THE STUDY OF RESISTANCE TO INSECTICIDE ACTIVE INGREDIENTS IN RELATION TO POPULATION SIZE IN THE GERMAN COCKROACH (*BLATTELLA GERMANICA*)

by

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Dedicated to my mom

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ABSTRACT

The German cockroach (*Blattella germanica*) is one of the most critical urban pests globally due to the health risks, such as asthma, it imposes on people. Insecticides are known to manage their large population sizes, but the rapid rate at which cockroaches develop resistance is a continuing problem. This can be expensive and time-consuming for both the consumer and the pest management professional (PMP) applying the treatment. Each cockroach population is unique because different strains have different resistance profiles, so resistance profiles must be considered. This thesis addressed this little-studied issue in a controlled laboratory setting. Cockroach strains from Indianapolis, Indiana, Danville, Illinois, and Baltimore, Maryland, were used. Fifteen insecticide active ingredients most used by consumers and PMPs were selected for testing in vial bioassays to establish resistance profiles. No choice and choice feeding assays with four currently registered bait products were performed to assess the impacts of competing food and circadian rhythms on bait resistance levels. Selected population growth characteristics were compared in virgin females and nymphs in each cockroach strain to determine if certain population traits were associated with insecticide resistance. The results indicate that emamectin benzoate is the most effective active ingredient in causing the highest mortality in all strains in vial bioassays. No choice assays confirmed vial assay results the best, with Optigard (emamectin benzoate) being the most effective bait in all strains. The time a female carries its egg case and takes for a nymph to become an adult was significantly different across all strains, suggesting possible fitness costs for higher-level multi-resistance. The results acquired from these studies can help develop rapid tests to use in the field based on the no choice feeding assay while also adding more information supporting current resistance and cross-resistance evolution theories.

CHAPTER 1. INTRODUCTION

1.1 German cockroach

German cockroaches (*Blattella germanica*) are a common household pest in low-income housing, and they also pose threats to tenant health. They are known to produce specific environmental allergens associated with deadly respiratory diseases. These deadly respiratory diseases include asthma and allergic rhinitis, and they also cause atopic dermatitis. Cockroach feces, body parts, and cast exoskeletons are the allergen trigger for these respiratory diseases (Celmeli et al. 2016). In 2019, China reported a fatal respiratory disease known as the 2019 novel coronavirus that has spread worldwide (Huang et al. 2020). While cockroaches may trigger fatal respiratory diseases for tenants living in low-income housing, it is currently unknown if cockroach allergens are associated with the 2019 novel coronavirus and the resulting condition known as "COVID-19." However, given the elevated infection and death rates in disadvantaged inner-city populations, cockroach allergens may be associated with greater COVID-19 risk (Yancy 2020). Cockroaches pose a respiratory threat, but they are also a known vector of *Salmonella typhimurium* and *Escherichia coli*, which are bacterial diseases that can become lethal when left untreated (Kopanic et al. 1994; Zurek and Schal 2004). There is also evidence that cockroaches contribute to house dust microbiomes that, in turn, worsen symptoms in sensitive asthmatic persons (O'Connor et al. 2018). Living with cockroaches has negative consequences on human health, and these pests have adapted unique behavioral mechanisms that allow them to reside with tenants.

Cockroaches can infest buildings by taking particular advantage of the structural features of low-income apartments. The primary movement mechanism is through the plumbing connections, such as the drains in sinks and bathtubs; therefore, if one apartment has an infestation, it is easy for nearby apartments and buildings to become infested (Runstrom and Bennett 1984). Cockroaches are also attracted to messy situations and clutter since they provide more food, water, and shelter. It is known that sanitation conditions correlate with cockroach populations (Schal 1988). Cockroaches are known to live for months without food (Durbin and Cochran 1985). However, cockroaches require water daily, which is often associated with plumbing and housekeeping practices in low-income housing situations (Appel 1991). Cockroaches are also very social creatures, and a small-sized apartment can house a large population of at least hundreds of cockroaches (Wang and Bennett 2009). However, it is currently unknown if the size of the cockroach population affects cockroach survival in apartments.

