with acetone, and 0.5 μL of a dilution was applied to the ventral surface of the cockroach between the metacoxae with a repeating micropipette (Hamilton Company, Reno, NV). At least 30 individuals were treated with each concentration. Following treatment, cockroaches were maintained in three groups of ten in 10 cm diameter petri dishes (Fisher Scientific, Pittsburgh, PA) and provisioned with rat chow and water. Cockroaches topically treated with fipronil or indoxacarb were monitored for mortality daily for 2 days, and those treated with hydramethylnon for 5 days. The greatest range of mortality for the AIs tested at given doses occurred at these two time points. Insects that could not right themselves within 30 s when flipped, and would exhibit erratic appendage movements, were considered dead. Values for LD_{50} and LD_{90} and their respective fiducial limits were estimated from probit analysis in Polo Plus (LeOra Software Company, Petaluma, CA).[26]

2.3 Effect of bait formulations on adult male survival

We provided male German cockroaches with ~0.5 g of one of three baits in a vial cap (Maxforce FC Magnum, 0.05% fipronil; Advion, 0.06% indoxacarb; Maxforce Pro Roach Killer, 2.15% hydramethylnon), plus rodent chow and water, for 1 week. The cockroaches were housed in glass jars (88 mm diameter, 95 mm height), with the inside rim coated with a thin layer of petroleum jelly/mineral oil to prevent escape. We recorded mortality daily and removed dead cockroaches. Five replicates, with 20 insects per replicate, were performed for each of the treatments.

2.4 Secondary toxicity to nymphs

After 1 week of bait exposure, all adults and bait were removed from the jars. We then placed 20 first-instar nymphs in each jar: ten of an insecticide-susceptible orange-body variant of Orlando Normal,[27] plus ten PR-712 nymphs from the same selection regime cohort initially evaluated in the bait experiment with adult males. The orange-body variant enabled us to distinguish effects on insecticide-susceptible and insecticide-resistant nymphs exposed to the same residues (feces, regurgitate), and thus served as a within-jar control. As a control for this color variant, both black-body (wild type) and orange-body Orlando Normal nymphs were exposed to deposits produced by black-body adult male Orlando Normal. Rodent chow and water were provided *ad libitum*.

2.5 Statistical analysis

LD_{50} and LD_{90} values for each strain–AI pair were calculated and compared using the lethal dose ratio test, whereby LD_{50} or LD_{90} values of different strains are significantly different from one another if the upper and lower 95% confidence intervals of the ratio do not contain 1 (PoloPlus program; LeOra Software Company).[26] We used a log-rank test to compare strains in the bait primary and secondary kill assays. A Sidak adjustment was used to account for multiple comparisons in primary kill (SAS 9.3; SAS Institute, Cary, NC). No adjustment was needed for comparisons of secondary kill because only two strains were compared. Resistance ratios (RRs) were calculated by dividing the LD_{50} and LD_{90} values of the PR-712 strain by the respective LD_{50} and LD_{90} values of the Orlando Normal susceptible strain.

3 RESULTS

3.1 Topical application of insecticides

Acetone alone did not cause any mortality. The PR-712 strain was significantly more resistant to fipronil, indoxacarb and hydramethylnon than the Orlando Normal susceptible strain (LD_{50} RR: 5.60, 23.21, 3.89; LD_{90} RR: 9.78, 391.3, 8.74 respectively) (Table 1). Continued lab selection of PR-712 increased fipronil, indoxacarb and hydramethylnon resistance (LD_{50} RR: 15.92, 13 375, 19.31; LD_{90} RR: 20.20, ~54 619, 350.9 respectively) (Table 1). LD_{90} could not be accurately estimated for the PR-712 lab-selected cockroaches treated with indoxacarb, because only 16% of the individuals died at the highest topical dose (150 µg 0.5 μL^{-1}), so an approximation was made, based on $\log_{10}$ dose and probit value regression.

3.2 Effect of bait formulations on adult male mortality

Unselected PR-712 males survived longer than Orlando Normal males when exposed to hydramethylnon bait (log-rank test: $\chi^2 = 14.6813$, $P = 0.0004$) but not Maxforce FC Magnum ($\chi^2 = 0.6850$, $P = 0.7924$) or Advion ($\chi^2 = 0.5389$, $P = 0.8450$) (Fig. 1).

