

# Efficacy of noviflumuron gel bait for control of the German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae) – laboratory studies

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**Abstract:** The insecticidal activity of a cockroach gel bait containing a chitin synthesis inhibitor, noviflumuron, was evaluated using laboratory and field strains of the German cockroach, *Blattella germanica* (L.). Noviflumuron gel bait (0.01–5 mg g<sup>-1</sup>) caused ≥90% nymphal mortality to laboratory and field strains of *B. germanica* in choice tests after 11 and 19 days of continuous exposure respectively. In 1 m × 1 m bioassay arenas, laboratory strain *B. germanica* population levels exposed to 5 mg g<sup>-1</sup> noviflumuron bait or 0.1 mg g<sup>-1</sup> fipronil gel bait were significantly lower than untreated population levels after 3 weeks and 1 week of exposure respectively. Various noviflumuron bait exposure periods (2, 4 and 7 weeks) caused similar population reductions, with a mean of 99.3 (±0.3)% at 7 weeks. Fipronil gel bait caused 100% population reduction at 2 weeks post-exposure. The control population increased 89.0% at 7 weeks. In a simulated kitchen experiment with mixed stage laboratory populations, cockroach trap catches decreased 96.8 (±2.0)% at 8 weeks in the 0.5 mg g<sup>-1</sup> noviflumuron bait treatment. The trap catches in the control increased 506.5 (±493.7)% during the same period. Trap catch reduction by 0.1 mg g<sup>-1</sup> fipronil gel bait reached 100% at 4 weeks. Noviflumuron bait caused significantly lower nymph/total ratios to *B. germanica* populations in bioassay arenas from 2 weeks after exposure, demonstrating its effectiveness as a control agent for *B. germanica* with a pattern of activity similar to that expected from a chitin synthesis inhibitor.

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**Keywords:** *Blattella germanica*; noviflumuron; fipronil; gel bait; efficacy

## 1 INTRODUCTION

The German cockroach, *Blattella germanica* (L.), continues to be a major indoor pest in many parts of the world despite the availability of effective modern insecticides. A survey indicated that 50% of low-income residences in Gary, IN, USA were infested by *B. germanica* during 2002–2004 (Wang C *et al.*, unpublished). Cockroaches not only contaminate food, they also cause allergic reactions. Cockroach allergens are some of the most prominent allergens in inner-city homes.<sup>1,2</sup> People can become sensitised to proteins produced by cockroaches. A recent study showed that even low levels of exposure to cockroach allergens are a risk factor for cockroach sensitisation.<sup>3</sup> Exposure to cockroach allergens has been reported to be among the most important risk factors in severe asthma symptoms and mortality for children living in inner cities.<sup>4,5</sup>

The prevalence of *B. germanica* is partly due to its relatively small size, high level of fecundity and shorter development time than other cockroach pest species.<sup>6</sup> Besides its biological characteristics, *B. germanica* is also notorious for its ability to develop physiological and behavioural resistance to insecticides.<sup>7,8</sup> Over the years, numerous chemicals

and formulations have been developed to combat cockroach infestations. These products were often effective for control of *B. germanica* at the beginning, but failed after an extended period of use owing to the development of resistance and/or were withdrawn owing to safety concerns. High levels of *B. germanica* resistance to all major neurotoxins have been reported.<sup>9</sup> Recently, some *B. germanica* populations were reported to have developed high levels of resistance to gel baits (Maxforce, Maxforce FC, Avert, Pre-Empt) after repeated applications.<sup>10–12</sup> This calls for more selective use of available tools and the development of new chemistries and formulations to slow down or circumvent the development of cockroach resistance.

New chemicals, especially those in novel categories that exhibit different insecticidal mechanisms, are particularly beneficial in insecticide resistance management. Noviflumuron is an insect growth regulator (IGR) in the class of benzoylphenyl ureas.<sup>13</sup> This compound inhibits insect chitin synthesis and therefore disrupts the moulting of nymphs and embryo development. Cockroach nymphs ingesting enough noviflumuron will die before or during the moulting process. Adult cockroaches exposed to noviflumuron

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fail to produce viable eggs, hence their reproductive potential is reduced.<sup>14</sup>

Previous studies indicated that noviflumuron formulated as a suspension concentrate, dust or gel bait can effectively suppress *B. germanica* populations under laboratory and field conditions.<sup>15–17</sup> These promising results prompted us to investigate noviflumuron's insecticidal activity. In this study we report results on (1) the concentration–mortality response of *B. germanica* to a noviflumuron gel bait under laboratory conditions, (2) the differential response of laboratory and field strains of *B. germanica* to a noviflumuron gel bait, (3) the effect of exposure period on control efficacy and (4) the comparative efficacy of noviflumuron gel bait and a commercial gel bait for control of *B. germanica* populations.

