Effects of Continuous Exposure of German Cockroach Nymphs to Various IGR and Neurotoxic Gel Baits

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ABSTRACT

German cockroaches are an invasive urban pest that pose a significant public health threat due to disease, allergens, and trauma leading to entomophobia. Standard insecticide control methods have become less effective due to developed resistance. Integrated pest management may be the key in sustainable control practices. In this study, the impact of two insect growth regulators, Novaluron and Pyriproxyfen, in standalone baits and in IGR-Indoxacarb mixture baits were tested alongside standalone Indoxacarb baits on German cockroach nymphs. Results showed that Novaluron, Pyriproxyfen, and IGR-Indoxacarb mixture baits caused significant mortality and sterilization in German cockroach nymphs, making them promising for use in future field work.

INTRODUCTION

German cockroaches, also known as *Blattella germanica,* are a flightless domestic species of cockroach from East and Southeast Asia that are now located worldwide in human dwellings (Bonnefoy et al., 2009). *B. germanica* especially thrive in heated homes that contain food and shelter. They have a fast reproduction rate, ranging from 3-4 generations per year depending on temperature. Their development time from egg to adult is around 103 days (Gondhalekar et al., 2021). *B. germanica* are 1 of 24 invasive cockroach species in the USA and can cause serious infestations in homes, posing significant threats to the health of homeowners (Bonnefoy et al., 2009). *B. germanica* are often found in low-income households and apartments where they can cause a variety of issues for residents including mental trauma leading to entomophobia as well as negative impacts faced due to social stigma from others (Gondhalekar et al., 2021). They also can cause physical health problems such as transferring harmful pathogens to humans through contact with food and food handling surfaces. *B. germanica* molt skins, feces and body parts are a significant allergen (Bonnefoy et al., 2009). A single gravid female cockroach can produce a maximum average of 9.6 fecal pellets per day. During their remaining lifetime, they can produce between 25,000 to 50,000 units of allergens while males produce 2000 to 3000. Large scale infestations can therefore have a tremendous impact on the health of people living in an infested home. One issue we are currently facing is the increased resistance to standard control that *B. germanica* has evolved. Currently, *B. germanica* rank as the second most resistant urban pest, with known resistance to 42 active pesticide ingredients (Hou et al., 2021). Indoxacarb is a neurotoxic insecticide that is usually used in the control of *B. germanica*, but as of late, *B. germanica* has

begun developing resistance to it. Scharf et al., (2022) assessed the resistance of current field populations to Indoxacarb. When compared with a susceptible lab strain known as J-wax and a resistant strain known as Oviedo in a no choice feeding bioassay, where the cockroaches only had access to Indoxacarb baits, there was significant difference in mortality. While by the third day of the bioassay the susceptible strain had experienced 100% mortality, the resistant strain saw less than 20% mortality throughout a 21-day period. Because of this resistance, long term suppression of infestations may need the use of alternative pest management strategies. Two insect growth regulators, Novaluron and Pyriproxyfen show promise as possible integrated pest management strategies. Novaluron, developed by Makhteshim-Agan Ltd. in Israel, is a novel chitin synthesis inhibitor that disrupts cuticle formation and ecdysis in cockroaches, leading to molting failure (Cutler & Scott-Dupree, 2007). It belongs to the benzoyl phenyl ureas group and is a larvicide that impacts a broad range of insect orders such as Lepidoptera, Coleoptera, Homoptera, Hymenoptera and Diptera (Kostyukovsky & Trostanetsky, 2006). While its mode of action isn't well known, Novaluron works similarly to other insecticides in its group. Developed by Sumitomo Chemical Co., Ltd., Pyriproxyfen is a juvenile hormone analog that mimics real growth hormones in an insect, leading it to continuously molt past its final instar into supernumerary nymphs (Koehler & Patterson, 1991 and Sullivan & Goh, 2008). It also is known to cause sterilization and prevent embryo development in *B. germanica*. Pyriproxyfen has been more actively used in urban pest management compared to Novaluron and may benefit from the addition of Novaluron in urban pest control. In some studies, evidence has shown that while initially more costly than standard insecticides, IGRs were more effective in controlling *B. germanica* populations, with subsequent treatments of standard insecticides becoming more expensive than IGR treatments in the long run (Miller & Meek, 2004). However, on their own, IGRs take a significant amount of time to control *B. germanica* infestations compared to standard insecticides. As such, there may be promise in using IGRs alongside standard insecticides to make control with them more effective. Both Novaluron and Pyriproxyfen have a different mode of action from Indoxacarb, which is imperative to combat resistance. In this study, the objectives were to research the effects of IGR and Indoxacarb mixture baits and products that only contain IGRs or Indoxacarb on a field representative strain. My hypothesis was that the IGR baits mixed with Indoxacarb would cause the most effective mortality due to the combination of neurotoxic insecticide and IGRs either killing or preventing the growth and reproduction of *B. germanica*.

