Research Article

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Effects of novaluron ingestion and topical application on German cockroach (*Blattella germanica*) development and reproduction

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Abstract

BACKGROUND: Insect growth regulators disrupt insect development and reproduction. Chitin synthesis inhibitors (CSIs) allow the insect to grow normally, but because chitin is an essential component of the cuticle, formation of a new cuticle and ecdysis are prevented and the insect dies. CSIs can also kill embryos by disrupting their normal development. We evaluated the potential utility of novaluron in bait formulations against the German cockroach (*Blattella germanica* L.).

RESULTS: The minimum novaluron intake that interfered with molting and reproduction was assessed by exposing nymphs and adult females to novaluron. Results showed that 1 day of feeding on 0.1% novaluron was sufficient to disrupt molting in nymphs and prevent adult females from developing viable oothecae. The long-term effects on gravid females were investigated by feeding females 0.1% novaluron for different 5-day intervals during successive stages of gestation. Results demonstrated that gravid females fed novaluron during any period of gestation were able to produce viable eggs. To determine if ingestion of novaluron affected mating success and fertility of adult males, males were fed novaluron and then allowed to mate with untreated virgin females. Males that fed on novaluron successfully mated, and the females produced viable oothecae. Finally, direct comparisons revealed that novaluron is equally effective by ingestion and topical application.

CONCLUSIONS: Novaluron caused mortality in nymphs and interfered with ootheca production in adult females, but only before they formed an ootheca. It successfully reduced German cockroach populations in cages and has potential to be incorporated in cockroach baits.

Keywords: insect growth regulator; chitin synthesis inhibitor; novaluron; cockroach; reproduction; sterility

1 INTRODUCTION

Insect growth regulators (IGRs), also termed insect growth disruptors, have been used in pest management since the 1980s. These chemicals interfere with physiological and biochemical processes that are essential for normal growth, development, and reproduction in insects.[1,2] IGRs are compatible with integrated pest management (IPM) programs because they are specific to the target pest species, have minimal effects on beneficial insects, and have unique modes of action that are different from conventional broad-spectrum (neurotoxic) insecticides.[1–3] IGRs are grouped according to their mode of action, as chemicals that either mimic key insect hormones (juvenile hormone analogs [JHAs], ecdysone agonists) or interfere with cuticle formation (chitin synthesis inhibitors [CSIs]).

Several CSIs (all benzoylphenyl ureas [BPUs]) have been studied for their potential in cockroach management. Diflubenzuron,[4,5] penfluron,[4] triflumuron,[6–8] chlorfluazuron,[5,9] UC 84572,[5] flufenoxuron,[10] lufenuron,[11,12] and noviflumuron[13–15] have been shown to be effective in reducing German cockroach (*Blattella germanica* L.) populations.[16] They disrupt the deposition of chitin in the newly synthesized cuticle, causing the new exoskeleton to weaken and resulting in nymphs dying during ecdysis.[4–7,9,11,12,17] In cockroach embryos, CSIs disrupt cuticle formation, which prevents hatching of neonates.[2,3,18]

Novaluron is also a BPU. The specific mode of action of novaluron is not well understood, but the general mechanisms are similar to other BPUs.[19] Novaluron has been used to manage various insect pests, including crop pests such as *Spodoptera littoralis*, *Bemisia tabaci*[20] and *Leptinotarsa decemlineata*,[21] structural pests (termites),[22,23] medically important mosquitoes,[24–27] and stored product beetle pests.[28] Novaluron has demonstrated activity primarily through ingestion, but was shown to disrupt ecdysis and interfere with embryogenesis by contact as well.[19] The US Environmental Protection Agency,[29] Canadian Pest Management Regulatory Agency,[30] Food and Agriculture Organization, and World Health Organization[31,32] consider novaluron a low risk to

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the environment and nontarget organisms, and value it as an important option for IPM that should decrease reliance on organophosphate, carbamate and pyrethroid insecticides.

The overall goal of this project was to evaluate the potential utility of novaluron in bait formulations for management of German cockroach populations. We hypothesized that novaluron, like other CSIs, will cause death of nymphs during ecdysis and interfere with reproduction in adults. First, we assessed its effects on the structure of a mixed-stage cockroach population containing early and late instar nymphs and on the structure of a population containing only adult males and females. Next, we determined the minimal novaluron intake that would interfere with molting in nymphs and reproduction in adults. We then assessed the effects of novaluron ingestion by gravid females on their offspring and whether ingestion of novaluron by males affected their mating success and fertility. Finally, we conducted dose–response studies to directly compare the bioactivity of novaluron through ingestion and topical application.

2 MATERIALS AND METHODS

2.1 Cockroaches

The cockroaches used in these experiments were from a standard insecticide-susceptible strain of *B. germanica* (Orlando Normal = American Cyanamid, collected in a Florida apartment over 70 years ago). They were reared on food pellets (Purina 5001 Rodent Diet, PMI Nutrition International, St. Louis, MO, USA) and water was provisioned in cotton-stoppered vials. Cockroach colonies were maintained at 27 ± 1 °C, 40–70% relative humidity, and L:D = 12:12 h photoperiod.