## 2 MATERIALS AND METHODS

### 2.1 Insects and gel baits

Two *B. germanica* strains were tested. The Jwax strain is a standard susceptible strain maintained in the laboratory for over 30 years. It was originally collected before the widespread use of synthetic organic insecticides. The Muncie strain was collected from eight apartments in Muncie, IN, USA, 1.5 months prior to the experiment. These apartments had received a variety of pyrethroid spray, bait and boric acid dust applications by residents and management staff over a period of 5 years before collection. Both strains were reared in the laboratory on Harlan Teklad™ rodent diet (Harlan Teklad, Madison, WI, USA). Cockroaches were maintained in 40.5 cm × 28.0 cm × 14.5 cm plastic boxes in walk-in environmental chambers at 26°C, 60% relative humidity (RH) and 12/12 h light/dark photoperiod. The noviflumuron gel bait was provided by Dow AgroSciences LLC (Indianapolis, IN, USA). A fipronil cockroach gel bait (0.1 mg AI g<sup>-1</sup>, Maxforce FC, Bayer Environmental Science, Raleigh, NC, USA) was purchased from a commercial distributor.

### 2.2 Concentration–mortality response of laboratory and field *Blattella germanica* to noviflumuron gel bait

Twenty Jwax or Muncie strain small nymphs (2nd–3rd instar) were transferred to individual plastic boxes (18.7 cm × 13.3 cm × 9.5 cm) after brief anaesthetisation by carbon dioxide. Each box contained a water vial and a 10 cm × 10 cm cardboard 'tent' as harbourage. After 1 day of acclimatisation, food (3 cm × 3 cm of white bread and one piece of rodent chow) and ~0.8 g of noviflumuron gel bait were added to the boxes. The bait was dispensed into a small plastic weigh container. Noviflumuron concentrations in the baits were 0, 0.01, 0.1, 1 and 5 mg g<sup>-1</sup>. Each concentration (treatment) was replicated three times for each strain. The cockroaches had access to the treatment continuously during the experiment.

Dead cockroaches were counted and removed every 1–3 days until day 21.

### 2.3 Comparative efficacy of noviflumuron and fipronil cockroach gel baits against *Blattella germanica* populations in 1 m × 1 m arenas

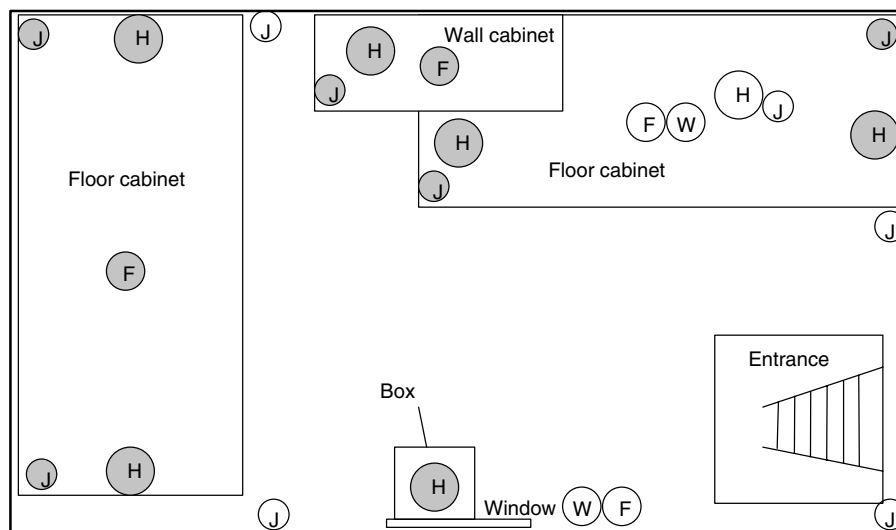
Bioassay arenas (1 m × 1 m) were constructed from Plexiglas™ sheets (walls) and 2 cm thick particle board (floor) with a white painted surface. A thin layer of petroleum jelly + mineral oil (1 + 2 by volume) was applied to the arena walls 5–10 cm above the arena floor to prevent cockroaches from escaping. Each arena contained two harbourage units at the centre, two food placements (one piece of rodent chow, peanut oil soaked in filter paper, and grape jelly) and two water vials located at opposite corners. Each harbourage unit consisted of eight plywood panels (9 cm × 10 cm) separated by 5 mm spacers. Five hundred Jwax strain cockroaches were released into each arena in the following age structure: 250 small nymphs (2nd–3rd instar), 125 large nymphs (4th–5th instar), 65 adult males and 60 non-gravid females. They were allowed to acclimatise for 2 days. Dead and weak cockroaches were replaced with healthy cockroaches immediately before the introduction of baits.