Continuous laboratory selection with each bait extended the survival of PR-712 relative to Orlando Normal (fipronil bait: $\chi^2 = 8.3912$, $P = 0.0113$; indoxacarb: $\chi^2 = 42.6871$, $P < 0.0001$; hydramethylnon: $\chi^2 = 127.2$, $P < 0.0001$) and unselected PR-712 (indoxacarb bait: $\chi^2 = 29.4652$, $P < 0.0001$; hydramethylnon: $\chi^2 = 34.2846$, $P < 0.0001$) (Figs 1B and C) but not for fipronil ($\chi^2 = 4.2812$, $P = 0.1112$) (Fig. 1A).

3.3 Secondary toxicity to nymphs

The orange-body within-jar controls did not differ from the black-bodied insecticide-susceptible strain when exposed to secondary excretions from black-body Orlando Normal adult males fed fipronil- or indoxacarb-containing cockroach bait

Table 1. Toxicity, by topical application, of fipronil, indoxacarb or hydramethylnon to adult males of a field-collected (PR-712) and a laboratory insecticide-susceptible strain of *Blattella germanica*

Strain[a]	Insecticide	*n*	Slope (± SE)	LD_{50} (95% CI) (μg g^{-1})[b]	LD_{90} (95% CI) (μg g^{-1})[c]	χ^2 (df)	RR_{50}[d]	RR_{90}[e]
Orlando Normal	Fipronil	150	7.59(±1.4)	0.04 (0.04–0.05)[f]	0.06 (0.05–0.07)[f]	1.999 (2)	–	–
PR-712 unselected	Fipronil	180	3.07(±0.41)	0.22 (0.12–0.33)[g]	0.60 (0.39–1.81)[g]	9.749 (4)	5.6	9.78
PR-712 fipronil selected	Fipronil	180	4.59(±0.72)	0.64 (0.50–0.99)[h]	1.21 (0.84–4.61)[h]	4.491 (3)	15.93	20.2
Orlando Normal	Indoxacarb	330	6.21(±0.91)	3.8 (3.2–4.5)[f]	6.1 (5–14.3)[f]	28.659 (8)	–	–
PR-712 unselected	Indoxacarb	210	0.9(±0.12)	88.2 (9.3–323.2)[g]	2387.2 (559.7–442 224.3)[g]	11.760 (4)	23.21	391.34
PR-712 indoxacarb selected	Indoxacarb	210	0.66(±0.22)	50 839 (9318–134 000 000)[h]	~33 317 865 (147 493.7–NA)[h]	3.008 (4)	13 374.73	~54 619.45[i]
Orlando Normal	Hydramethylnon	180	2.82(±0.3)	19 (14.2–24.8)[f]	54.3 (39.8–85.9)[f]	6.830 (6)	–	–
PR-712 unselected	Hydramethylnon	210	1.59(±0.19)	74 (23.7–186.2)[g]	474.7 (188.2–5512.5)[g]	11.099 (4)	3.9	8.74
PR-712 hydramethylnon selected	Hydramethylnon	180	0.75(±0.16)	367 (186.4–793.2)[h]	19 054 (4961.9–422 880)[h]	1.989 (3)	19.32	350.9

[a] Strain weights: Orlando Normal 52.95 mg $male^{-1}$; PR-712 unselected 54.27 mg $male^{-1}$; PR-712 fipronil selected 53.72 mg $male^{-1}$; PR-712 indoxacarb selected 51.90 mg $male^{-1}$; PR-712 hydramethylnon selected 56.82 mg $male^{-1}$.
[b] LD_{50} values with 95% confidence intervals; values in μg AI g^{-1} insect.
[c] LD_{90} values with 95% confidence intervals; values in μg AI g^{-1} insect.
[d] Resistance ratio at LD_{50}. $RR_{50} = LD_{50}$ value of PR712 strain/LD_{50} value of Orlando Normal strain.
[e] Resistance ratio at LD_{90}.
[f,g,h] Significant, based on non-overlap of the 95% confidence intervals among strains within the insecticide applied.
[i] The LD_{90} value cannot be accurately determined owing to high-level resistance.