2.2 Insect growth regulator

Novaluron-supplemented diets were prepared by adding the appropriate amount of novaluron (98.5%, Control Solutions-ADAMA, Pasadena, TX, USA), dissolved in 20 mL of acetone, to 100 g of ground rodent chow. The slurry was thoroughly mixed and acetone was allowed to evaporate from the novaluron-chow mix at least overnight. For most studies novaluron-containing diets were prepared at the following concentrations: 0% (acetone control), 0.0001%, 0.001%, 0.01%, 0.025%, 0.1%, and 0.5% (1, 10, 100, 250, 1000, and 5000 ppm). For the comparison of ingested and topically applied novaluron, we used ground rodent chow that contained 0% (acetone control), 0.0002%, 0.002%, 0.02% and 0.2% (2, 20, 200, and 2000 ppm) novaluron. To generate small food particles, rodent chow in this experiment was ground in a micro-mill grinder with stainless steel blade and grinding chamber (Bel-Art, South Wayne, NJ, USA).

2.3 Population studies

To test the effects of various novaluron concentrations on the demographic structure of German cockroach populations, no-choice and two-choice assays were conducted in rectangular cages (18.7 × 13.3 × 9.5 cm, T-79, Althor Products, Windsor Locks, CT, USA), each containing an egg carton shelter and a cotton-stoppered water vial. Adults and nymphs were separated in different cages to allow us to monitor adult emergence in cages of nymphs, and each concentration was tested separately in no-choice assays and two-choice assays. Each adult cage (three replicates per treatment) contained 10 males (>7 days old) and 10 females (0–2 days old). Each nymph cage (three replicates per treatment) contained 50 early instars (first to third) and 30 late instars (fourth and fifth). Cockroaches were placed into the cages, acclimated for 24 h with untreated rodent chow, and then the rodent chow was replaced by the appropriate novaluron-containing diet. For no-choice tests, 1–2 g of the appropriate diet (0% to 0.5% novaluron) was placed in a disposable plastic Petri dish (5.5 cm diameter, Falcon-Corning, Corning, NY, USA) and replaced as needed. For two-choice assays, 1–2 g of each diet was placed in two Petri dishes positioned equal distance from the shelter and water. The treatments included acetone control diet (0% novaluron) *vs* various concentrations of novaluron-supplemented diets. The 0% diet was also offered in a two-choice assay with untreated rodent chow (no acetone) to confirm that acetone did not affect the palatability of the diet. The demography of each cage (number in each stage alive) was recorded weekly.

One set of experiments (0.0001%, 0.001%, 0.01% novaluron) was conducted at 27 ± 1 °C. A second set of experiments (0%, 0.025%, 0.1% and 0.5% novaluron) inadvertently experienced a lower temperature, about 25 °C. Therefore, the general patterns may be compared across the full dose–response, but key developmental events (eclosion, ootheca formation, hatching) occurred sooner in the experiments conducted at 27 °C.

2.4 Minimal novaluron intake that interferes with molting in nymphs and reproduction in adults

To determine the minimal novaluron intake that would interfere with molting, no-choice assays were conducted with first instars. Groups of 30 newly hatched nymphs were separated into cages (T-29 Althor) with water, shelter, and 1 g of 0.1% novaluron-supplemented chow. Nymphs were allowed to feed for 0 h (control group), 24, 48, or 72 h, and then the diet was replaced with untreated ground rodent chow. Mortality and molting success were recorded daily for 14 days. There were four replicates per treatment.

A similar experiment was conducted using adult females to determine the minimal novaluron intake that would interfere with reproduction. Groups of 10 adult females (newly eclosed 0 days old) were placed into cages (T-29 Althor) with water, shelter, and ground rodent chow. The females were allowed to feed on 1 g of 0.1% novaluron-supplemented chow for 0 h (control group), 24, 48 or 72 h. The treated diet was then replaced with 1 g of untreated ground rodent chow and 10 adult males (2 weeks old, virgin) were added to each cage for mating. To ensure that females were exposed to treated diets during their maximal food consumption and allowed to mate at the same age, females were presented the diets at different ages. Thus, females in the 24 h group were given novaluron between days 4 and 5, females in the 48 h group were given novaluron between days 3 and 5, and females in the 72 h group were given novaluron between days 2 and 5. All females in all groups were 5 days old when males were added. The number of females that formed oothecae was recorded. The females were monitored for another 21 days to determine if the embryos were viable. There were four replicates per treatment.

2.5 Effects of novaluron on gravid females

Unlike most oviparous cockroaches (such as *Periplaneta americana* and *Supella longipalpa*), female *B. germanica* retain the ootheca for the entire period of embryogenesis. We tested whether novaluron ingested by females after ootheca formation would affect the maturing embryos. Groups of 20 adult females (5 days old) were placed in six cages (T-29 Althor) and 20–22 adult males (8–15 days old) were added to each cage. Females were

monitored daily and when an ootheca was visible (day 0), the female was placed singly in a disposable plastic Petri dish (9 mm diameter, Falcon-Corning) with 0.3 g rodent chow, water, and shelter. Individually housed females were placed into groups and each group was exposed to novaluron during a different 5-day interval during gestation. The rodent chow in the Petri dish was replaced with 0.1% novaluron-chow for a 5-day interval during gestation, as follows: no exposure to novaluron (control group), exposure to novaluron on days 0 to 5, 5 to 10, 10 to 15, and 15 to 20 post oviposition. We also included a group of gravid females exposed to novaluron for the entire gestation period, days 0 to 20. There were 20 individually housed females per group. Females were then monitored and deformed or aborted oothecae were noted. When a female aborted or hatched the ootheca, it was moved to a new Petri dish with untreated chow, water, shelter, and a fresh male (>8 days old). The males were removed when the female formed an ootheca and the females were again monitored for viability of the embryos within each ootheca.