Five treatments were included: untreated control, 0.1 mg g<sup>-1</sup> fipronil cockroach gel bait and 5 mg g<sup>-1</sup> noviflumuron gel bait with exposure periods of 2, 4 and 7 weeks. The inclusion of various exposure periods allowed us to examine whether the removal of bait after exposure for 2 and 4 weeks would affect the result. The experimental units were observed for 7 weeks after treatment. Each treatment was replicated three times. The bait was provided in 1.7 ml centrifuge vials each containing ~1 g of bait. Four vials were placed in each arena, one in each corner. Extra vials of bait were left in a petri dish in the same room for aging. When a bait vial became empty, it was replaced with a vial containing same-aged bait so that cockroaches had continuous access to the bait.

The numbers of live cockroaches were counted weekly until 7 weeks. The harbourages were disassembled during each counting to obtain accurate numbers. They were recorded as the following: male adult, female adult, large nymph and small nymph. Dead cockroaches in the arenas were removed after each counting. The experimental units were maintained in a room at 27°C, 38% RH and 12/12 h light/dark photoperiod.

### 2.4 Comparative efficacy of noviflumuron and fipronil cockroach gel baits against *Blattella germanica* populations in simulated kitchens

This experiment was designed to compare the effectiveness of noviflumuron bait with that of a commercial bait product for the control of *B. germanica* populations in conditions similar to field situations. The treatments were as follows: 0.1 mg g<sup>-1</sup> fipronil cockroach gel bait (Maxforce



**Figure 1.** A bird's eye view of the simulated kitchen. Shaded objects were located inside the cabinets (F, food; W, water; H, harbourage; J, jar trap for monitoring cockroach populations before and after treatment).

FC),  $0.5 \text{ mg g}^{-1}$  noviflumuron gel bait and untreated control. Each treatment was randomly assigned to a simulated kitchen. The kitchens were located in the basement of the Whistler Hall of Agricultural Research building at Purdue University. The environmental conditions were  $25.2\text{--}30.3^\circ\text{C}$ ,  $23.2\text{--}53.6\%$  RH and 12/12 h light/dark photoperiod. The kitchens ( $3.05 \text{ m} \times 1.83 \text{ m} \times 2.67 \text{ m}$ ) were made of wood panels. Their inner surfaces were painted white. The entrance was located on the roof of the kitchen. Petroleum jelly + mineral oil (1 + 2 by volume) was applied to the perimeter of the entrance to prevent cockroaches from escaping. Each kitchen had two cabinets ( $150 \text{ cm} \times 60 \text{ cm} \times 85 \text{ cm}$ ) on the floor and one cabinet ( $75 \text{ cm} \times 30 \text{ cm} \times 15 \text{ cm}$ ) on the wall (Fig. 1). Cardboard rolls and a cardboard box were provided as harbourages. Two water jars (476 ml each, with cotton wicks) and six food placements (each with peanut oil, grape jelly and rodent chow) were provided as water and food sources. Jwax cockroaches were released 6–7 days prior to bait placement. The age structure (small nymph/large nymph/adult male/non-gravid female/gravid female) of replicate 1 was 725:250:150:100:50. The age structure of replicates 2 and 3 was 700:250:150:50:100.