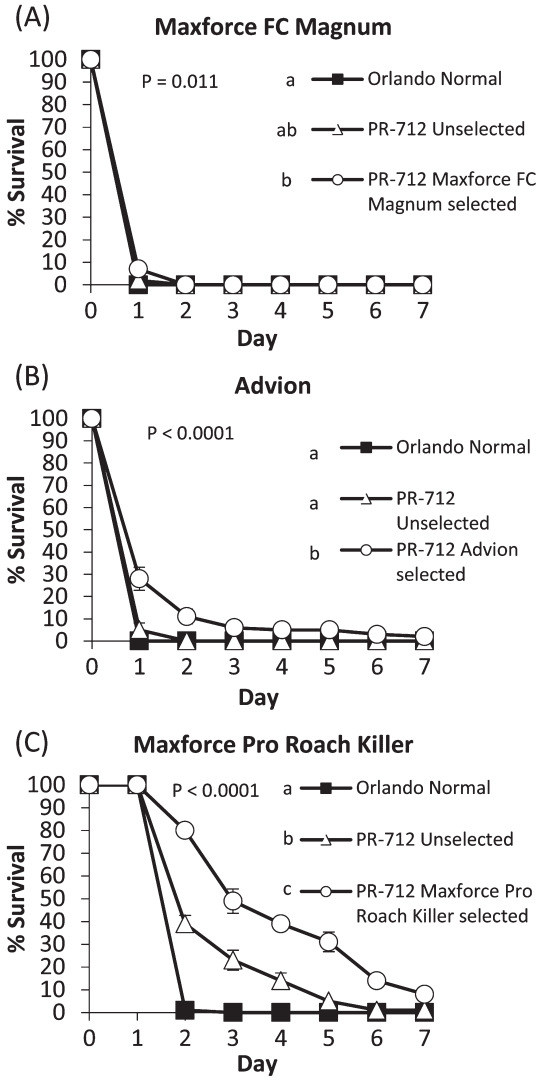

Figure 1. Survival of adult male *B. germanica* on three commercial bait products: (A) Maxforce FC Magnum cockroach bait (0.05% fipronil); (B) Advion cockroach bait (0.6% indoxacarb); (C) Maxforce Pro Roach Killer (2.15% hydramethylnon). *P*-values were determined by the log-rank test, with Sidak adjustment. *P*-values represent the overall differences among strains. Strains not sharing a lower-case letter (adjacent to legend) are significantly different from each other.

($\chi^2 = 0$, $P = 1.0$; $\chi^2 = 2.0222$, $P = 0.1550$) (Figs. 2A and B respectively), but the two lab susceptible genotypes differed in their secondary response to hydramethylnon ($\chi^2 = 6.4451$, $P = 0.0111$) (Fig. 2C). Nevertheless, the absolute differences between black- and orange-body cockroaches were relatively minor and justified the use of the latter in subsequent assays.

In all subsequent assays, nymphs were exposed to residues of adults of the same strain, so different amounts of residues might have been available in different treatments. Therefore, comparisons should be limited mainly to the two strains cohabiting the same jar. Nymphs of the PR-712 unselected strain survived significantly longer than nymphs of the Orlando Normal susceptible strain when exposed to secondary excretions from PR-712 unselected adults that had been fed fipronil-, indoxacarb- or hydramethylnon-containing bait (log-rank test: $\chi^2 = 12.2360$, $P = 0.0005$; $\chi^2 = 27.2406$, $P < 0.0001$; $\chi^2 = 40.4296$, $P < 0.0001$) (Figs. 2D, E and F respectively).