MATERIALS AND METHODS

5 treatments were used in this bioassay. These treatments were a CSI bait consisting of 0.1% Novaluron only, a JHA mimic bait of 0.1% Pyriproxyfen only, a Trio Bait known as Advion Trio consisting of 0.1% Novaluron + 0.1% Pyriproxyfen + 0.6% Indoxacarb, a neurotoxic insecticide known as Advion Evolution consisting of 0.6% Indoxacarb, and lastly the control blank bait that contained no insecticides. The strain used was a mixed field strain collected from various field locations in 2019 from the USA. These strains are susceptible to Indoxacarb. This mixed strain is the combination of 5 different strains collected: Missouri, NH1, Logansport, Fowler 1, and Fowler

2. The reason we decided to use a field representative population was because while a past study from Hamilton et al., (2020) showed the effectiveness of Novaluron in a lab susceptible strain, the mixed strain is more accurate to field populations and yet to be tested. Using a field representative strain will help us better understand how *B. germanica* in the field react to our treatments. 100 *B. germanica* nymphs were selected for each treatment. These nymphs were picked randomly from each strain and were mixed between small and large in an equal 1:1 ratio for each replication. Small nymphs were early 3rd instars while large nymphs were predominantly 4th instars.

Bioassays were conducted in a rectangular plastic Tupperware container with vented fabric lids (13 inches length, 9 inches width, and 4 inches height). Each treatment had 3 replicates with 100 nymphs per replicate. Prior to the start of the bioassay, nymphs were starved and given time to acclimate to their surroundings for 16-24 hours before the bioassay. Water and harborage consisting of cardboard tents were provided at all times. At the time of the bioassay, any dead or unhealthy nymphs were replaced with extras that underwent the same process. The bioassay conducted was a choice feeding bioassay where the nymphs had access to treatment baits as well as rodent food (Teklad rodent chow #8604). 2 grams of gel bait total in the form of 4 0.5-gram placements were provided alongside 4 placements of rodent food (Fig 1). If these baits were consumed, then more bait was provided to the cockroaches and the refilled amount was recorded. The bioassays were conducted at a temperature of 22–25°C, at 20–35% humidity and on a 10:14 light:dark cycle.

Observations were recorded 1 day after beginning, 3 days after beginning, and then weekly for 20 weeks after the start of the bioassay. Observations were taken on mortality, developmental defects such as molting failures, supernumerary nymph molts, adultoids, darkened nymphs and twisted wing adultoids. Reproduction and final weight would also be recorded. Each replicate was started at different timepoints between September and November 2021.