Ten baby food jar traps (6.5 cm tall, 4.5 cm opening, 124 ml volume) were placed in each kitchen for 24 h to obtain cockroach counts. Each jar contained one-ninth of a slice of white bread soaked with 3 ml of beer (Miller Lite®, Miller Brewing Co., Milwaukee, WI, USA). The upper inner surface of the jars had a thin layer of petroleum jelly + mineral oil (1 + 2 by volume) to prevent escape. Trapped cockroaches were released back into the kitchens after counting. Immediately after the initial trapping, one 1.7 ml centrifuge vial containing  $\sim 0.8 \text{ g}$  of bait was placed at the same location of the jar trap. Two additional vials were placed beside the cardboard box on the floor. Jar traps were deployed for 24 h every 7 days until 8 weeks.

The weight of the bait vials in each kitchen was measured weekly. Consumption of bait was calculated using the formula given by Wang *et al.*<sup>12</sup> Empty vials were replaced with vials containing same-aged bait. Cockroach food was replenished every 2–3 weeks. Dead cockroaches, cockroach skins and oothecae (full or empty) were removed from the kitchens every 4–7 days.

At the end of the 8 week period the remaining numbers of cockroaches in each kitchen were estimated by both jar traps and visual inspection. The kitchens (including the cabinets) were cleaned with disposable cloths and soapy water. They were then used for the next replicate of the experiment. Each treatment was replicated three times over time. For each replicate the kitchens were randomly assigned to the three treatments.

## 2.5 Statistical analysis

The time–mortality response data were analysed by Proc Probit in SAS.<sup>18</sup> Times for 50% ( $LT_{50}$ ) and 90% ( $LT_{90}$ ) mortality were estimated for each concentration and strain combination. Cockroach counts (log transformed) were analysed by mixed models with repeated measurements (Proc Mixed). Treatment means were compared using Fisher's least significant difference (LSD). Nymphal ratios (nymph/total) were calculated where the trap catch was  $\geq 10$ . The noviflumuron treatments were combined as one treatment, because some arenas had too few cockroaches after 4 weeks and these treatments had similar effects on the cockroaches. The nymphal ratios from the control and noviflumuron treatments were analysed by mixed models with repeated measurements (Proc Mixed). Bait consumption was log transformed prior to analysis of variance (ANOVA). Ratios of full (with dead embryos) oothecae to empty oothecae collected in the kitchens were square root transformed prior to ANOVA.

**Table 1.** Efficacy of noviflumuron gel bait against laboratory (Jwax) and field (Muncie) German cockroach small nymphs (2nd–3rd instar)

Strain	Rate (%)	$n^a$	Model parameters <sup>b</sup>		Lethal time (days)		Model fit		
			Intercept ( $\pm$ SE)	Slope ( $\pm$ SE)	LT <sub>50</sub> (95% FL)	LT <sub>90</sub> (95% FL)	$\chi^2$	DF	$P$
Jwax	0.001	649	-5.4 ( $\pm$ 0.6)	6.8 ( $\pm$ 0.6)	5.7 (5.1–6.1)	9.5 (8.9–10.3)	5.88	8	0.66
	0.01	616	-1.3 ( $\pm$ 0.3)	2.5 ( $\pm$ 0.3)	3.4 (2.3–4.5)	11.1 (9.1–13.7)	4.05	8	0.85
	0.1	649	-5.2 ( $\pm$ 0.6)	6.4 ( $\pm$ 0.6)	6.6 (6.0–7.1)	10.5 (9.8–11.5)	3.42	8	0.91
	0.5	605	-6.4 ( $\pm$ 0.8)	6.1 ( $\pm$ 0.6)	7.2 (6.5–7.7)	10.7 (10.0–11.7)	6.41	8	0.60
Muncie	0.001	935	-6.4 ( $\pm$ 0.8)	6.1 ( $\pm$ 0.6)	11.0 (10.0–11.7)	17.8 (16.7–19.3)	3.33	14	1.00
	0.01	969	-5.8 ( $\pm$ 0.5)	5.7 ( $\pm$ 0.4)	10.5 (9.7–11.0)	17.4 (16.5–18.7)	8.19	14	0.88
	0.1	867	-4.5 ( $\pm$ 0.5)	4.6 ( $\pm$ 0.4)	9.5 (8.5–10.3)	18.1 (16.7–19.9)	13.04	14	0.52
	0.5	969	-6.5 ( $\pm$ 0.6)	6.6 ( $\pm$ 0.5)	9.4 (8.8–9.9)	14.6 (13.9–15.6)	14.0	14	0.45

<sup>a</sup> Total number of trials with 51–60 cockroaches at each observation.