2.6 Effects of novaluron on males

To investigate if novaluron affected mating success and fertility of males, groups of 50 newly emerged adult males were fed 0% chow (acetone-treated) or novaluron-chow (0.1% or 0.5%) for 13 days. Before placing them with females, males were fed untreated rodent chow for 24 h to minimize transfer of novaluron to females either on the cuticle or in male feces. Groups of 30–40 newly emerged adult females were fed rodent chow for 5 days, then paired individually with one male in a Petri dish (9 mm diameter) with untreated rodent chow and water. The pairs were video-recorded for 3 h in a temperature-controlled (27 °C) room to confirm that mating occurred, and males were removed when an ootheca was formed. The phallomere of unmated males was inspected under a microscope for any abnormalities that might interfere with copulation. Females that formed an ootheca were kept in their individual Petri dishes and viability of the embryos within the oothecae was recorded.

2.7 Dose–mortality assays with ingested and topically applied novaluron

Newly emerged last instars (sex not determined) were housed in groups for 2 days with access to rodent chow and water. On day 3, each nymph was placed in a 9-cm Petri dish with water in a cotton-stoppered microcentrifuge tube and starved for 24 h. On day 4, each nymph was provided 5.0–5.2 mg of novaluron-supplemented ground rodent chow in a microcentrifuge tube cap. Nymphs rarely walked over the caps, which minimized the ground rodent chow being displaced. Only nymphs that consumed all the novaluron-containing rodent chow were retained. Thus, the dose of novaluron ingested was estimated as the novaluron concentration per mg multiplied by 5 mg, so the novaluron doses we tested were 0, 0.01, 0.1, 1, and 10 μg. On day 5, each nymph was offered fresh rodent chow *ad libitum* and molting success was assessed daily.

For topical applications of novaluron, nymphs were treated as above, but on day 4 each nymph received two successive topical applications (0.5 μL each) of novaluron in acetone (0, 0.01, 0.1, 1, and 10 μg). Application was made between the mesothoracic coxae, an area that is least accessible to grooming to minimize the ingestion of groomed novaluron.

We conducted similar experiments with adult females, as follows: Newly eclosed females were fed rodent chow in groups, starved on day 3 for 24 h, and offered 5 mg of novaluron-containing or control rodent chow. Only females that fully consumed the 5 mg of diet were retained. Some females received topical applications, as described for nymphs. On day 5 females were placed back in groups with fresh rodent chow, water, and sexually mature males (14–20 days old). Females were monitored daily, and as each female formed an ootheca the female was separated in a Petri dish with food and water and monitored daily for hatching or abortion of the egg case.

2.8 Statistics

Kaplan–Meier analysis was performed using JMP[33] to compare mortality of small and large nymphs in no-choice and two-choice assays for each novaluron concentration, and to compare mortality of first instars fed 0.1% novaluron for 0 h (control group), 24, 48, or 72 h. The effect of 0.1% and 0.5% novaluron on mating success and ootheca hatch was analyzed using binomial generalized linear models (GLM) conducted in R.[34] A *t*-test was conducted in Microsoft Excel to compare the number of hatched nymphs in the first ovarian cycle of females that mated with males fed 0% (control) and 0.5% novaluron.

3 RESULTS AND DISCUSSION

3.1 Population studies

We conducted two-choice and no-choice assays to determine the efficacy of novaluron at different concentrations. Additionally, the two-choice assays provided information on the relative palatability and acceptance of novaluron-supplemented diets, an important consideration for inclusion of novaluron in baits. The two-choice assays showed that *B. germanica* nymphs and adults fed on all novaluron diets, indicating that all concentrations were palatable. The mean number of nymphs in no-choice and two-choice assays are shown in Fig. 1 (0% acetone control, 0.0001%, 0.001%, and 0.01% novaluron) and Fig. 2 (0.025%, 0.1%, and 0.5% novaluron). Results for control cages were similar in no-choice assays (0% novaluron acetone control) and two-choice assays (0% novaluron acetone control *vs* untreated chow). In these cages, some nymphs matured to the adult stage within 15 days and neonates appeared in these cages 50 days after the start of the experiment (Fig. 1(A)).

The response to 0.0001% novaluron represented a dramatic transition from the response to 0% control treatments to responses to higher doses. In the nymph cages (Fig. 1), almost all the small nymphs died by day 8 in the no-choice and two-choice cages provisioned with 0.0001% novaluron. Some large nymphs survived to the adult stage, but only one produced an ootheca that hatched. At this concentration, there were significant differences in mortality for both small and large nymphs between no-choice and two-choice assays on day 8 (log rank test; small nymphs: $\chi^2 = 11.68$, df = 1, $P = 0.0006$; large nymphs: $\chi^2 = 26.05$, df = 1, $P < 0.0001$). At higher novaluron concentrations (0.001% and 0.01%), however, almost all the nymphs died by day 15 in both no-choice and two-choice assays (Fig. 1(C), (D)). The few nymphs that survived to the adult stage (2% of all nymphs on 0.001% novaluron and 1% of all nymphs on 0.01% novaluron) produced inviable oothecae. At higher concentrations (0.025%, 0.1%, and 0.5%) all the nymphs died by days 22–29, with small nymphs generally dying within 15 days and the larger nymphs taking longer (Fig. 2(A)–(C)). None of these nymphs were able to reach the adult stage and there were no differences between the no-choice and two-choice assays, except for small

