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Toxicity of isocycloseram, an isoxazoline insecticide, against laboratory and field-collected German cockroaches (Blattodea: Ectobiidae)

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Isocycloseram is a new insecticide in the isoxazoline class that targets insect GABA-gated chloride channels. In this study, we evaluated a cockroach gel bait formulation containing 1% isocycloseram against a susceptible strain (UCR) and 5 field-collected strains (WM, RG386, Ryan, CDR, and SY) of the German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae), and compared it with several commercial insecticide baits in the laboratory. Using the Ebeling choice box method, we also tested a residual deposit of an SC formulation of isocycloseram against the UCR, RG386, and Ryan strains. The isocycloseram bait was among the fastest-performing treatments against adult males (mean survival time: 0.9–2.7 days) and mixed stages and sexes (mean survival time: 1.4–5.4 days) across all strains. Secondary transfer effects of the bait were demonstrated in the UCR strain by exposing new adult males to individuals killed by direct bait treatment. Physiological resistance was not detected in the WM, CDR, and RG386 strains with topical treatment of a diagnostic dose (3× LD_{95}) of isocycloseram developed using the UCR strain. However, topical assays revealed resistance ratios (RR_{50}) of 1.6 and 3.0× in the Ryan and SY strains, respectively. The performance of a 0.05% isocycloseram residual application against the Ryan strain was improved with the addition of piperonyl butoxide.

Key words: Plinazolin, insecticide resistance, piperonyl butoxide, secondary transfer

Introduction

The German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae), is one of the most important indoor insect pests of public health importance worldwide (Lee and Wang 2021). Two significant health risks are associated with German cockroach infestation in homes: transmission of pathogenic microbes (also as vectors of antibiotic resistance genes) and producers of metabolites that could trigger allergies and asthma (Schal and DeVries 2021). The control of this species has relied heavily on the use of insecticides. Frequent usage and heavy reliance on insecticides have led to the development of widespread resistance in the German cockroach (Scharf and Gondhalekar 2021, Lee et al. 2022a).

Adopting new insecticides for German cockroach management requires caution because of widespread resistance in the species. Resistance management strategies such as rotations and mixtures are often recommended to ensure proper control and to preempt further resistance development (Scharf and Gondhalekar 2021). Because implementing these methods depends on the availability of insecticides across different modes of action, introducing compounds with unique mechanisms is vital to continue using common resistance management strategies.

Isocycloseram, also known as Plinazolin, belongs to the novel class of isoxazolines (Cassayre et al. 2021). It is categorized under the IRAC Mode of Action Group 30 along with meta-diamides (GABA-gated chloride channel allosteric modulators). It is active at low rates against a broad spectrum of arthropod pests such as Lepidoptera, Coleoptera, Thysanoptera, and Diptera (Blythe et al. 2022, Palumbo 2022a, 2022b, Buzza and Alyokhin 2023). To date, no commercial insecticides for *B. germanica* share the same mode of action as isocycloseram.

However, even the judicial introduction of compounds with categorically different modes of action can lead to resistance issues due to the German cockroach's preexisting or fast-developing cross-resistance mechanisms (Fardisi et al. 2019). For example, despite having decades separating their usages, the *Rdl* mutation of the GABA chloride channel was a source of early fipronil resistance because of prior selection from cyclodiene treatment (Kristensen et al. 2005). Broad families of detoxifying enzymes, such as the P450 monooxygenases and esterases, collectively play a crucial role in resistance toward many insecticides and can be a source of preexisting resistance or quickly develop in response to exposure (Hawkins et al. 2019, Lee et al. 2022b, Scharf et al. 2022, Tisgratog et al. 2023). Thus, evaluating isocycloseram against strains of German cockroaches that are resistant to different bait toxicants is necessary to predict its potential in the field.

In this study, we conducted 4 experiments: (i) Evaluation of a 1% isocycloseram bait formulation developed by Syngenta against adult males and mixed stages + sexes of the susceptible UCR strain and 5 field-collected strains (WM, RG386, Ryan, CDR, and SY) in comparison with commercial bait products in laboratory assays. (ii) Investigation of the secondary transfer effects using the UCR strain by exposing adult males to cockroaches killed by the bait. (iii) Contact toxicity of isocycloseram with topical applications on the UCR strain and determine the diagnostic dose to apply to field-collected strains to monitor for resistance. Lastly, (iv) the efficacy of isocycloseram residual spray was assessed using Ebeling choice boxes, and the performance of isocycloseram on the Ryan strain was further investigated with the addition of piperonyl butoxide (PBO).

Materials and Methods

Cockroach Strains

The strains WM, RG386, Ryan, CDR, and SY originated from field populations collected from 2018 to 2020 in California and kept in laboratory conditions of 24 ± 2 °C, 30%–50% RH, and 12:12 L:D photoperiod (Lee et al. 2022a). The WM, RG386, Ryan, CDR, and SY strains are resistant to insecticides including deltamethrin, fipronil, clothianidin, indoxacarb, DDT, and dieldrin (Lee et al. 2022a, 2022b), and have not been selected with insecticides while in the laboratory. The UCR strain, originally from the Orlando-normal strain, is a laboratory-susceptible strain that has been reared for >40 years without insecticide exposure. All strains were reared in 121-liter garbage bins equipped with electrical barriers and provided dog food (Purina Dog Chow, Nestlé Purina Petcare, St. Louis, MO, USA), cardboard harborages, and water ad libitum.