<sup>b</sup> The intercept and slope parameters are for models in which the independent variable is the natural logarithm of the exposure time (days).

### 3 RESULTS

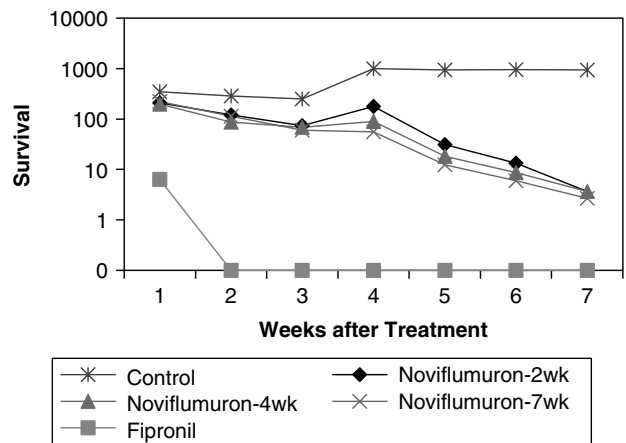
#### 3.1 Concentration–mortality response of laboratory and field *Blattella germanica* to noviflumuron gel baits

Noviflumuron bait-induced mortality of Jwax and Muncie strain cockroaches occurred 2 and 7 days after exposure respectively. This was independent of the noviflumuron concentration in the bait. All concentrations caused a high level of mortality ( $\geq 90\%$ , corrected by Abbott<sup>19</sup>) to Jwax and Muncie strains at 20 days. The mortality of the Muncie strain occurred much more slowly than that of the Jwax strain, as indicated by its much larger LT<sub>50</sub> and LT<sub>90</sub> values compared with the Jwax strain (Table 1).

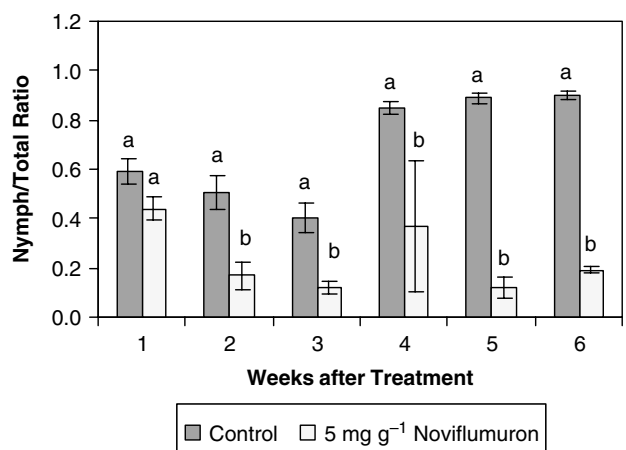
#### 3.2 Comparative efficacy of noviflumuron and fipronil cockroach gel baits against *Blattella germanica* populations in 1 m $\times$ 1 m arenas

The 5 mg g<sup>-1</sup> noviflumuron gel bait (4 and 7 week exposure treatments) caused significantly lower population counts ( $P < 0.05$ , LSD) than the untreated control at 3–7 weeks after exposure (Fig. 2). The 2 week exposure treatment caused significantly lower population counts than the control at 4–7 weeks after exposure. The three noviflumuron treatments resulted in similar population reductions at 7 weeks ( $P > 0.05$ , LSD). The mean number of surviving cockroaches for the three noviflumuron treatments at 7 weeks was 3.3 ( $\pm 1.3$ ). Fipronil gel caused significantly lower population counts than the control 1 week after exposure ( $t = 5.1$ , degrees of freedom (DF) = 64,  $P < 0.001$ ). The mean number of cockroaches in the fipronil treatment was 6.3 ( $\pm 2.7$ ) after 1 week of exposure. No live cockroaches existed after 2 weeks of exposure to fipronil. Compared with fipronil, the effect of noviflumuron on laboratory strain *B. germanica* was much slower. The untreated control population increased 89.0% at 7 weeks as a result of reproduction by the females.