1.2 History of insecticide resistance

While German cockroach populations can quickly infest and thrive in apartments, it is possible to eliminate them with different methods. One of the standard methods to eliminate cockroaches is insecticides, which are chemicals used to control insects (Stephenson et al. 2006). However, there are many underlying mechanisms that cockroaches have evolved in order to develop resistance to various insecticides (Cochran 1995; Scharf and Gondhalekar 2021). Cockroaches can exhibit behavioral resistance to insecticides by avoiding baits that could potentially harm them (Wang et al. 2004). Physiological resistance in cockroaches includes changes in their biochemical composition. A well-known studied enzymatic mechanism in cockroaches is Cytochrome P450s, and cockroaches that exhibit an increased expression of cytochrome P450s are resistant to various insecticides (Scharf et al. 1998; Gondhalekar et al. 2012, 2016). Another physiological mechanism that is well-studied in cockroaches is the sodium channel, and cockroaches that have the *kdr* mutation are known to be resistant to various insecticides (Chai and Lee 2010; DeVries et al. 2019).

The first significant pesticide that was able to eliminate German cockroach populations successfully was DDT, and it was discovered in the 1940s as a synthetic organic insecticide. Another type of synthetic organic insecticide called cyclodienes (i.e., dieldrin) was also around in the 1940s. Unfortunately, cockroaches could develop an insecticide resistance to DDT and cyclodiene insecticides, and researchers were challenged with developing insecticides to combat cockroach population resistance (Cochran et al. 1952; Matsumara and Hayashi 1966).

In the 1960s, insecticide companies released a new generation of insecticides, including organophosphate and carbamate insecticides. These insecticides successfully eliminated cockroach populations but only for a short time. Eventually, the cockroaches developed a resistance to organophosphate and carbamate insecticides, and a newer generation of insecticides was released (Bennett and Spink 1968; Collins 1973). During the 1970s, pyrethroid and abamectin pesticides were discovered, and they successfully controlled cockroach populations for many years. The cockroaches later developed insecticide resistance to pyrethroids and abamectin, and researchers were challenged with developing insecticides with better environmental characteristics while still providing control (Cochran 1987, 1994).

Researchers successfully developed newer insecticides with better environmental characteristics in the 1990s, and these newer classes included phenylpyrazoles (fipronil) and neonicotinoids (imidacloprid). It has been reported that cockroaches have developed resistance to phenylpyrazoles (Holbrook et al. 2003; Gondhalekar and Scharf 2012). Later in the 2000s, insecticides such as indoxacarb were released with even better environmental characteristics. Gondhalekar et al. (2013) reported resistance to indoxacarb in German cockroach populations. Overall, German cockroaches have a history of becoming resistant to almost every insecticide that has been released. However, insecticide resistance levels for current and newly registered products in 2022 for German cockroaches are yet to be determined.

1.3 Insecticide resistance today

It is known that German cockroaches have shown resistance to 43 active ingredients (AIs) (Whalon et al. 2022). There are many ways to study physiological insecticide resistance. However, the glass vial bioassay method is better because it works based on cockroach tarsal contact with insecticide residues and ingestion via tarsal grooming (Scharf et al. 1999). Diagnostic concentration (DC) bioassays are concentrations that kill 90 or 99% of susceptible individuals, and they do not require as much effort and insects as the conventional resistance ratio method (Gondhalekar et al. 2013). Fardisi et al. (2017) conducted an experiment to determine DCs for 14 insecticide AIs from a susceptible lab strain known as the JWax-S strain and from Danville, Illinois, and Indianapolis, Indiana, field-collected resistant strains. The researchers determined susceptibility profiles by comparing percent mortalities between the field strains and the JWax-S strains. They were able to satisfy their two objectives: to create a valuable resource for pesticide applicators and discover the AIs with the lowest resistance levels. To determine the DCs for the 14 AIs, Fardisi et al. (2019) utilized the pre-resistance-monitoring data by selecting insecticide products with the lowest resistance levels in apartment complexes. They found that these single AI treatments successfully eliminated cockroach populations while other insecticides with higher resistance levels failed (even those included in the AI mixture products). However, more data needs to be collected on insecticide resistance for AIs available over the counter and for AIs available for professional use. Looking at both professional and over-the-counter AIs is necessary because of the significant potential for cross-resistance between products (Fardisi et al. 2019).

While the authors could eliminate cockroach populations with single AI treatments, they also discovered that rotation treatments could reduce selection pressure when no or low-resistance bait insecticides were used (Fardisi et al. 2019). It is common to combine Integrated Pest Management (IPM) techniques with bait insecticides to achieve success by causing high mortality rates in German cockroach populations (Wang and Bennett 2006). There are many different IPM practices, and the most common practices to control German cockroach populations are sanitation and structural modifications. Kaakeh and Bennett (1997) discovered that sticky traps and vacuuming were effective IPM sanitation techniques for controlling German cockroach populations. As for structural modifications, it is simple to control the movement of cockroaches from one apartment to another by sealing holes or cracks in the walls where cockroaches typically take shelter (Wang and Bennett 2006). While IPM practices may control cockroach populations, Wang et al. (2018) reported that many residents who have cockroach infestations are unaware of them. Residents who typically have poor sanitation conditions are more likely to tolerate cockroaches.