Insecticides and Chemicals

The following baits (% active ingredient) were used in bait assays: isocycloseram bait provided by Syngenta (1%, Syngenta Crop Protection LLC, Greensboro, NC, USA), Advion Cockroach Gel Bait (0.6% indoxacarb, Syngenta Crop Protection LLC, Greensboro, NC, USA), Advion Evolution Cockroach Gel Bait (0.6% indoxacarb, Syngenta Crop Protection LLC, Greensboro, NC, USA), Alpine Cockroach Gel Bait Rotation 1 (0.5% dinotefuran, BASF Corporation, Research Triangle Park, NC, USA), Maxforce FC Magnum Roach Gel Bait (0.05% fipronil, Bayer Environmental Science, Research Triangle Park, NC, USA), Siege 2% Gel Bait (2% hydramethylnon, BASF Corporation, Research Triangle Park, NC, USA), Optigard Cockroach Gel Bait (0.1% emamectin benzoate, Syngenta Crop Protection LLC, Greensboro, NC, USA), and Vendetta Cockroach Gel Bait (0.05% abamectin, MGK Company, MN, USA). Isocycloseram technical material (≥90% to <100%, Syngenta Crop Protection LLC, Greensboro, NC, USA) was used in topical assays. An isocycloseram residual formulation (SC 400) (Syngenta Crop Protection LLC, Greensboro, NC, USA) and piperonyl butoxide (PBO) (90% tech., Sigma–Aldrich, St Louis, MO, USA) were used in the choice box assay.

Experiment 1a: Performance of Cockroach Gel Baits

The performance of the following cockroach gel baits described above was evaluated: 1% isocycloseram, Advion Evolution Cockroach Gel Bait, Alpine Cockroach Gel Bait Rotation 1, Maxforce FC Magnum Roach Gel Bait, Optigard Cockroach Gel Bait, and Siege 2% Gel Bait. Ten adult male cockroaches were acclimatized for 2 days in the test arena (27.5 cm × 20 cm × 9 cm) with dog food, a cardboard harborage, a distilled water source, fluon on the inner arena wall surface to prevent escape, and a sheet of filter paper covering the arena bottom (to increase the traction of cockroach locomotion). A 0.3 g placement of bait was placed on a weigh boat and introduced into the arena, establishing a choice test. Controls were not provided with a bait application. Each bait was replicated 3 times. Mortality was observed every 2 h for 2 days and then every 12 h until 14 days. Cockroaches were considered dead when they could not move or turn upright within 2 min when touched with forceps (Lee et al. 1996, Chai and Lee 2010). Survivorship was calculated using the Kaplan–Meier method and compared with log-rank tests in SPSS version 28 (IBM Corporation, Armonk, NY, USA).

Experiment 1b: Performance of Isocycloseram Bait Against Mixed Stages and Sexes

Ten adult males, 10 nongravid adult females, and 20 nymphs (third–fourth instar, mixed sex) of a cockroach strain (UCR, RG386, or Ryan) were acclimatized for 2 days in a test arena (30.5 cm × 47 cm × 30.5 cm) with dog food, a cardboard harborage, distilled water, and petroleum jelly on the walls to prevent escape. A 1.0 g bait application (1% isocycloseram, Advion Cockroach Gel Bait, Alpine Cockroach Gel Bait Rotation 1, Maxforce FC Magnum Roach Gel Bait, or Vendetta Cockroach Gel Bait) in a weigh boat was introduced into individual arenas, establishing a choice test of an insecticide bait and dog food. Each bait was tested independently. Controls were not provided with a bait application. Each bait was replicated 4 times. Mortality was recorded at selected time intervals for 5 days (susceptible strain) or 14 days (resistant strains). The UCR strain was observed for a shorter period due to faster responses to the treatments. Cockroaches were considered dead when they were unable to move or turn upright within 2 min after being gently probed with a pair of forceps. Mortality of all stages and sexes was pooled, and survivorship was calculated with the Kaplan–Meier method and compared with log-rank tests in SPSS version 28.

Experiment 2: Secondary Transfer Effects of Cockroach Gel Baits

The secondary transfer effects of 5 cockroach gel baits were evaluated using the UCR strain: 1% isocycloseram, Advion Evolution Cockroach Gel Bait, Alpine Cockroach Gel Bait Rotation 1, Maxforce FC Magnum Roach Gel Bait, and Vendetta Cockroach Gel Bait. Ten adult males were introduced into an arena (28 cm × 15 cm × 11 cm) with a water source, a folded cardboard harborage, dog food, and petroleum jelly on the inner walls to prevent escape. After 24 h of acclimatization, 1 g of bait was introduced, and mortality was recorded every 12 h up to 7 days. The cockroaches were considered dead when they were immobile and unresponsive when

gently touched with forceps to ensure that the slight movement from moribundity would not affect the secondary exposure group. Controls were not provided bait. Each treatment was replicated 5 times. When all cockroaches were dead, the bait was removed, and a new set of ten adult males was introduced into the same arena containing a water source, a folded cardboard harborage, dog food, and the dead cockroaches. As defined above in this section, mortality was recorded every 12 h up to 7 days using the same criterion.