The noviflumuron bait treatments caused significantly lower cockroach nymphal ratios than the control from 2 weeks ( $P < 0.05$ , LSD) (Fig. 3). At 4 weeks the

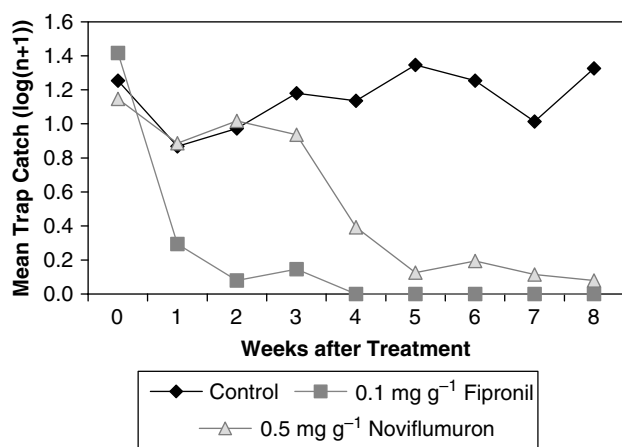


**Figure 2.** Effect of 5 mg g<sup>-1</sup> noviflumuron gel bait and 0.1 mg g<sup>-1</sup> fipronil gel bait on German cockroach (Jwax strain) populations in 1 m  $\times$  1 m arenas. Five hundred cockroaches were exposed to noviflumuron bait for 2, 4 and 7 weeks and fipronil bait for 7 weeks.



**Figure 3.** Effect of 5 mg g<sup>-1</sup> noviflumuron gel bait on German cockroach (Jwax strain) population structure in 1 m  $\times$  1 m arenas. Bars with different letters indicate significant differences ( $P < 0.05$ ).

emergence of large numbers of small nymphs caused an increase in nymphal ratios in some of the arenas and a large variance in the mean nymphal ratio. After 4 weeks, nymphal ratios in the noviflumuron treatments remained significantly lower than that of the control.



**Figure 4.** Effect of 0.5 mg g<sup>-1</sup> noviflumuron gel bait and 0.1 mg g<sup>-1</sup> fipronil gel bait on German cockroach (Jwax strain) populations in simulated kitchens.

### 3.3 Comparative efficacy of noviflumuron and fipronil cockroach gel baits against *Blattella germanica* populations in simulated kitchens

The 0.5 mg g<sup>-1</sup> noviflumuron gel bait caused significantly (at  $\alpha = 0.10$ ) greater trap catch reduction than the control 4 weeks after treatment ( $t = 2.01$ ,  $DF = 26$ ,  $P = 0.055$ ) (Fig. 4). The mean trap catch reduction was 82.6 ( $\pm 7.5$ )% at 4 weeks. Visual inspection at 8 weeks after treatment showed that the mean number of live cockroaches in the 0.5 mg g<sup>-1</sup> noviflumuron-treated kitchens was 37 ( $\pm 7$ ). All the surviving cockroaches were adults. Fipronil gel caused significantly (at  $\alpha = 0.10$ ) greater trap catch reduction than the control 1 week after treatment ( $t = 1.73$ ,  $DF = 26$ ,  $P = 0.096$ ). At 4 weeks, only one replicate in the fipronil treatment contained live cockroaches. At 8 weeks the mean trap catch in the control increased 506.5 ( $\pm 493.7$ )%. Visual inspection showed that the remaining numbers of cockroaches in the control ranged from 600 to 1100.

No nymphs were found in the noviflumuron-treated kitchens 5 weeks after treatment (based on jar trap counts). The majority of the remaining adult gravid females carried black or deformed oothecae. The mean ratio of unhatched oothecae to hatched oothecae collected from the noviflumuron-treated kitchens (0.66 ( $\pm 0.27$ )) was significantly higher than that from the control kitchens (0.10 ( $\pm 0.02$ )) ( $F = 7.84$ ,  $DF = 1, 4$ ,  $P = 0.049$ ).

Cockroaches consumed significantly more noviflumuron bait than fipronil bait ( $F = 6.73$ ,  $DF = 1, 2$ ,  $P = 0.015$ ). Mean consumption of 0.5 mg g<sup>-1</sup> noviflumuron and 0.1 mg g<sup>-1</sup> fipronil was 22.46 ( $\pm 4.19$ ) and 0.50 ( $\pm 0.19$ ) g per kitchen respectively.