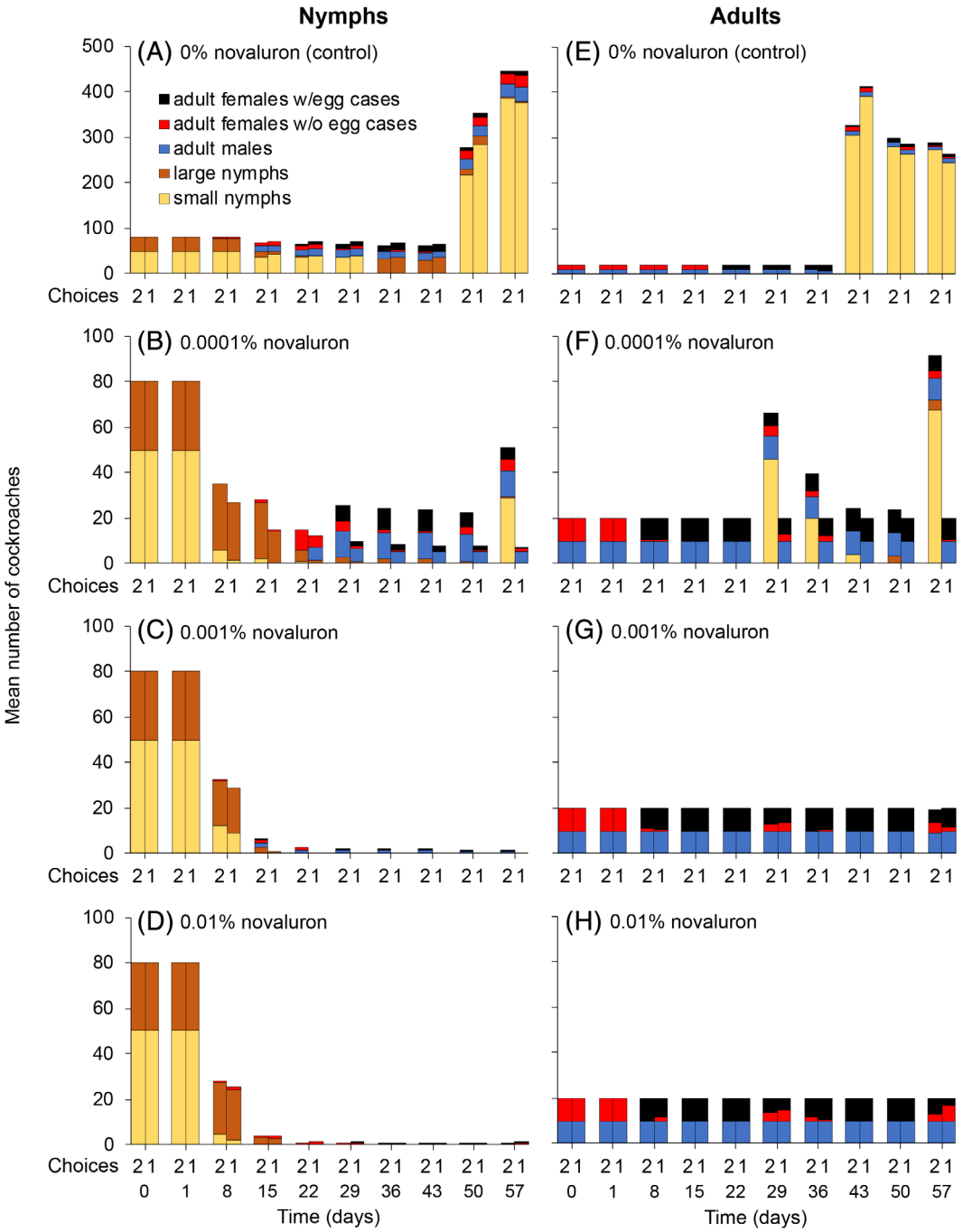

Figure 1. Mean number of cockroaches in two-choice (2 on the *x* axes) and no-choice (1 on the *x* axes) assays with 0% (control), 0.0001%, 0.001%, and 0.01% novaluron. Each of three replicates per treatment started with either nymphs (A–D: 50 early and 30 late instars) or adults (E–H: 10 males and 10 females). Note that the 0.0001%, 0.001%, and 0.01% novaluron treatments were conducted at 27 °C, whereas the control (0% novaluron) was inadvertently run at a lower temperature of about 25 °C.

nymphs at 0.025% novaluron (log rank test, $\chi^2 = 62.4$, df = 1, $P < 0.0001$). Nymphs either died within their exoskeleton or while attempting to molt. These results are consistent with the effect of other BPUs in *B. germanica*, where nymphal mortality was highest during ecdysis and mortality occurred when the emerging nymphs were unable to separate from the exuviae.[5,16]

Adult females in the adults-only control (0% novaluron) cages developed oothecae after 22 days and they hatched within 43 days of the start of the experiment (Fig. 1(E)). The delayed oviposition and hatching were attributed to the lower temperature of *c.* 25 °C in this treatment. The 0.0001% novaluron treatments were conducted at 27 °C and thus oothecae were observed as early as day 8 and some hatched by day 29 (Fig. 1 (F)). Adult females in the two-choice assays with 0.0001% novaluron were able to produce viable embryos, again demonstrating a 50% dilution of novaluron by untreated chow at this threshold concentration. In the no-choice assays, however, adult females developed oothecae, but all the embryos were inviable and