Experiment 3: Topical Toxicity of Isocycloseram

The topical toxicity of isocycloseram on the UCR, Ryan, and SY strains was measured using a range of doses, causing ~5%–95% mortality. The doses applied to the UCR strain were 0.01, 0.013, 0.019, 0.025, 0.037, and 0.05 µg/insect. The doses applied to the Ryan strain were 0.01, 0.015, 0.024, 0.0375, and 0.05 µg/insect. The doses applied to the SY strain were 0.01, 0.015, 0.024, 0.0375, 0.05, and 0.075 µg/insect. These doses were prepared by serially diluting technical-grade isocycloseram in acetone. Adult males were briefly (<30 s) anesthetized with CO_2, and a 0.5 µl of the known dose of isocycloseram was applied to the abdominal sternites using an Isco Model M microapplicator (Instrumentation Specialties, Seward, NE, USA). Cockroaches were provided food, water, and harborage after the treatment, and mortality was scored at 72 h. Cockroaches were considered dead when they were immobile and unresponsive when touched with forceps. Ten individuals were used per replicate, and each dose was replicated 8–12 times. Controls were treated with acetone. The data were fitted to a probit model using PoloPlus (LeOra Software LLC, Petaluma, CA, USA) to generate the lethal doses: LD_{50} and LD_{95}. Adult males of the UCR, Ryan, and SY strains (n = 30–36) were killed with HCN gas (~20-min exposure) and weighed with a microbalance (Sartorius AG, Göttingen, Germany) to determine whether average mass significantly affected the proportional doses received per strain.

The LD_{95} of the UCR strain was 0.047 µg/insect (see Table 4). A diagnostic dose using the 3× LD_{95}, 0.14 µg/insect, was used to assess the susceptibility of field strains based on the method described by Lee et al. (2022a) (Georghiou and Mellon 1983, Mota-Sanchez et al. 2008). The UCR strain was also tested to confirm that the diagnostic dose causes complete mortality in the susceptible strain. Adult males were anesthetized with CO_2, and the diagnostic dose was applied to the first or second abdominal sternites with the above-mentioned microapplicator. Cockroaches were kept with food, water, and harborage, and mortality was recorded at 72 h posttreatment. Strains that had ≥10% survivors (Ryan and SY strains) were further examined by conducting topical assays and probit analysis to determine their LD_{50} values in an identical manner as the UCR strain.

Experiment 4: Performance of Isocycloseram Residual Formulation (400 SC) Using Ebeling Choice Boxes

The performance and potential field efficacy of residual treatment of isocycloseram were assessed with the Ebeling choice boxes (Ebeling et al. 1966). The boxes were constructed from white pine drawer siding (30.5 cm × 9.5 cm) with a tempered Masonite floor. The tops were covered in plexiglass and divided into 2 equal-sized compartments, light and dark, with the dark side covered with a piece of Masonite to prevent light from entering. A small hole at the top center of the divider allowed the cockroaches to move between the light and dark compartments. Panels of unpainted wood (30.8 cm × 15.2 cm × 0.8 cm) were sprayed with 3 ml of 0.05% isocycloseram aqueous preparation (0.011 mg/cm^2) using an airbrush (Master Hi-Flow AllPurpose, TCP Global, San Diego, CA, USA) and dried for 24 h. Another set of panels was sprayed with 3 ml of 0.05% isocycloseram and allowed to dry for 1 h, then sprayed with 3 ml of 0.5% PBO in acetone solution (0.11 mg/cm^2). Isocycloseram-PBO treated panels were allowed to dry for 24 h.

An isocycloseram-treated panel was placed on the floor of the dark compartment, and 20 adult male cockroaches of the UCR, RG386, or Ryan strains were confined in the light compartment for 5–6 h before being allowed to move freely throughout the box. Three replicates per strain were conducted for each treatment and untreated control. The Ryan strain was tested with isocycloseram + PBO treated panels and isocycloseram alone. Experiments were run under a photoperiod of 12:12 h (L:D), and the number of dead and alive cockroaches in the light and dark compartments was recorded for 14 days. The performance index (PI) was calculated to assess the combined effects of mortality and repellency, with a PI of 100 indicating complete mortality and no repellency, a PI of 0 indicating no mortality and no repellency, and a PI of −100 indicating no mortality and complete repellency (Rust and Reierson 1978):

$$\mathrm{PI} = \left\{1 - \left(\frac{\text{Total number alive} + \text{Number alive in light side}}{\text{Total number dead} + \text{Total number}}\right)\right\} \times 100$$

Results

In 5 of 6 strains, the 1% isocycloseram bait performed comparably or superior to the other baits against adult males. No difference in survivorship was detected in the UCR strain between 1% isocycloseram and Maxforce FC Magnum, Advion Evolution, and Optigard. However, Alpine performed faster, and Siege performed slower than isocycloseram (Fig. 1A). For the WM, RG386, Ryan, and CDR strains, 1% isocycloseram, along with Advion Evolution and Optigard (except for CDR), had the greatest impact on survivorship (Fig. 1B–E). For the SY strain, Alpine caused a comparable decrease in survivorship compared to 1% isocycloseram, although Alpine treatments resulted in incomplete mortality (90%), and 1% isocycloseram killed all insects (Fig. 1F; Table 1). There were significant differences ($P < 0.05$) in survivorship between the UCR strain and resistant strains treated with the 1% isocycloseram bait, with a mean survival time of 0.9 days for the UCR strain and 1.2–2.7 days for the resistant strains (Table 1; Supplementary Fig. S1). Except for the Ryan strain, which ended at 96.7% mortality by 14 days, the 1% isocycloseram bait completely killed the adult males of every strain within the 14-day evaluation period (Table 1).

When treating mixed stages and sexes of the UCR strain, 1% isocycloseram showed comparable performance to Alpine and Maxforce FC Magnum but was more efficacious than the Advion Evolution and Vendetta baits (Fig. 2A). The 1% isocycloseram bait was the fastest performing bait against RG386 mixed stages (Fig. 2B; Table 2). The 1% isocycloseram bait, Advion Evolution, and Maxforce FC Magnum were most efficacious against the Ryan mixed stages based on survivorship (Fig. 2C). None of the treatments caused complete mortality in any strain, and mortality from 1% isocycloseram ranged from 82.6% to 95.7% (Table 2).