RESULTS

After the 20-week period, the survivorship rates were analyzed (Fig. 2). A matching letters report shows that Pyriproxyfen and Novaluron both had similar results to each other while Trio and Indoxacarb also had similar results to each other. The control was not similar to any treatments. At week 20, Novaluron reached a mortality rate of 99% mortality while Pyriproxyfen reached 90%. Both the Trio bait and Indoxacarb bait reached 90% to 100% mortality between week 1 and 3. During weeks 4 through 11, the mortality in the blank treatment was high, approaching around 50% to 60%. It is unknown if this high insect mortality in the control treatment had been due to natural developmental mortality, unaccounted for factors, contamination, or caused by consumption of the blank bait. However, the control nymphs were fertile and were able to reproduce, leading to an increase in survivorship after week 11. By week 20, survivorship reached 178%, growing to populations higher than the initial 100 nymphs in the starting population. The

control treatment was the only treatment able to reproduce, and despite their initial high mortality rate, their ability to reproduce is a crucial factor in comparing their treatment to the others. This was because the control treatment nymphs molted into fertile adopts that reproduced (Tbl. 1, Fig. 3). Novaluron and Pyriproxyfen nymphs could not reproduce due to molting caused deaths, molting into supernumerary nymphs, or molting into adultoids that were sterile. Sterile adultoids were identified by the presence of wing twisting on the adultoid. Advion Evolution and Trio did not reproduce due to death of the population. No surviving nymphs from the Novaluron and Pyriproxyfen treatments molted into normal adults and were sterile (Tbl. 1, Fig. 4, Fig. 5).

Bait consumption was also recorded during the trial period (Fig. 6). When nymphs would completely consume bait, the refill would be noted, allowing us to measure how much they had eaten over the 20-week period. Pyriproxyfen consumed the most bait followed by the control, then Novaluron, and then a tie between Trio and Indoxacarb. Pyriproxyfen ranged from 14g to 20g, the blank bait from 12g to 13.5g, and the Novaluron treatment from 9.5g to 12g. Due to high mortality observed with the Advion Evolution and Trio treatments, bait consumption was always less than 2 grams per replicate. Trio and Indoxacarb treatment groups did not consume past the initial baits given to them, providing evidence that the baits were highly effective in controlling the population.

The weight of the supernumerary nymphs and adultoids were taken after the 20-week period for the Novaluron, and Pyriproxyfen treatments while the average weight was taken of regular adults from the control (Fig. 7). Pyriproxyfen nymphs averaged a weight of 0.1098g, followed by Novaluron at 0.1012g, and lastly the control at 0.0824g. These weights provide evidence that the nymphs in the Pyriproxyfen treatment continued to grow past the usual instar stages into supernumerary nymphs or adultoids.

DISCUSSION

Our results showed that both CSIs and JHAs prevented normal adult formation in all three replicates. All nymphs either died during nymph to nymph molts, nymph to adult molts, molted into supernumerary instars, or molted into sterile adultoids with twisted wings. The results of the mortality were significant, providing promise that these results could be useful to future studies on IGRs. However, IGRs took a much longer period to kill field representative cockroaches while the Trio and Indoxacarb treatments could kill much faster. Pyriproxyfen consuming the most bait could be a sign that it takes longer to succumb to complications from JHAs. This is because they are eating and continuing to grow, while nymphs from Novaluron ate less because they died faster from molting defects. Similarly, Trio and Indoxacarb ate the least, meaning that they were controlled at the fastest rate due to high mortality. Compared to Hamilton et al., (2020) who reported that it took 8 weeks to reach 100% mortality in lab strain cockroaches with Novaluron, our field representative population took 13-20 weeks to reach around 90-95% mortality. This can provide some insight into how the biology of field populations may be different from the biology of the susceptible lab strain that was tested by Hamilton et al. All cockroaches in the IGR bioassays