## 4 DISCUSSION

Results from this study indicate that noviflumuron gel bait with  $\geq 0.01$  mg g<sup>-1</sup> AI is highly effective against *B. germanica* nymphs. Both 0.5 and 5 mg g<sup>-1</sup> concentrations can provide effective control of

*B. germanica* populations. Noviflumuron gel bait was palatable to both laboratory and field strains of *B. germanica*. Even when alternative food resources were present, cockroaches consumed enough active ingredient to be killed.

The differential time–mortality response of the laboratory and field strains to noviflumuron bait might be due to differences in their diet history and/or differing development speed. A chitin synthesis inhibitor only kills nymphs prior to or during moulting. Field strains generally have longer periods between moults than laboratory strains.<sup>20</sup> Therefore nymphs of a field strain will take longer to die than laboratory strain nymphs. Diet history influences the feeding behaviour of *B. germanica*. Our preliminary studies showed that cockroaches reared on a mixed diet (rodent diet, peanut butter, fruit jelly) consumed much less gel bait than those reared on a single diet (rodent diet) based on 3 day consumption data. Mixed food resources provide more balanced nutrients to cockroaches. The Muncie strain was exposed to much more diverse food in the field than the laboratory strain. It had also been exposed to cockroach gel baits which had similar physical characteristics and probably similar ingredients to the noviflumuron gel bait. This exposure history may have caused slower consumption of noviflumuron bait in the Muncie strain and contributed to its slower time–mortality response to the bait.

In the 1 m  $\times$  1 m arena experiment, large numbers of small nymphs emerged at 4 weeks. Although noviflumuron bait was removed in two of the treatments (2 and 4 week exposure treatments), similar levels of *B. germanica* population reduction were achieved at 7 weeks after initial exposure. The additional mortality after removal of the bait suggested that secondary kill (mortality through exposure to noviflumuron-killed cockroaches and/or cockroach faeces) was an important factor in the mortality of *B. germanica*. Secondary kill with cockroach baits as a result of coprophagy, necrophagy and cannibalism in *B. germanica* has been reported.<sup>21–23</sup> These studies demonstrated that exposure to dead cockroaches and faeces produced by bait-treated cockroaches could induce significant mortality to the cockroaches that were not exposed to the toxic bait. Smith *et al.*<sup>16</sup> showed that 100% nymphal mortality occurred after exposure to cockroach faeces collected from noviflumuron bait-treated cockroaches at 28 days. The secondary kill of noviflumuron bait through coprophagy was similar to that of hydramethylnon gel bait (Maxforce Roach Bait Gel). The secondary kill effect of noviflumuron bait through necrophagy and cannibalism is not clear. Further studies on this subject are warranted for better understanding of the insecticidal activity of this bait matrix.

The large ratios of unhatched oothecae to hatched oothecae collected in the noviflumuron-treated kitchens suggest a strong ovicidal effect of noviflumuron. This effect was also supported by

the existence of large numbers of females carrying darkened or deformed oothecae. The ovicidal effect contributed to noviflumuron's overall good population suppression in simulated kitchens. This property is especially beneficial for long-term suppression of *B. germanica*, which has a short reproductive cycle relative to other cockroach species. The smaller difference in speed of control between noviflumuron and fipronil gel bait in the kitchen experiment compared with the arena experiment was probably due to the failure of the gravid females to produce nymphs in the simulated kitchen experiment. The slower activity of noviflumuron against *B. germanica* populations compared with conventional insecticides is an inherent characteristic of IGRs. The inclusion of a conventional insecticide in the management programme may be helpful for immediate suppression of cockroach populations.

Routine application of conventional insecticides for cockroach management is declining as a result of cockroach resistance development and environmental concerns. The unique mode of action of noviflumuron provides an advantage over conventional insecticides in long-term management of cockroach infestations. It is also less likely to induce cockroach resistance. There have been no reports of cockroach resistance to IGR products, although they have been on the market for many years. As people become more concerned with their indoor environments, insecticide exposure and insecticide resistance, this new chemical has the potential to become an important addition to the available tools for *B. germanica* management.

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