In the secondary transfer effect experiment, the 1% isocycloseram bait resulted in the fastest decrease in survivorship for the UCR strain adult males under direct exposure, resulting in a mean survival time of 1.3 days (Fig. 3A; Table 3). Survivorship under secondary transfer conditions was also lowest for isocycloseram-exposed cockroaches, with a mean survival time of 1.5 days vs. 1.8–2.9 days with the other baits (Fig. 3B; Table 3).

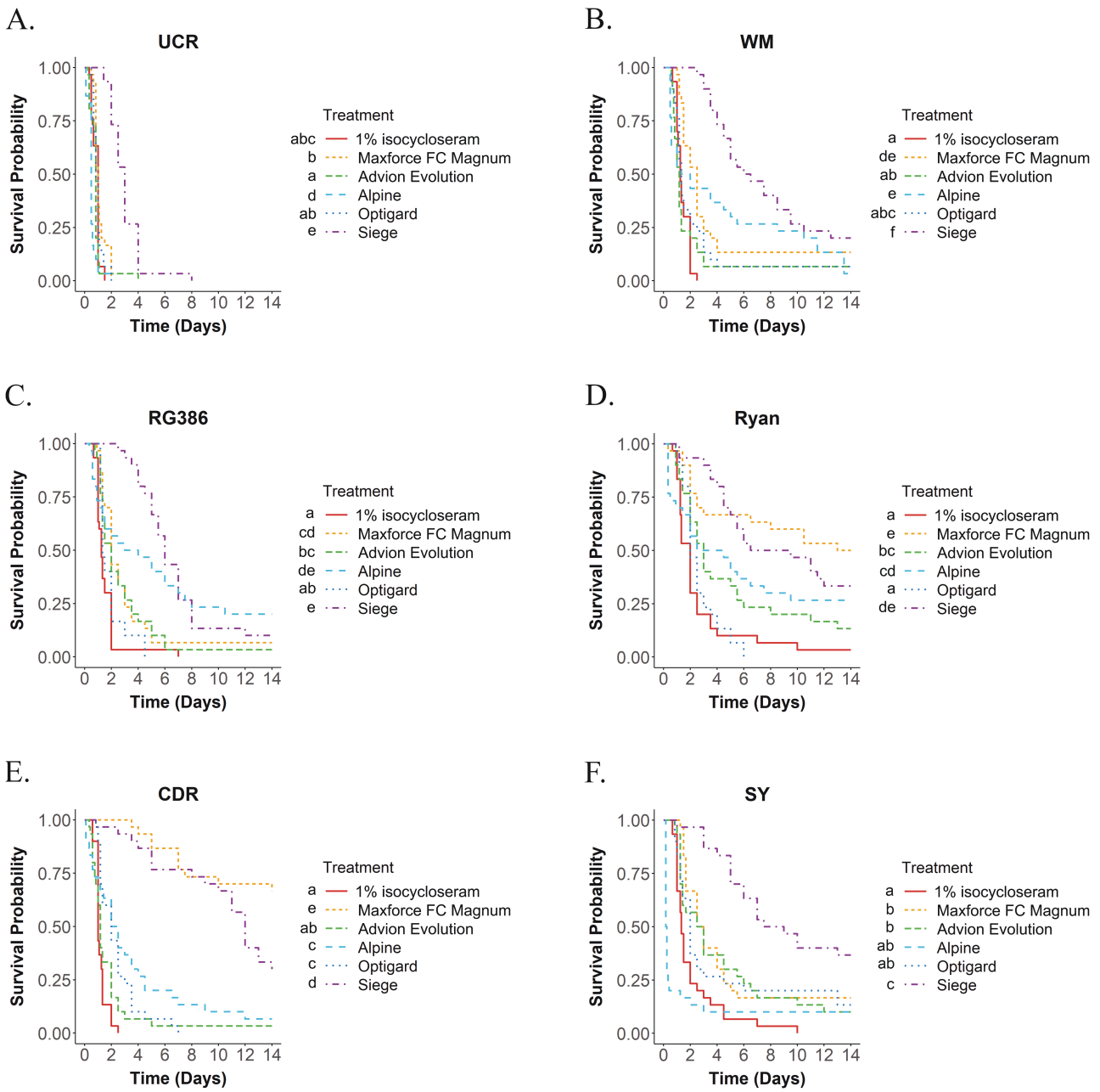

Fig. 1. Survivorship of adult male cockroaches of the A) UCR, B) WM, C) RG386, D) Ryan, E) CDR, and F) SY treated with baits. Different letters by the figure legend indicate significant differences between treatments (log-rank test; $\alpha = 0.05$).

Topical applications of isocycloseram on the UCR adult males resulted in a 72 h LD_{50} of 0.015 (0.013–0.017) µg/insect and LD_{95} of 0.047 (0.040–0.059) µg/insect (Table 4). Mortality of the field strains from topical applications of the diagnostic dose (3 × LD_{95}) (0.14 µg/insect) was 97.5%–100% for WM, CDR, and RG386 strains and 90% for the Ryan and SY strains. The LD_{50} of isocycloseram for Ryan strain adult males was 0.022 (0.019–0.024) µg/insect, resulting in a resistance ratio (RR_{50}) of 1.6 (1.4–1.7) (Table 4). The LD_{50} of isocycloseram for SY strain adult males was 0.042 (0.036–0.048) µg/insect, resulting in an RR_{50} of 3.0 (2.6–3.6) (Table 4).