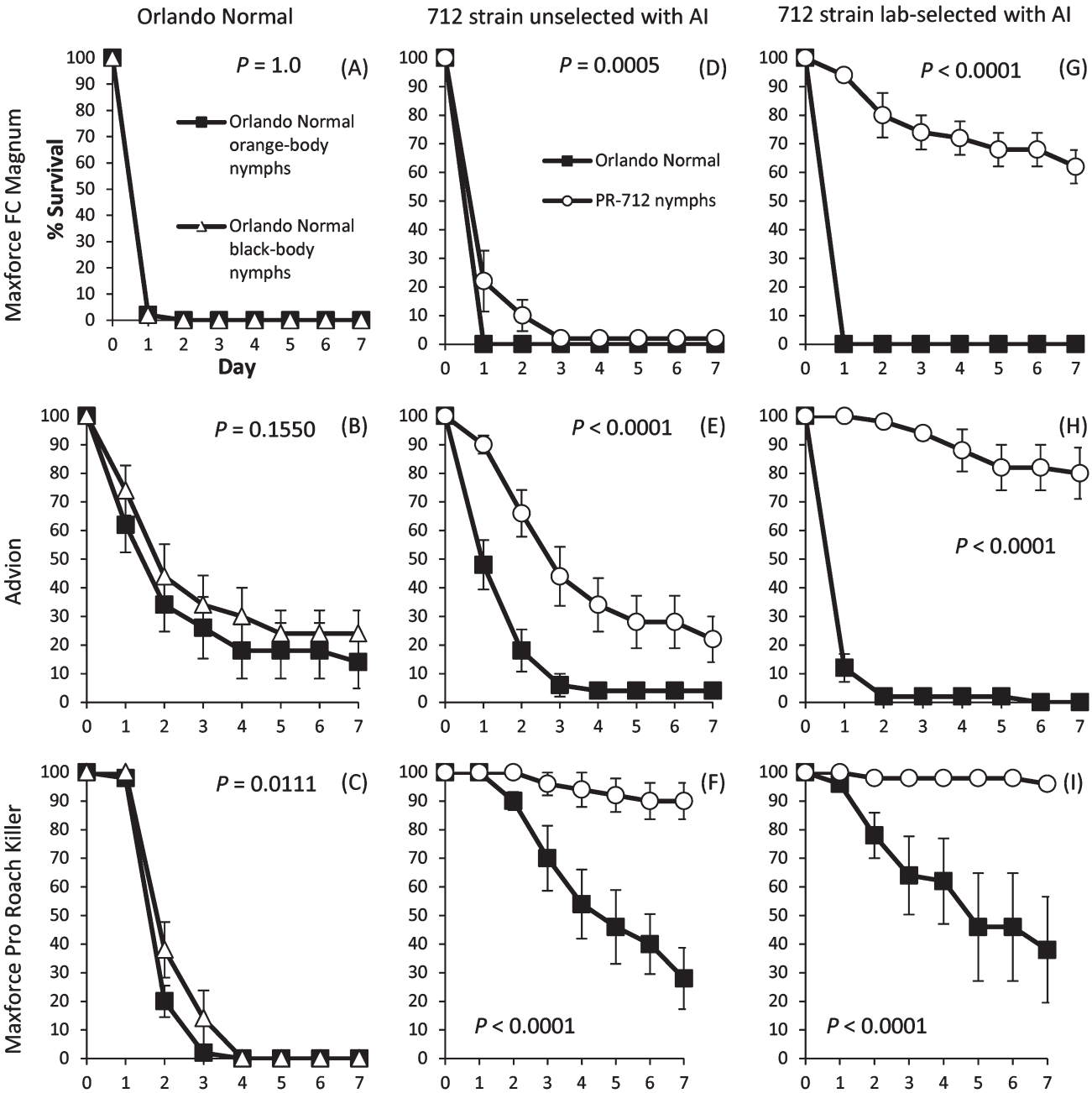

Figure 2. Survival of Orlando Normal and PR-712 nymphs continuously exposed to excreta from Orlando Normal or PR-712 males that had been fed one of three commercial baits. (A) to (C) Survival of Orlando Normal black-body and orange-body nymphs on excreta from black-body Orlando Normal adult males; (D) to (F) survival of Orlando Normal orange-body nymphs and unselected PR-712 nymphs on the excreta of unselected PR-712 adult males; (G) to (I) survival of Orlando Normal orange-body nymphs and bait-selected PR-712 nymphs on the excreta of selected PR-712 adult males. *P*-values were determined by the log-rank test.