were sterile, which is useful for population control. When using IGRs with Indoxacarb or other insecticides, we may be able to provide better control of populations than any standalone bait. IGRs may be useful in inhibiting growth of nymphs that are resistant to the initial dose of neurotoxic insecticide, so that nymphs who survive are unable to reproduce and pass on their genes. Not only is this important for control in general but being unable to reproduce can also help reduce resistance genes passed down through the offspring. It also makes it more difficult for *B. germanica* to develop resistance, as they would need to evolve resistance to three different chemicals rather than one. It was unclear whether my hypothesis was supported by the data. The Trio bait did as well as the standalone Indoxacarb bait. This was because an Indoxacarb susceptible strain was used for this research. Seeing that IGRs were effective in population control, the next step would be to run this bioassay again on an Indoxacarb resistant population. This would better allow us to see if there are differences between the Trio bait and the standalone Indoxacarb bait on cockroach mortality. IGRs are also known to disrupt the growth of embryos in adult cockroaches (Hamilton et al., 2020). A possible future experiment would be to test these IGR baits on adult *B. germanica* and assess how they impact cockroach reproduction. Lastly, it may be worthwhile to also use other species of cockroaches such as brown-banded or American cockroaches, some of which have different life cycle durations and therefore could be impacted differently by IGRs. Currently, CSIs like Novaluron are mostly used in management of agriculture pests (Kostyukovsky & Trostanetsky, 2006). However, their impact on *B. germanica* show promise as an additional IGR to combine with JHAs like Pyriproxyfen and neurotoxic insecticides like Indoxacarb for a more effective bait. As well as this, the data provides evidence that CSIs may be faster in killing cockroaches than JHAs. Short term treatments are crucial in urban pest management. Homeowners desire control of populations to be immediate, as *B. germanica* can continue to cause public health concerns and discomfort during the months it may take for IGRs to wipe out populations. Effective use of integrated pest management should also not solely rely on IGRs and insecticide bait. Multiple tactics should be integrated to control *B. germanica*. Outreach to homeowners can be effective in controlling populations by advising the public to maintain clean spaces (Gondhalekar et al., 2021). They can do this by cleaning out food scraps, throwing away garbage, caulking leaky pipes, and removing possible harborage for cockroaches to shelter. Regular vacuuming can also pick up cockroaches if they are scared from their harborage. Aside from picking up cockroaches, vacuuming can greatly help remove feces and casted molts that are serious allergens. Sticky traps can be used to monitor populations, which can allow pest control officials to assess the severity of an infestation and whether populations are growing or declining. Novel IPM strategies such as the use of RNA interference and heat treatments are also being studied. Desiccant dusts like diatomaceous earth may also be useful in cutting the cuticles of *B. germanica*, leading to fluid loss and death (Hosseini et al., 2014). As *B. germanica* continue to evolve resistance to standard insecticides, new integrated pest management strategies become imperative to use alongside insecticides to combat resistance. In a growing urban world, pest infestations may become increasingly prevalent. As such, the need to develop novel methods to sustainably control infestations is crucial to the health of our society.

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Figure 1. Interior view of a nymph bioassay arena with 4 placements of gel bait and rodent diet along with cardboard harborage and a water source.