The UCR and RG386 strains had similar slopes in the choice box assay. They reached PIs of 100, indicating complete kill (Fig. 4). Isocycloseram alone did not cause complete mortality for the Ryan strain, with a PI of ~69.4. At the same time, the combination of PBO and isocycloseram resulted in complete mortality (Fig. 4). The lack of negative PI suggests that there was no repellency detected in the isocycloseram residual treatment (Supplementary Table S1).

Discussion

When tested against adult male German cockroaches of susceptible and field-collected strains, the 1% isocycloseram bait showed comparable performance to the other the gel baits evaluated. Despite overt resistance toward some baits, such as Siege, Alpine, and Maxforce FC Magnum, the performance of the isocycloseram bait was not similarly compromised, indicating a lack of cross-resistance to the isocycloseram bait, supporting similar findings of previous studies on other insect species (Sun et al. 2023). The strain-wise comparison

Table 1. Survival time and mortality of adult male cockroaches treated with baits

Strain	Treatment	Mean survival time (days)	95% CI	% Total mortality
UCR	1% isocycloseram	0.9	0.8–1.0	100.0
	Maxforce FC Magnum	1.1	1.0–1.3	100.0
	Advion Evolution	0.9	0.6–1.1	100.0
	Alpine	0.5	0.4–0.7	100.0
	Optigard	1.0	0.8–1.1	100.0
	Siege	3.0	2.6–3.4	100.0
	Control	—	—	0.0
WM	1% isocycloseram	1.4	1.2–1.6	100.0
	Maxforce FC Magnum	3.7	2.3–5.2	86.7
	Advion Evolution	2.1	1.0–3.3	93.3
	Alpine	4.4	2.7–6.2	96.7
	Optigard	2.4	1.3–3.6	93.3
	Siege	7.6	6.1–9.0	80.0
	Control	—	—	0.0
RG386	1% isocycloseram	1.5	1.2–2.0	100.0
	Maxforce FC Magnum	3.1	2.0–4.2	93.3
	Advion Evolution	2.8	1.9–3.7	96.7
	Alpine	5.4	3.6–7.2	80.0
	Optigard	1.9	1.5–2.3	100.0
	Siege	6.7	5.6–7.8	90.0
	Control	—	—	2.5
Ryan	1% isocycloseram	2.7	1.7–3.7	96.7
	Maxforce FC Magnum	9.3	7.3–11.2	50.0
	Advion Evolution	5.0	3.4–6.6	86.7
	Alpine	5.7	3.8–7.7	73.3
	Optigard	2.7	2.2–3.1	100.0
	Siege	8.6	7.0–10.3	66.7
	Control	—	—	5.0
CDR	1% isocycloseram	1.2	1.0–1.3	100.0
	Maxforce FC Magnum	11.7	10.3–13.1	33.3
	Advion Evolution	1.8	1.0–2.7	96.7
	Alpine	3.6	2.2–5.0	93.3
	Optigard	2.4	1.8–2.9	100.0
	Siege	10.4	8.9–11.9	70.0
	Control	—	—	7.5
SY	1% isocycloseram	2.1	1.4–2.9	100.0
	Maxforce FC Magnum	4.6	3.1–6.2	83.3
	Advion Evolution	4.4	2.9–5.9	90.0
	Alpine	1.8	0.3–3.3	90.0
	Optigard	4.3	2.6–6.0	86.7
	Siege	9.0	7.4–10.6	63.3
	Control	—	—	5.0

revealed a temporal delay in mortality between the resistant strains and the UCR strain, indicating that slower performance may be observed when treating field populations (Table 1; Supplementary Fig. S1). Nonetheless, complete mortality was consistently reached within 14 days for all strains (except 96.7% for Ryan).

Tests on mixed stages and sexes of the UCR, RG386, and Ryan strains showed that the isocycloseram bait retained its comparative efficacy relative to commercial competitor baits as one of the fastest-acting treatments (Fig. 2). However, unlike in the bioassays solely treating adult males, there were survivors at the end of all the exposure periods irrespective of strain or bait. For the UCR strain, this is likely due to the 5-day observational period instead of 14 days for the other strains. For the RG386 and Ryan strains, reoccurring incomplete mortality runs a risk of resistance propagation and treatment failure. Recommended reapplication intervals for conventional baits ranges from 2 to 4 weeks, which would contribute to eliminating the remaining cockroaches (Appel and Rust 2021). The tendency to have a fraction of survivors was not a unique issue for the isocycloseram bait alone and was observed for all bait treatments.

When tested against adult males of UCR strain, the isocycloseram bait showed secondary transfer effects as with the other bait products, with every treatment exerting complete kill within 7 days via secondary exposure. Because cockroaches are not guaranteed to interact directly with bait applications in the field, demonstrating that they can be killed through exposure to treated conspecifics provides additional utility (Appel and Rust 2021). Also, in both direct treatment and secondary transfer experiments, the isocycloseram bait maintained its advantage as the fastest-acting bait, suggesting a high level of bioavailability from carcasses, physically transferred residues, feces, and/or vomitus of poisoned cockroaches (Buczkowski et al. 2001, 2008).