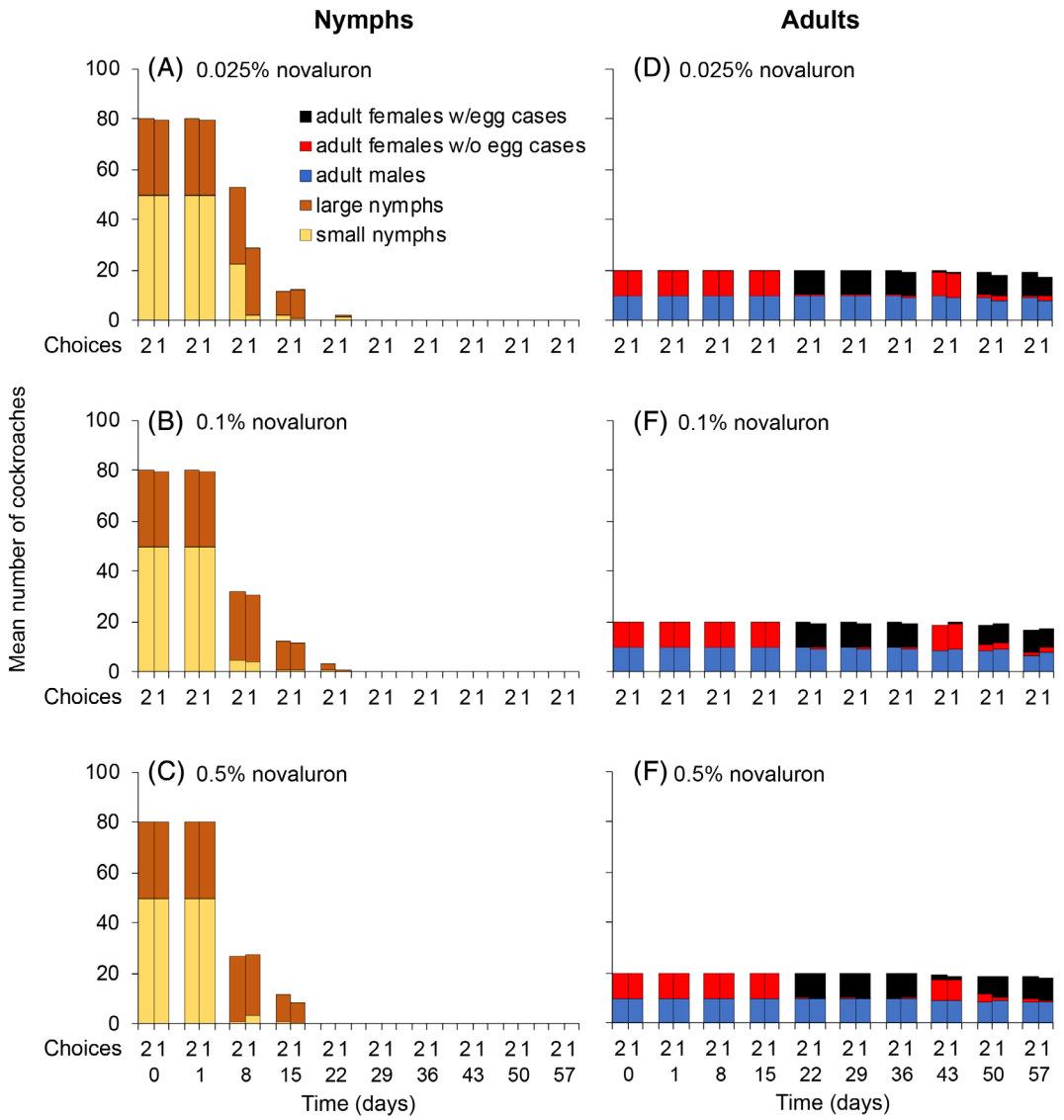

Figure 2. Mean number of cockroaches in two-choice (2 on the *x* axes) and no-choice (1 on the *x* axes) assays with 0.025%, 0.1%, and 0.5% novaluron. Each of three replicates per treatment started with either nymphs (A–C: 50 early and 30 late instars) or adults (D–F: 10 males and 10 females). These assays were conducted at about 25 °C.

turned black. In all other adult cages provisioned with ≥0.001% novaluron in both no-choice and two-choice assays, all females produced only inviable oothecae (Figs 1(G),(H); 2(D)–(F)). There was no significant adult female or male mortality in any of the treatment cages, as expected, because CSIs do not cause adult mortality in most insects,[1–3] including *B. germanica*.[16]

Overall, these results indicate that novaluron was palatable to nymphs and adults even at relatively high concentrations (0.5%). Although novaluron is not an adulticide, it causes high nymphal and embryo mortality, suggesting that it would be effective in baits for managing German cockroach populations.

3.2 Minimal novaluron intake that interferes with molting

We performed no-choice behavior tests using first instars to determine the minimal duration of exposure to 0.1% novaluron that would interfere with molting. The duration of the first instar is approximately 6–8 days, so we exposed neonates to novaluron for 1, 2 or 3 days. There was 100% survival of nymphs in the control cages (0% novaluron), second instars were observed around day 6, and all first instars molted to second instars by day 8 (Fig. 3). No second instars were found in any of the treatment cages. In four replicate assays of all treatments, 100% of first instars failed to molt and died around days 8–9 (Fig. 3). Overall mortality was significantly higher in all the treatment groups than in the controls (log rank test, $\chi^2 = 391$, df = 3, $P < 0.0001$). Thus, only 1 day of feeding on 0.1% novaluron (followed by feeding only on untreated chow) was sufficient to disrupt ecdysis and kill all the nymphs.