Laboratory selection with baits further decreased mortality of PR-712 nymphs exposed to residues of adult males; these nymphs always survived significantly longer than Orlando Normal nymphs, regardless of bait (Maxforce FC Magnum: $\chi^2 = 87.7925$, $P < 0.0001$; Advion: $\chi^2 = 106.5$, $P < 0.0001$; hydramethylnon bait: $\chi^2 = 38.8107$, $P < 0.0001$) (Figs. 2G, H and I respectively).

4 DISCUSSION

The PR-712 strain was collected from an apartment where repeated treatments with insecticidal baits failed to provide adequate cockroach control. We report three novel findings from experiments with this field-collected strain of *B. germanica*: (1) the first evidence for hydramethylnon resistance in any insect; (2) rapid elevation in both hydramethylnon and indoxacarb resistance in response to selection; (3) reduced hydramethylnon, indoxacarb and fipronil secondary mortality in nymphs, suggesting a novel mechanism of selection for insecticide resistance.

4.1 Resistance to active ingredients

Our findings with topical applications of insecticides indicate that control failures were, at least in part, attributable to resistance

to a broad spectrum of AIs. Cockroaches have developed resistance to every organic insecticide within several years of intensive usage, going back to DDT, regardless of its formulation.[6] Resistance has been documented even to the most recent introductions of new AIs.[21] Surprisingly, however, despite its widespread and intensive usage in commercial cockroach baits for over 30 years, resistance to hydramethylnon has remained low, with a RR less than 1.5.[16,22–25] The field-collected PR-712 strain exhibited RR_{50} and RR_{90} of four- and ninefold respectively, and after artificial selection these values increased to 19- and 351-fold respectively (Table 1). This rapid increase in resistance following 2 years of continuous artificial selection indicates that the allele(s) underlying hydramethylnon resistance were present in this population and selection elevated their frequency. Moreover, stability of hydramethylnon resistance in the PR-712 strain suggests that reversion to susceptibility in the absence of selection would be slow. Although specific records of hydramethylnon use are unavailable, hydramethylnon-based baits presumably selected on this population some time prior to 2010.

The PR-712 population was exposed to intensive applications of Advion (indoxacarb) and Maxforce FC Magnum (fipronil) gel baits between 2010 and 2012, and not surprisingly, this strain exhibited resistance to both AIs (Table 1), showing that this is a multiresistant strain. As with hydramethylnon, topical assays revealed that continuous artificial selection on PR-712 significantly increased resistance (RR_{50}) to fipronil from six- to 16-fold and resistance to indoxacarb from 23- to >10 000-fold compared with the susceptible strain. Such high levels of resistance, based on topical applications, would be expected to impede pest control efforts.