The LD_{50} of the UCR strain at 72 h was 0.015 µg/insect, making isocycloseram a moderately active compound compared to other conventional neurotoxic insecticides, being less toxic than deltamethrin and fipronil (LD_{50} = 0.0046 and 0.0013 µg/insect, respectively), but

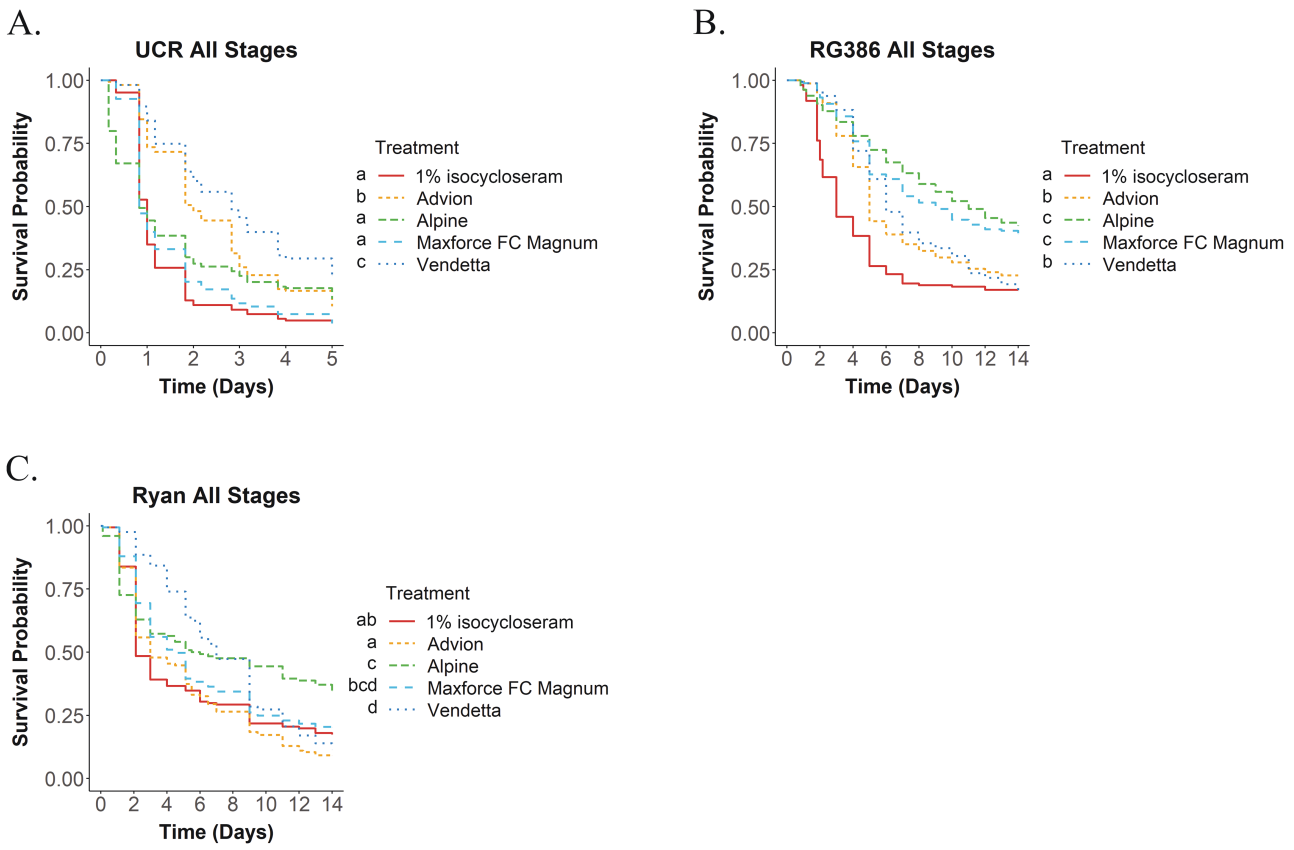

Fig. 2. Survivorship of mixed stages and sexes of the A) UCR, B) RG386, and C) Ryan strains treated with baits. Different letters by the figure legend indicate significant differences between treatments (log-rank test; $\alpha = 0.05$).

Table 2. Survival time and mortality of mixed stages and sexes treated with baits

Strain	Treatment	Mean survival time (days)	95% CI	% Total mortality[a]
UCR	1% isocycloseram	1.4	1.2–1.5	95.7
	Advion	2.5	2.2–2.7	77.3
	Alpine	1.7	1.5–2.0	86.6
	Maxforce FC Magnum	1.5	1.3–1.7	96.3
	Vendetta	3.0	2.7–3.2	89.5
	Control	–	–	10.0
RG386	1% isocycloseram	5.1	4.5–5.8	83.0
	Advion	7.1	6.4–7.8	77.1
	Alpine	9.5	8.8–10.2	57.7
	Maxforce FC Magnum	9.0	8.2–9.7	60.9
	Vendetta	7.7	7.0–8.3	83.2
	Control	–	–	4.4
Ryan	1% isocycloseram	5.4	4.5–6.1	82.6
	Advion	5.3	4.6–5.9	90.8
	Alpine	7.5	6.5–8.5	65.3
	Maxforce FC Magnum	6.3	5.5–7.0	79.6
	Vendetta	7.6	7.0–8.3	88.5
	Control	–	–	2.6

[a]Mortality at 14 days for RG386 and Ryan, 5 days for UCR.

more toxic than indoxacarb and clothianidin (LD_{50} = 0.1100 and 0.0199 µg/insect, respectively) (Lee et al. 2022a). Topical treatments with the 3× LD_{95} of the UCR strain (diagnostic dose) caused high mortality (>97.5%) in the WM, CDR, and RG386 strains, suggesting a lack of contact resistance towards isocycloseram (ffrench-Constant and Roush 1990). The Ryan and SY strains had 10% survivors from the diagnostic dose and were 1.6 and 3.0× less sensitive based on topical application, respectively (Table 4). Although low levels of insensitivity do not necessarily cause observable problems with treatment efficacy, it is a sign that alleles that decrease susceptibility are present in a subset of the population (ffrench-Constant and Roush 1990).