3.3 Minimal novaluron intake that interferes with reproduction in females

To determine the minimal duration of ingestion of 0.1% novaluron that would disrupt reproduction in females, we performed no-choice tests with newly eclosed females. Females were exposed to 0.1% novaluron for 1, 2 or 3 days before day 5, and

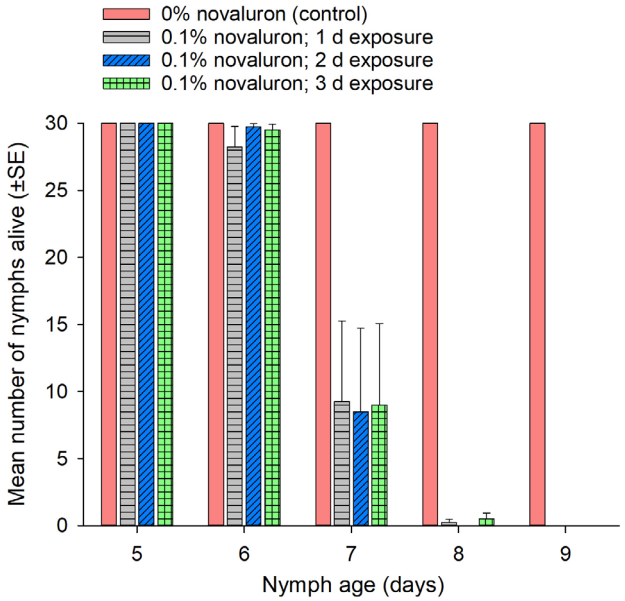

Figure 3. Mean (±SEM) number of nymphs alive on days 5–9 after first instars were fed 0.1% novaluron in rodent chow for 0 (control), 1, 2 or 3 days. There were four replicates for each treatment.

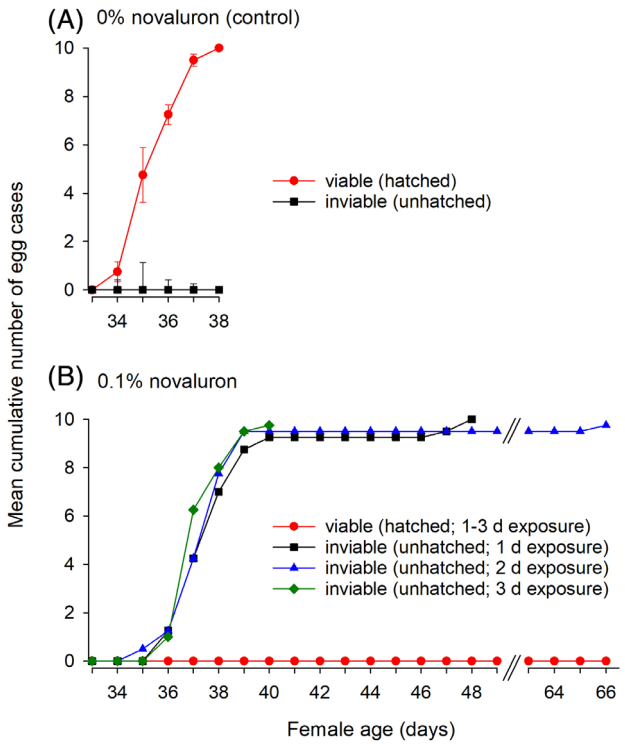

Figure 4. Mean cumulative number of viable and inviable oothecae produced by female cockroaches. Females were either (A) not exposed to novaluron (control) or (B) exposed to 0.1% novaluron for 1, 2 or 3 days. In (A) ±SEM are shown. There were four replicates for each group.

then allowed to mate with untreated males. In the control group, females developed oothecae by day 8, retained them for approximately 21 days, and 100% of the embryos successfully hatched between days 34 and 38 (Fig. 4(A)). In all the treatment groups, females developed oothecae that turned dark brown or black, deformed, and produced no nymphs; nevertheless, females retained these inviable oothecae for approximately 25 days (Fig. 4(B)). Overall, females were unable to develop viable oothecae and produce nymphs after only 1 day of feeding on 0.1% novaluron.

These observations are consistent with the ovicidal activity of other BPUs, where the disruption of cuticle formation causes developing embryos to fail to hatch.[1–3] Ovicidal effects have also been shown in adult German cockroaches fed triflumuron,[5,7,8] UC 84572, chlorfluazuron, diflubenzuron,[5] and noviflumuron.[15] These studies determined that the darkened appearance of the ootheca reflected embryonic mortality, which likely occurred during the last 2 days when the chitinous exoskeleton of the pharate first instar was forming.[5]

German cockroach females carry their ootheca externally during embryonic development and drop it immediately prior to hatch, or the eggs may hatch while the ootheca is still attached to the female. The anterior end of the ootheca is permeable, which allows transport of water and other materials from the female to the developing eggs.[35] Moreover, the chorion, which envelopes each of the embryos, might also provide for distribution of liquid.[36] This raised the possibility that xenobiotics, including insecticides, ingested by the gravid female might be transferred to the ootheca and affect the embryos.

To assess the effects of novaluron on gravid females, we divided the 21-day gestation period into 5-day intervals and fed cohorts of females 0.1% novaluron chow for 5 days during all phases of gestation. In the control group (no exposure to novaluron), 95% of the oothecae hatched (Table 1). Similarly, in all treatment groups (exposed to novaluron on days 0 to 5, 5 to 10, 10 to 15, and 15 to 20 post oviposition) 95–100% of the oothecae hatched. All the oothecae of females exposed to novaluron for the entire gestation period (days 0 to 20) hatched, showing that novaluron, at this relatively high concentration, does not affect the embryos within the ootheca carried by females.

After they dropped their oothecae, all females were transferred to new Petri dishes and provided a new male. All of the females formed oothecae. In their second ovarian cycle, they were again exposed to the same novaluron treatments as in the first ovarian cycle. All females in the control group (no exposure to novaluron) hatched their oothecae, and in all other treatments (females exposed to 0.1% novaluron on days 0 to 5, 5 to 10, 10 to 15, 15 to 20 or 0 to 20 post oviposition) 90 to 100% of the oothecae hatched (Table 1).