However, bait formulations may be efficacious even in moderately resistant populations. The difference in mortality between insecticide-susceptible Orlando Normal and PR-712 was much less in bait feeding tests than from topical application, as most adult PR-712 died by the end of the bait feeding trials. The disparity between topical application and ingestion results may be related to two major issues. Firstly, many modern bait AIs are much more toxic by ingestion than when topically applied, and some are activated by gut enzymes. Gondhalekar *et al.*[21] reported a lower RR to indoxacarb when a *B. germanica* field strain ingested the AI compared with topical exposure, possibly a consequence of post-ingestive activation.[28] Secondly, ingestion can deliver a massive dose of AI that often can overcome low to moderate resistance. For example, LD_{50} of fipronil was 2.12 ng per Orlando Normal male ($LD_{90} = 3.18$ ng). Ingesting 2.5 mg of bait (0.05% AI), the typical daily intake of adult males,[29] would deliver 1250 ng of fipronil, or almost 600-fold the LD_{50} and nearly 400-fold the LD_{90}. This amount of AI would be sufficient to overcome the 16-fold resistance even of our artificially selected PR-712 strain. Likewise, the corresponding Orlando Normal estimates for hydramethylnon are: topical $LD_{50} = 1.0$ µg ($LD_{90} = 2.9$ µg); bait (2.15% AI) would deliver 53.8 µg of AI, which is much more than necessary to kill the hydramethylnon-selected PR-712 cockroaches. For indoxacarb, however, these estimates reveal that ingestion of even large amounts of AI are not likely to overcome indoxacarb resistance. The topical LD_{50} for Orlando Normal is 201 ng indoxacarb $male^{-1}$ ($LD_{90} = 323$ ng); the Advion bait (0.06% AI) would deliver 1500 ng of AI, ~7.5-fold the LD_{50} dose. Thus, it is unlikely that the ingested dose would overcome a RR_{50} of 23.2 in the unselected strain, and >10 000 in the artificially selected PR-712 strain. We emphasize two important points regarding comparisons of topical applications and ingestion. Firstly, we provided bait in excess, allowing continued *ad libitum* ingestion for the 7 day duration of the experiment. Thus, whereas faster-acting AIs (e.g. fipronil, indoxacarb) incapacitate the cockroach and probably limit it to a single meal, slower-acting AIs (e.g. hydramethylnon) may allow multiple meals – and more AI ingestion – before death. Secondly, these comparisons assume similar toxicodynamics for ingested and topically applied AIs, but more of the ingested than surface-applied AI is expected to be metabolized *in vivo*, leaving a smaller percentage of ingested AI to reach the target site compared with topical application, where AI bypasses the harsh digestive tract. Nevertheless, the huge amounts of ingested AIs compared with topically applied AIs, discussed above, are expected to compensate for any losses due to AI metabolism. Overall, these estimates predict that under field conditions, where cockroach populations are high and bait is often limited, highly or moderately resistant cockroaches may not ingest sufficient bait to succumb to a lethal dose of insecticide.

4.2 Secondary kill of nymphs

Our secondary-kill assay was designed to expose both Orlando Normal and PR-712 first-instar nymphs to equal amounts of insecticide residues produced by either Orlando Normal or PR-712 males. To distinguish these two cohabiting strains, we used orange-body mutants of the susceptible Orlando Normal strain. The orange-body and black-body nymphs responded similarly to fipronil- and indoxacarb-containing residues. Surprisingly, however, black-body nymphs exhibited significantly delayed mortality relative to orange-body nymphs on hydramethylnon-containing excretions from adult males. Reasons for this disparity, including the possibility that orange-body nymphs consume more feces than black-body nymphs, will be investigated in future research.

Nevertheless, the differences between orange- and black-body nymphs are minor compared with the differences between orange-body nymphs and PR-712 nymphs. In all instances we examined, the mortality of Orlando Normal orange-body nymphs was significantly faster and greater than that of unselected and selected PR-712. Although this pattern is largely attributable to multiresistance of PR-712 nymphs to fipronil, indoxacarb and hydramethylnon, we cannot rule out that the two strains differed in their consumption of the AI-containing adult excretions or their post-ingestive processing.

Commercial baits and AIs can also vary in secondary kill characteristics. Translocation of hydramethylnon and indoxacarb largely occurs via coprophagy,[8,10] whereas fipronil acts secondarily via emetophagy and contact.[12] In our study, all adults were removed before the nymphs were introduced, so horizontal translocation of AI through mechanisms other than coprophagy was most likely very limited, although surface contact of nymphs with toxic excretions was possible. Metabolic differences and differences in pre-ingestive sensory preferences of nymphs could also alter the lethality of excretions to nymphs. It is possible that coprophagy is more pronounced in laboratory colonies, and German cockroaches in the field (including PR-712) rely less on coprophagy, resulting in PR-712 nymphs ingesting less feces (AI) than Orlando Normal nymphs. Additionally, the two strains may differ in their qualitative preferences for adult feces. First-instar nymphs offered equal amounts of adult male and female feces perform better (more likely to molt to the second stadium) on female feces.[15] Although no study has yet determined whether nymphs exhibit preferences for ingesting male versus female feces, it is conceivable that differential preferences of the two strains contributed to differences in mortality.