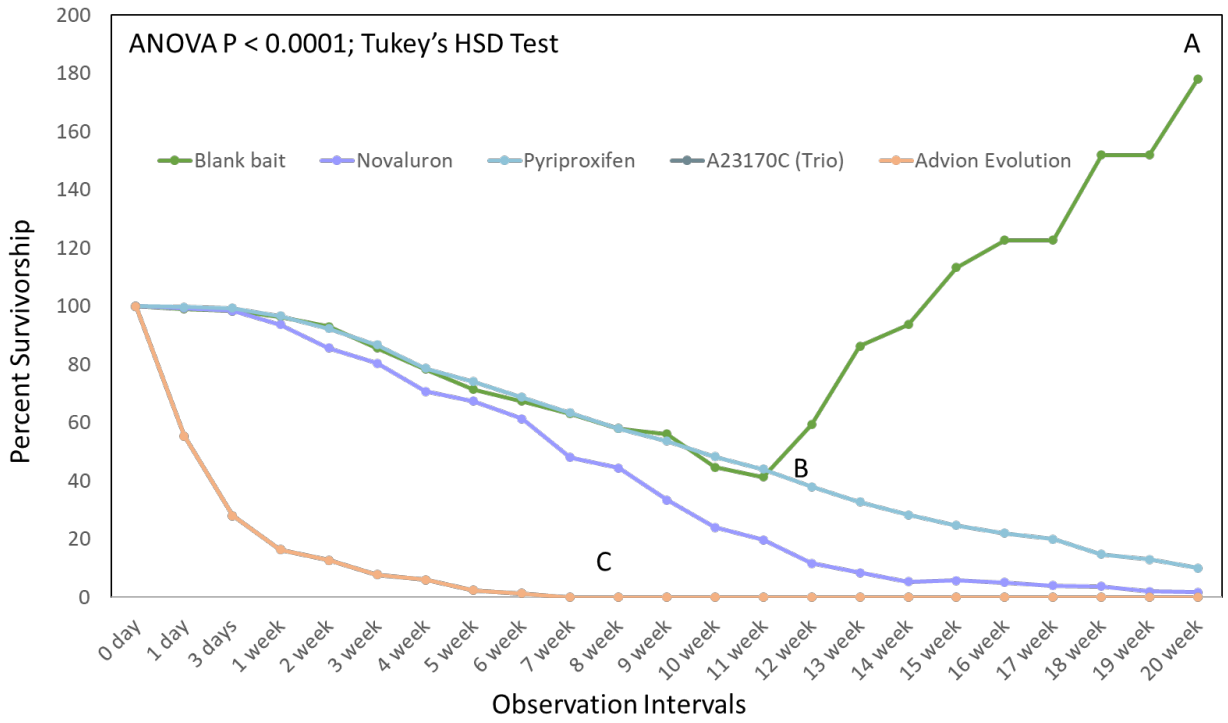

Figure 2. Percent survivorship of 5 treatments over the 20-week period. Note that the Trio bait and Advion Evolution overlap with each other. Matching letters report shows that Novaluron and Pyriproxyfen had similar results to each other, and the Trio bait and Advion Evolution bait had similar results to each other. The blank bait did not have similar results to any other treatment.

Treatment	Percentage of normal nymph to adult molts		
	Rep 1 (thru week 20)	Rep 2 (thru week 20)	Rep 3 (thru week 20)
Control (Blank bait)	90% of surviving nymphs molted to adults between 4 to 8 weeks	90% of surviving nymphs molted to adults between 4 to 8 weeks	80% of surviving nymphs molted to adults between 4 to 8 weeks
0.1% Novaluron bait	None	None	None
0.1% Pyriproxifen bait	None	None	None
A23170C (Advion Trio)	None	None	None
Advion Evolution	<5%	None	<3%

Table 1. The percentage of normal nymph to adult molts. 80-90% of the blank bait molted into normal adults between weeks 4 to 8. In rep 1 and 3, Advion Evolution saw the successful adult

molts of less than 5% of nymphs. All replicates for Novaluron, Pyriproxyfen and Advion Trio did not see any successful adult molts.

Figure 3. Control treatment nymphs molted into normal, fertile adults and reproduced. Left image: Three gravid female cockroaches. Right image: F2 generation nymphs.

Figure 4. Nymphs from the Pyriproxyfen treatment. Left image: Adultoids with twisted wings and supernumerary nymphs. Right image: Nymphs that died due to molting complications.

Figure 5. Nymphs from the Novaluron treatment. Left image: Adultoids with twisted wings. Right image: Nymphs that died due to molting complications