Overall, females exposed to novaluron during oocyte maturation produced inviable embryos, whereas those exposed after the first or second oothecae were formed were unaffected, showing high egg viability and hatch. This reversible sterility was also reported in the Colorado potato beetle, where the majority of eggs produced by females that continuously fed on novaluron-treated foliage failed to hatch, but after a recovery period females resumed laying viable eggs.[37] Thus, feeding on novaluron during the egg maturation period is necessary to cause ovicidal effects in females. These results suggest that CSIs are delivered to the eggs during vitellogenesis, when German cockroach females feed extensively to provision the eggs and produce the ootheca.[38–40] These observations are consistent with a previous study showing that females that were fed BPUs during egg maturation in the first ovarian cycle or immediately after the first ootheca hatched produced inviable oothecae.[18]

Experiments with radiolabeled compounds indicated that the anterior escutcheon region of the ootheca is permeable not only

Table 1. Percentage of viable embryos within oothecae produced by gravid females exposed to 0.1% novaluron during different 5-day intervals and the full 20-day gestation period in the first and second ovarian cycles ($n = 20$ for all treatments)

Treatment	% Viable embryos ± SE	
	First ovarian cycle	Second ovarian cycle
Control (no novaluron)	95 ± 4.9	100 ± 0.0
0.1% novaluron days 0–5	100 ± 0.0	100 ± 0.0
0.1% novaluron days 5–10	100 ± 0.0	100 ± 0.0
0.1% novaluron days 10–15	95 ± 4.9	90 ± 6.7
0.1% novaluron days 15–20	100 ± 0.0	95 ± 4.9
0.1% novaluron days 0–20	100 ± 0.0	95 ± 4.9

Table 2. Percentage of successful matings between novaluron-treated males and untreated virgin females, and percentage hatch of the resulting oothecae

Novaluron concentration	*n*	% Mated ± SE	% Hatched (of mated) ± SE
0% (control)	30	73.3 ± 8.1 a	95.5 ± 4.4 a
0.1%	17	88.2 ± 7.8 ab	93.3 ± 6.4 a
0.5%	30	96.7 ± 3.3 b	100 ± 0.0 a

Within each column, percentages followed by the same letter are not significantly different ($P > 0.05$; GLM).

to water but also to low-molecular-weight water-soluble materials.[36] Novaluron has relatively low solubility in water and a high molecular weight,[19,31,41] suggesting that it might be transferred to the oocyte bound to hemolymph proteins, but not to eggs already oviposited into an ootheca. Additionally, gravid females feed less compared to other cockroach life stages,[39] so there might have been insufficient intake of the active ingredient to affect the embryos.

3.4 Effects of novaluron on males

The effects of novaluron on male mating success and fertility were investigated by treating newly eclosed males, allowing them to mate with normal untreated females, and assessing the females' fertility. In all treatments (males fed 0%, 0.1% or 0.5% novaluron chow) 73.3% to 96.7% of the males successfully mated (Table 2). While the effect of novaluron concentration on mating was significant (GLM, $P = 0.026$), there was only a significant difference in mating success between the males fed 0% and 0.5% novaluron (GLM, $z = 2.15$, df = 74, $P = 0.0319$), and no significant differences between the other groups (GLM; 0% and 0.1% novaluron: $z = 1.17$, df = 74, $P = 0.243$; 0.1% and 0.5% novaluron: $z = 1.27$, df = 74, $P = 0.285$). This trend was the opposite from what was expected as significantly more novaluron-treated males mated than control males. Other stimulatory effects have been reported for novaluron, including an increase in fecundity of young adult Colorado potato beetles exposed to novaluron[42] and greater mass of second instar larvae from eggs treated with novaluron than larvae from control eggs.[43] Hatching rate in the mated females ranged from 93.3% to 100%, indicating that high concentrations of novaluron fed to adult males did not affect their fertility. The effect of novaluron concentration on ootheca hatch was not significant (GLM, $P = 0.295$) and there were no significant differences between any of the groups (GLM; 0% and 0.1% novaluron: $z = 0.279$, df = 63, $P = 0.781$; 0% and 0.5% novaluron: $z = 0.005$, df = 63, $P = 0.996$; 0.1% and 0.5% novaluron: $z = 0.005$, df = 63, $P = 0.996$). Also, there was no significant difference between the number of nymphs that hatched from oothecae resulting from matings with males fed 0% and 0.5% novaluron in chow in the first ovarian cycle (*t*-test, $t = 2.01$, df = 47, $P = 0.171$).

Triflumuron, chlorfluazuron, hexafluron, and UC 84572 were also found not to inhibit male spermatogenesis or spermatophore formation, and were not transferred to females in sufficient amounts during copulation to cause ovicidal effects.[18] However, 0.5% noviflumuron was reported to cause complete sterility in both the first and second ovarian cycles of untreated virgin females that were mated with treated males.[15] This could have occurred through mating, other physical contact, or from horizontal transfer of contaminated male feces to females, as shown for the German cockroach (noviflumuron)[44] and other insect species (diflubenzuron).[45] In our study, males were exposed to novaluron for 13 days, then untreated rodent chow for 24 h before contact with females. This procedure likely minimized transfer of novaluron through noncopulatory routes, such as cuticular contact or in feces.

Since cuticle deposition continues even in the adult stage, we considered that exposure to novaluron might deform or weaken the male genitalia. We examined the phallomere of males that failed to mate; we found no abnormalities, consistent with other reports on the effects of CSIs. Overall, novaluron did not affect mating success and fertility of males, and the effects of novaluron were not passed from males to females.