We found that only ~70% of the insecticide-susceptible nymphs died within 7 days on residues from PR-712 adults fed hydramethylnon bait (Figs. 2F and I), whereas 100% of these nymphs died when exposed to residues from Orlando Normal adults fed the same bait (Fig. 2C). It is possible that PR-712 adults ingested less bait than Orlando Normal during the same time period, suggesting either lower general food intake or a sensory avoidance of the bait. Glucose-averse cockroaches avoid glucose-containing baits,[30] and our preliminary evidence suggests that a small fraction of the PR-712 population is sugar averse,[31] possibly contributing to lower ingestion of baits. In addition, it is also possible that one of several mechanisms that underlie hydramethylnon resistance in PR-712 cockroaches is the catabolism and inactivation of hydramethylnon in the digestive tract. Both mechanisms would result in less AI in the adult feces and lower mortality of nymphs exposed to adult feces. Nevertheless, the striking differences between the Orlando Normal and PR-712 nymphs, and especially the artificially selected PR-712 line, support the conclusion that multi-AI resistance was the primary factor that significantly lessened secondary kill in PR-712 nymphs.

The horizontal transfer of AIs has potential advantages and disadvantages.[7] In the short term it results in secondary kill and presumably amplifies the direct effects of the AI, although it is important to note that all the evidence for these secondary effects comes from laboratory and mesocosm studies and not from efficacy trials with field populations. On the other hand, we provide empirical support for the idea that translocation of AIs can expose low or moderately resistant populations to sublethal doses of AIs, selecting for and causing a rapid increase in the frequency of resistance alleles, as discussed by Gressel.[32] Coupled with other mechanisms that produce sublethal exposure to AIs in moderately resistant cockroaches (e.g. ingestion of less bait, glucose and other nutrient aversions), exposure through contact and coprophagy to sublethal amounts of AI in conspecific feces may constitute an important mechanism that accelerates the development of insecticide resistance. This mechanism may counteract or even negate the advantages of secondary kill inherent to some bait products.