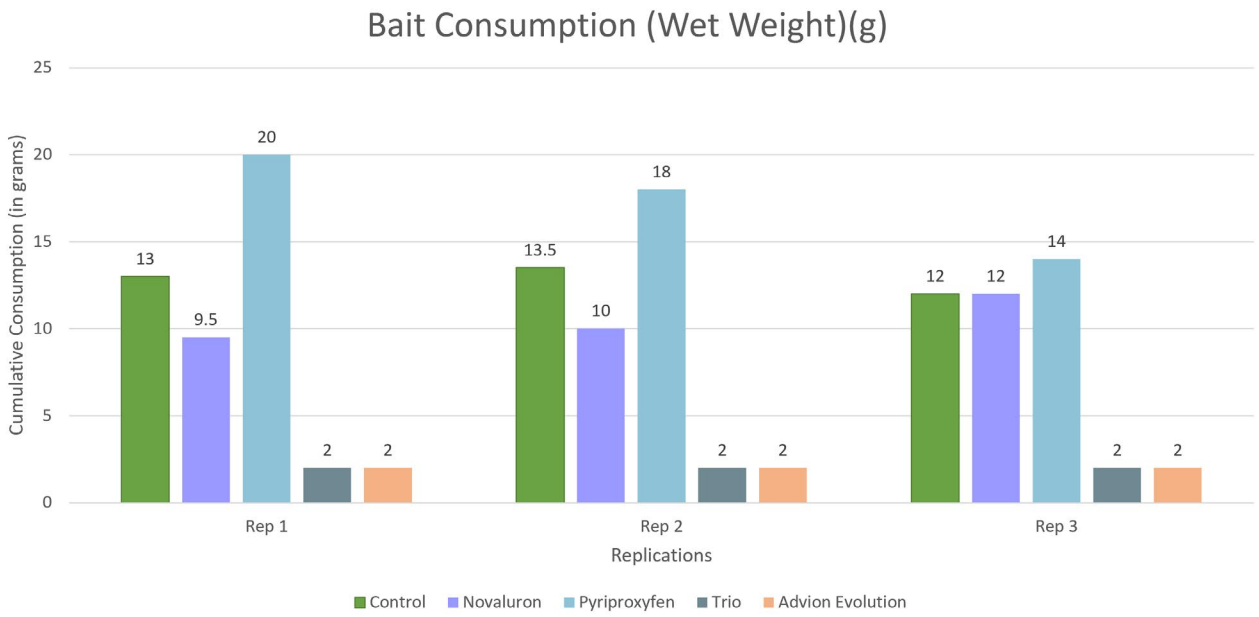

Figure 6. Wet weight bait consumption of nymphs over the 20-week period.

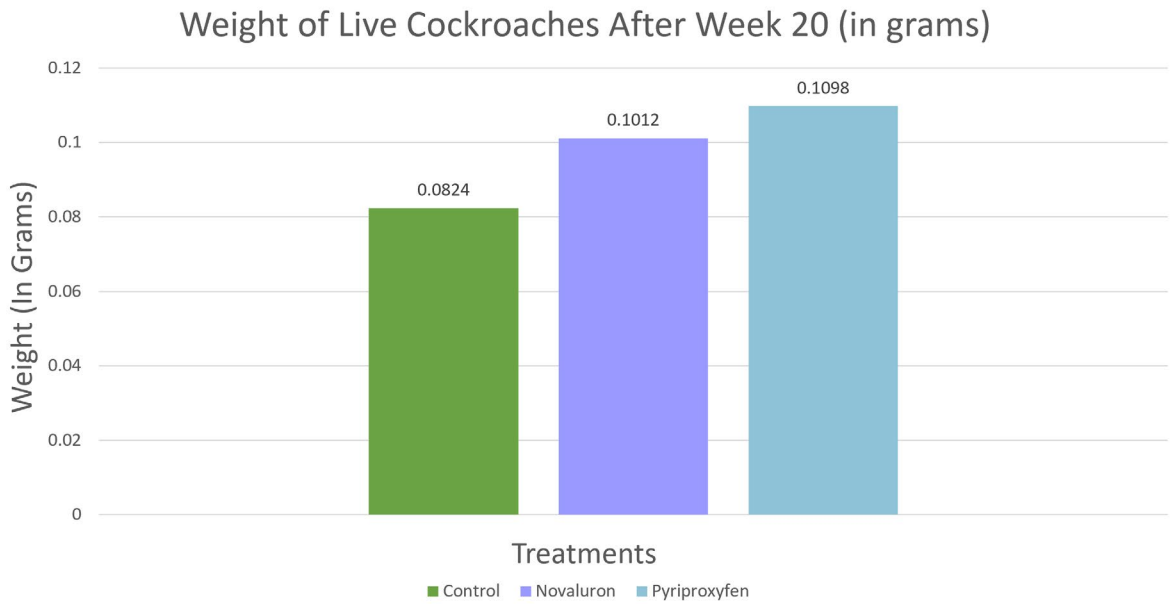

Figure 7. Average weight of live cockroach nymphs after the 20-week period.

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