3.5 Effectiveness of novaluron by ingestion *vs* topical application

In crop protection, CSIs are most often applied to foliage as residual insecticides. Pest management indoors is more reliant on baits, which are generally safer, more bioavailable, effective, require less AI, and result in less translocation.[46,47] Our comparison of ingestion and topical application demonstrated that both routes of delivery of novaluron are similarly effective in nymphs and adult females (Fig. 5). Although in nymphs 0.1 µg of novaluron was more effective by topical application (62% mortality) than by ingestion (14% mortality) (Fig. 5(A)), in females nearly all oothecae failed to hatch with either mode of delivery of novaluron (Fig. 5(B)). In both nymphs and adults 100% efficacy was demonstrated with 1 µg of novaluron by either topical application or ingestion.

These results confirm that in our experiments ingestion was the primary mode of novaluron delivery to nymphs and adult females. Although it is possible that cockroaches were also exposed to novaluron through contact, especially in assays that delivered the novaluron-augmented food in small Petri dishes, the dose–response results suggest that most of the novaluron was ingested. While topical application of novaluron was as effective as ingestion, cockroaches would pick up only a small fraction of the ingested dose by contacting the bait.

3.6 Chitin synthesis inhibitors in German cockroach management

Insect growth regulators that have been registered for German cockroach control are mostly JHAs. Although CSIs have been

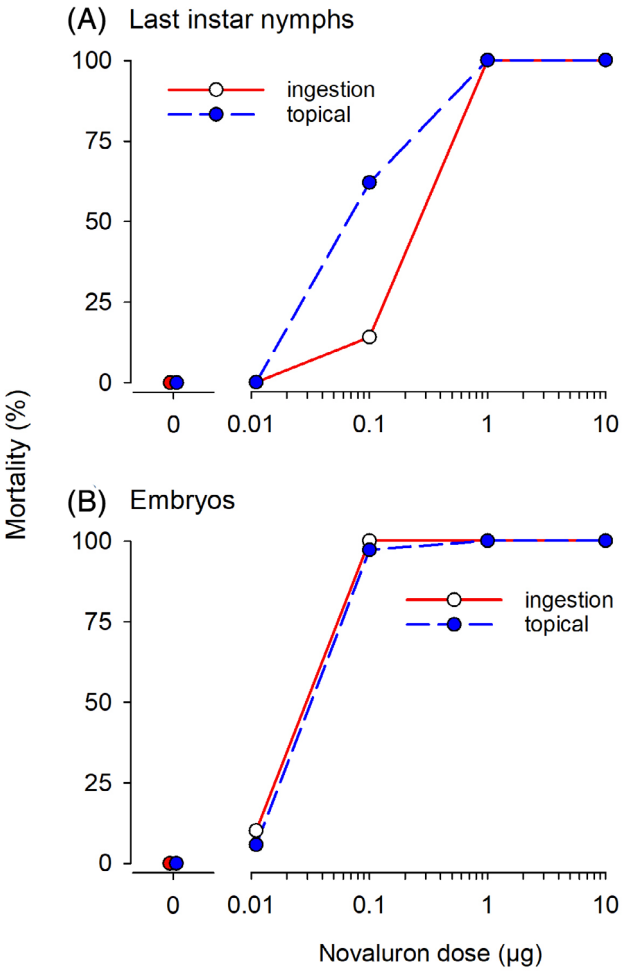

Figure 5. Comparisons of ingested and topically applied novaluron in last instars and adult females. Last instars (A) and adult females (B) were fed 5 mg rodent chow that delivered a known dose of novaluron or topically treated with a known dose of novaluron in 1 µL acetone (delivered as two successive 0.5 µL applications). Number of cockroaches assayed was 21 nymphs and 18–35 adult females per dose.

shown to be effective in managing cockroach populations, they are mainly used to manage crop and forest pests, and to our knowledge have not been commercialized for cockroach control. An experimental gel bait containing noviflumuron was consumed by cockroaches even when alternative food sources (white bread and rodent chow) were present, and more than a fipronil-based cockroach gel bait,[14] showing that baits with CSIs may be competitive with other insecticidal bait products. Novaluron has similar insecticidal activity to noviflumuron and was shown in our studies to be palatable, suggesting that this active ingredient could also be incorporated into cockroach baits. Novaluron in a bait formulation has already shown success controlling subterranean termite infestations,[22] so it is plausible that it might also be effective in managing *B. germanica* infestations.

German cockroaches have developed resistance to active ingredients in some gel bait products, so there is a need to incorporate insecticides with diverse modes of action and formulations into IPM programs to delay and mitigate the evolution of insecticide resistance. Novaluron's unique mode of action, low mammalian toxicity, and efficacy even on IGR-resistant agricultural pests[19–21,48] make novaluron a particularly compelling insecticide for bait development. A primary need is to formulate novaluron into attractive and palatable baits and evaluate its efficacy on field populations of *B. germanica*, likely in combination with adulticides.

Novaluron, like other CSIs, might be effective in preventing the growth of cockroach populations by causing mortality of nymphs and inhibiting adults from producing viable oothecae. Assuming extensive feeding on the bait, suppression of cockroach populations might be achieved faster with CSIs than with JHAs[5,16] because CSIs affect all stages that molt (including embryos) whereas JHAs mainly target the imaginal molt. CSIs also can be used in combination with other active ingredients that serve as adulticides, so that all life stages of the German cockroach are targeted. Moreover, CSIs can be incorporated into bait rotation schemes to manage insecticide resistance.

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CONFLICT OF INTEREST

Parts of this project were supported by Bayer CropScience, and AK is an employee of Bayer CropScience. JH, AW-K, and CS have no other conflict to declare.

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