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REFERENCES

1 Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P *et al.*, The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N Engl J Med* **336**:1356–1363 (1997).
2 Arruda LK, Vailes LD, Ferriani VPL, Santos ABR, Pomes A and Chapman MD, Cockroach allergens and asthma. *J Allergy Clin Immunol* **107**:419–428 (2001).
3 Zurek L and Schal C, Evaluation of the German cockroach (*Blatella germanica*) as a vector for verotoxigenic *Escherichia coli* F18 in confined swine production. *Vet Microbiol* **101**:263–267 (2004).
4 Ahmad A, Ghosh A, Schal C and Zurek L, Insects in confined swine operations carry a large antibiotic resistant and potentially virulent enterococcal community. *BMC Microbiol* **11**:1471–2180 (2011).
5 Jalil N, Amir K, Hasan MKS, Mahdi M, Monireh M and Atefeh B, Cockroaches' bacterial infections in wards of hospitals, Hamedan city, west of Iran. *Asian Pacif J Trop Dis* **2**(5):381–384 (2012).
6 Rust MK, Owens JM and Reierson DA, *Understanding and Controlling the German Cockroach.* Oxford University Press, New York, NY (1995).
7 Schal C, Cockroaches, in *Mallis Handbook of Pest Control*, ed. by Hedges S and Moreland D. GIE Media, Richfield, OH, pp. 150–291 (2011).
8 Silverman J, Vitale GI and Shapas TJ, Hydramethylnon uptake by *Blattella germanica* (Orthoptera: Blattellidae) by coprophagy. *J Econ Entomol* **84**:176–180 (1991).
9 Bayer BE, Pereira RM and Koehler PG, Differential consumption of baits by pest blattid and blattellid cockroaches and resulting direct and secondary effects. *Entomol Exp Applic* **145**:250–259 (2012).
10 Buczkowski G, Scherer CW and Bennett GW, Horizontal transfer of bait in the German cockroach: indoxacarb causes secondary and tertiary mortality. *J Econ Entomol* **101**:894–901 (2008).
11 Gahlhoff JE, Miller DM and Koehler PG, Secondary kill of adult male German cockroaches (Dictyoptera: Blattellidae) via cannibalism of nymphs fed toxic baits. *J Econ Entomol* **92**(5):1133–1137 (1999).
12 Buczkowski G and Schal C, Emetophagy: fipronil-induced regurgitation of bait and its dissemination from German cockroach adults to nymphs. *Pest Biochem Physiol* **71**:147–155 (2001).
13 Kopanic RJ and Schal C, Relative significance of direct ingestion and adult-mediated translocation of bait to German cockroach (Dictyoptera: Blattellidae) nymphs. *J Econ Entomol* **90**:1073–1079 (1997).
14 Kopanic RJ and Schal C, Coprophagy facilitates horizontal transmission of bait among cockroaches (Dictyoptera: Blattellidae). *Environ Entomol* **28**:431–438 (1999).
15 Kopanic RJ, Holbrook GL, Sevala V and Schal C, An adaptive benefit of facultative coprophagy in the German cockroach (*Blattella germanica*). *Ecol Entomol* **26**:154–162 (2001).
16 Schal C, Sulfluramid resistance and vapor toxicity in field-collected German cockroaches (Dictyoptera: Blattellidae). *J Med Entomol* **29**(2):207–215 (1992).
17 Holbrook GL, Roebuck J, Moore CB, Waldvogel MG and Schal C, Origin and extent of resistance to fipronil in the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). *J Econ Entomol* **96**:1548–1558 (2003).
18 Wang C, Scharf ME and Bennett GW, Behavioral and physiological resistance of the German cockroach to gel baits (Blattodea: Blattellidae). *J Econ Entomol* **97**:2067–2072 (2004).
19 Wang C, Scharf ME and Bennett GW, Genetic basis for resistance to gel baits, fipronil, and sugar-based attractants in German cockroaches (Dictyoptera: Blattellidae). *J Econ Entomol* **99**:1761–1767 (2006).
20 Gondhalekar AD and Scharf ME, Mechanisms underlying fipronil resistance in a multiresistant field strain of the German cockroach (Blattodea: Blattellidae). *J Med Entomol* **49**:122–131 (2012).
21 Gondhalekar AD, Song C and Scharf ME, Development of strategies for monitoring indoxacarb and gel bait susceptibility in the German cockroach (Blattodea: Blattellidae). *Pest Manag Sci* **67**:262–270 (2010).
22 Hollingshaus JG, Inhibition of mitochondrial electron transport by hydramethylnon: a new amidinohydrazone insecticide. *Pestic Biochem Physiol* **27**:61–70 (1987).
23 Koehler PG and Patterson RS, Toxicity of hydramethylnon to laboratory and field strains of German cockroach (Orthoptera: Blattellidae). *Fla Entomol* **74**(2):345–349 (1991).
24 Ajjan I and Robinson WH, Measuring hydramethylnon resistance in the German cockroach, *Blattella germanica* (L.). Proc 2nd Int Conf – Urban Pests, Edinburgh, UK (1996).
25 Valles SM and Brenner RJ, Variation in hydramethylnon susceptibility among insecticide-resistant German cockroaches (Blattodea: Blattellidae). *J Econ Entomol* **92**:617–623 (1999).
26 Robertson JL, Savin N, Preisler HK and Russell RM, *Bioassays with Arthropods.* CRC Press, Boca Raton, FL (2007).
27 Ross MH and Cochran DG, A body colour mutation in the German cockroach. *Nature* **165**:518–519 (1962).

28 Wing KD, Andaloro JT and McCann SF, Indoxacarb and the sodium channel blocker insecticides: chemistry, physiology, and biology, in *Insect Control: Biological and Synthetic Agents*, ed. by Gilbert LI and Gill SS. Academic Press/Elsevier, London, UK, pp. 35–53 (2010).
29 Hamilton RL and Schal C, Effects of dietary protein levels on reproduction and food consumption in the German cockroach (Dictyoptera: Blattellidae). *Ann Entomol Soc Am* **81(**6):969–976 (1988).
30 Silverman J and Bieman DN, Glucose aversion in the German cockroach. *J Insect Physiol* **39**:925–933 (1993).
31 Wada-Katsumata A, Silverman J and Schal C, Changes in taste neurons support the emergence of an adaptive behavior in cockroaches. *Science* **340**:972–975 (2013).
32 Gressel J, Low pesticide rates may hasten the evolution of resistance by increasing mutation frequencies. *Pest Manag Sci* **67**:253–257 (2010).