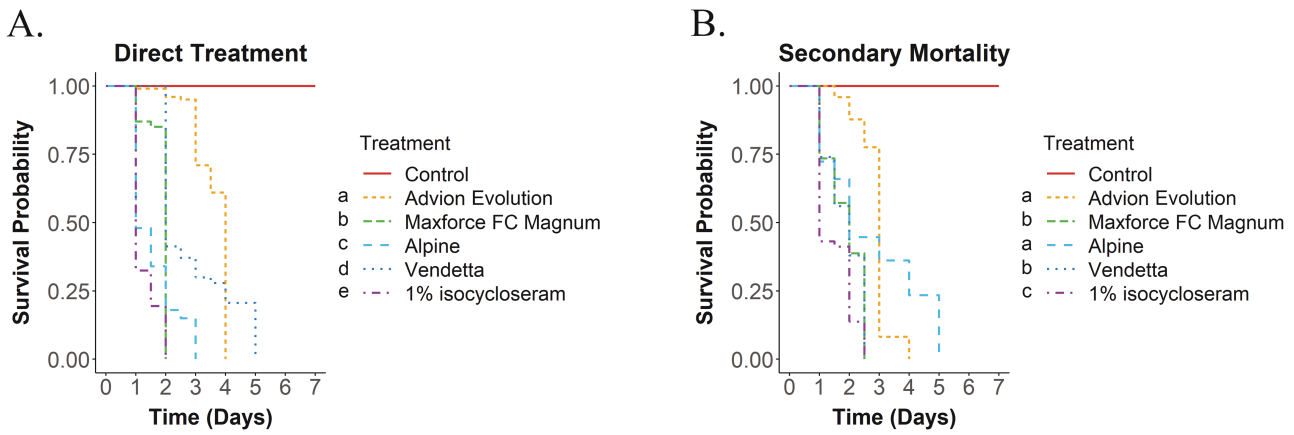

Fig. 3. Survivorship of UCR adult males from A) direct bait treatment and B) secondary mortality exposure. Different letters by the figure legend indicate significant differences between treatments (log-rank test; $\alpha = 0.05$).

Table 3. Survival time and mortality of the UCR strain adult males from direct bait treatment and secondary mortality exposure

Exposure type	Treatment	Mean survival time (days)	95% CI	% Total mortality at 7 days
Direct	Control	—	—	0.0%
	Advion Evolution	3.6	(3.5–3.7)	100.0%
	Maxforce FC Magnum	1.9	(1.8–1.9)	100.0%
	Alpine	1.6	(1.4–1.7)	100.0%
	Vendetta	2.9	(2.6–3.1)	100.0%
	1% isocycloseram	1.3	(1.2–1.4)	100.0%
Secondary	Control	—	—	0.0%
	Advion Evolution	2.9	(2.7–3.0)	100.0%
	Maxforce FC Magnum	1.9	(1.7–2.0)	100.0%
	Alpine	2.7	(2.3–3.2)	100.0%
	Vendetta	1.8	(1.7–2.0)	100.0%
	1% isocycloseram	1.5	(1.3–1.7)	100.0%

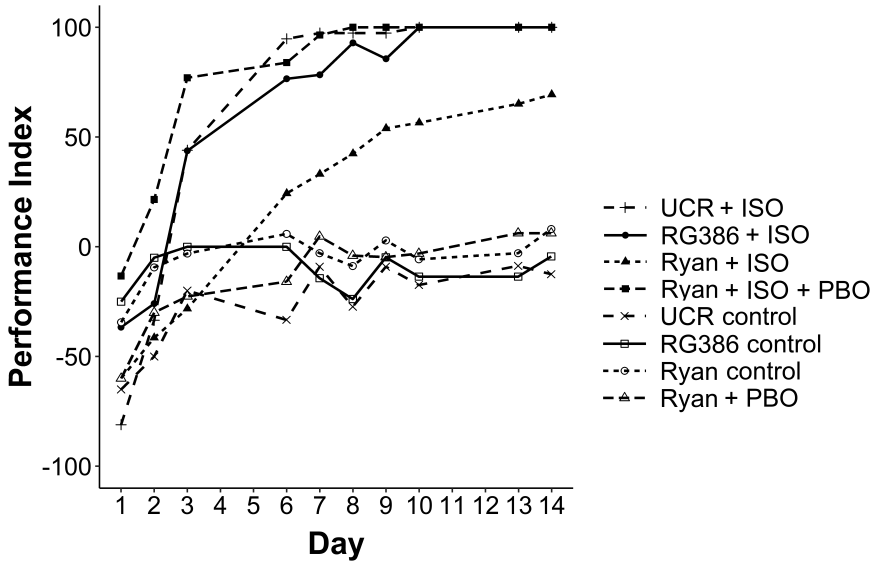

Fig 4. Response of the UCR, RG386, and Ryan adult males exposed to treated panels (0.05% isocycloseram [ISO], 0.5% piperonyl butoxide [PBO], and/or no treatment [control]) in the choice box assay.

Variation in susceptibility to isocycloseram among field-collected populations with known resistance to various classes of insecticides suggests that establishing proper rates to control all individuals in cockroach populations will minimize the chance for the escape of more tolerant individuals, which may lead to the development of a resistant population. Such variations in susceptibility were most clearly reflected in the choice box assay, in which the UCR and RG386 strains reached PIs of 100 when exposed to panels treated

Table 4. Topical toxicity of isocycloseram on the UCR, Ryan, and SY adult males at 72 h

Strain	*n*	LD_{50} (95% CI) (µg/insect)[a]	LD_{95} (95% CI) (µg/insect)	Slope	SE	χ^2 (df)	RR_{50}[b] (95% CI)
UCR	601	0.015 (0.013–0.017)	0.047 (0.040–0.059)	3.317	0.322	1.250 (4)	—
Ryan	290	0.022 (0.019–0.024)	0.065 (0.053–0.091)	3.456	0.403	2.515 (3)	1.6 (1.4–1.7)
SY	310	0.042 (0.036–0.048)	0.175 (0.127–0.288)	2.637	0.313	0.590 (4)	3.0 (2.6–3.6)

[a]Average mass of adult males: UCR (52.37 ± 1.0 mg), Ryan (53.18 ± 0.8 mg), SY (53.73 ± 0.8 mg); $P = 0.61$, Kruskal–Wallis test.
[b]Resistance ratio: LD_{50} (µg/insect) of field-collected strain divided by LD_{50} (µg/insect) of the UCR strain.

with isocycloseram alone, but the Ryan strain was only partially killed with ~70% mortality (Fig. 4). Because the Ryan strain did not reach a PI of 100, it was additionally tested using PBO + isocycloseram (Fig 4). The combination of insecticide and synergist increased the potency of the isocycloseram-treated panel, implying the negation of P450 activity in the Ryan strain (Fig. 4). We were unable to collect enough cockroaches of the SY strain to conduct similar comparisons due to the slow development of this strain. Appropriate doses should be identified to ensure that all field strains can be killed to prevent resistance selection when using isocycloseram.

Isocycloseram is classified under IRAC Group 30, which designates GABA-gated chloride channel modulation as its primary mode of action. GABA receptors are reportedly affected by 2 other registered insecticide classes for German cockroach control: phenylpyrazoles (fipronil), for which GABA receptors are the primary target, and avermectins, for which some lesser interactions with the channel can occur (Yu 2014, Casida and Durkin 2015). Although all resistant strains used in the present study have evolved high frequencies of the *Rdl* mutation (A302S) on the GABA chloride channel that confers fipronil resistance (Lee et al. 2022b), they remained mostly susceptible to isocycloseram. This was similar to the findings of Blythe et al. (2022) in which unaltered isocycloseram susceptibility was documented in *Drosophila melanogaster* Meigen expressing the A301S mutation. This phenomenon was attributed to the distinct binding sites of fipronil and isocycloseram. In contrast, *D. melanogaster* with L280C mutations (generated via gene editing) were less sensitive to topically applied isocycloseram, but this mutation has not been found in field populations (Ozoe et al. 2024). Unlike fipronil, target-site alterations associated with avermectin resistance have never been documented in *B. germanica*. Nonetheless, an abamectin resistance-conferring mutation in *Plutella xylostella* (L.) did not have an effect on isocycloseram toxicity, providing some evidence that target-site insensitivity has not developed simultaneously toward both compounds through currently identified mechanisms (Sun et al. 2023).

The impact of PBO on the performance of isocycloseram-treated panels for the Ryan strain provides evidence for P450-mediated detoxification pathways for this insecticide in *B. germanica* and may explain the slightly reduced susceptibilities of Ryan and SY (Table 4). This result supports the documentation of PBO increasing the sensitivity of *D. melanogaster* to topically applied isocycloseram (Ozoe et al. 2024). Because isocycloseram had never been used to control German cockroaches before these strains were collected from field sites (circa 2020), this is not a result of isocycloseram selection. The diverse P450 enzymatic family in *B. germanica* detoxifies a wide range of insecticides, and preexisting pathways may have been co-opted to target isocycloseram through pleiotropic mechanisms (Harrison et al. 2018, Hawkins et al. 2019). Because these mechanisms already exist, improper use of such compounds may quickly lead to resistance through additional elevation or alteration of P450 activity (Scharf et al. 2022). Like other insecticides, resistance management strategies such as rotating or mixing will theoretically benefit the longevity of isocycloseram by preventing continuous selection pressure.

The present study demonstrates that 1% isocycloseram bait is an effective new formulation against German cockroaches when directly compared to other bait products. Although bait performance cannot solely be attributed to the active ingredient in the formulation due to other vital inert components such as phagostimulants, the topical applications revealed negligible differences in response between the UCR (susceptible), WM, CDR, and RG386 strains toward isocycloseram. While the Ryan and SY strains were less sensitive to topical application, the bait formulation remained among the most effective for these strains (Fig 1D and F). With its consistent performance across different strains, isocycloseram is a promising bait toxicant for the control of the German cockroach.

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Conflict of Interest

David Cox is affiliated with Syngenta Crop Protection. Syngenta Crop Protection provided the isocycloseram bait and residual insecticide and partially funded the study.

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[supporting], Methodology [supporting], Project administration [supporting], Resources [supporting], Writing—review & editing [equal]), Michael Rust (Data curation [supporting], Formal analysis [supporting], Investigation [equal], Methodology [equal], Resources [supporting], Supervision [supporting], Writing—review & editing [supporting]), and Chow-Yang Lee (Conceptualization [lead], Funding acquisition [lead], Methodology [equal], Project administration [lead], Resources [lead], Software [equal], Supervision [lead], Writing—original draft [equal], Writing—review & editing [equal])

Supplementary Material

Supplementary material is available at *Journal of Economic Entomology* online.

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