1.4 Insecticide resistance and population size

Farmers have constantly been challenged with insect damage when growing crops. There has always been an arms race between farmers spraying pesticides and the insects developing resistance to them. Insects, typically small in size, can have larger populations; therefore, mutation rates per insect would be multiplied by a more significant population size to give a higher total mutational supply that allows them to develop insecticide resistance (Messer and Petrov 2013). Using computer simulations, Caprio and Tabashnik (1992) discovered that insecticide resistance in insects evolves faster when the population size increases per field. Sisterton et al. (2004) confirmed this with insect resistance in transgenic crops with the interaction between pink bollworm (*Pectinophora gossypiella*) and *Bacillus thuringiensis*. While there is plenty of data about insect resistance to insecticides and transgenic crops in fields, little data is known on the size of field populations concerning insecticide resistance in German cockroaches.

The characteristics of populations are essential to comprehend why a population is the size that it is. Population fecundity, the number of offspring produced by an organism over time, and mortality rates are essential characteristics determining population growth. Typically, a population grows fast when it exhibits more fecundity and lower mortality rates (Peters and Barbosa 1977). This is commonly observed in humans (Bongaarts 2009). The inverse trend is usually observed when insecticide resistance is involved in a population. In the codling moth (*Cydia pomonella*), it is known that insecticide resistance is higher when it exhibits low fecundity and high mortality rates (Boivin et al. 2001). This suggests that the population has a slow population growth. However, the extent to which population growth rate correlates with insecticide resistance in German cockroaches is unknown.

The current thesis can be divided into two approaches. These approaches are intended to compare cockroach strains having a wide range of susceptibility levels in different bioassays for (1) understanding which assays are better for resistance monitoring purposes and (2) for investigating possible links between founding population sizes and cross-resistance levels. The research's broader objective is to understand better the relationship between *B. germanica* populations and insecticide resistance. The overall hypothesis of the study is that cockroach resistance will be higher for cockroaches sampled from larger field populations than for cockroaches sampled from smaller field populations.

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CHAPTER 2. CONDUCTING RESISTANCE ASSAYS IN FOUR GERMAN COCKROACH STRAINS

2.1 Introduction

The German cockroach, *Blattella germanica*, is an urban pest commonly found in urban structures such as hotels and apartments. They are often considered a health pest due to their association with respiratory diseases by leaving their feces, body parts, and cast exoskeletons in their dwellings (Celmeli et al. 2016). Specifically, there is a strong connection between residents having asthma and residents that live with heavy cockroach infestations (Wang et al. 2008; Celmeli et al. 2016; Do et al. 2016). Finally, there may be an association between German cockroaches and viral diseases like COVID-19. The 2019 novel beta coronavirus, commonly known as COVID-19, is a respiratory disease with fatal symptoms of fever, cough, and fatigue that has changed the world in the past few years (Huang et al. 2020). Researchers recently discovered that children and adults are more likely to test positive for COVID-19 if they have asthma from indoor environmental triggers such as mold and cockroaches (Finkas et al. 2022; Harada et al. 2022). There is a need to control cockroach infestations now more than ever.

Pest management professionals (PMPs) typically control German cockroach infestations with bait insecticides. Bait insecticides are an effective control measure within integrated pest management (IPM) programs (Gondhalekar et al. 2021). Baits have been recognized as an effective control method for over 200 years after Cowan (1865) first documented them for use in 1865. Bait insecticides are excellent for PMPs since they are relatively inexpensive to make and purchase (Schal and Hamilton 1990; Wang and Bennett 2006). However, there have been many reports that German cockroaches are becoming resistant to newer insecticide baits and are becoming more difficult to control (Gondhalekar and Scharf 2012; Gondhalekar et al. 2013; Ko et al. 2016; Lee et al. 2022). Insecticide resistance is not a new trend limiting control of German cockroach infestations. German cockroaches are known to be resistant to nearly all insecticides. Specifically, they are resistant to at least 43 active ingredients (AIs) (Whalon et al. 2022). More than ever, studying insecticide resistance is a priority for helping to assure the success of IPM programs.

PMPs commonly control most insect pests with insecticides. In German cockroaches, it must be understood that different populations of cockroaches demonstrate different resistance profiles depending on the selection history of the cockroaches (Scharf et al. 1997). Insect behavior and insect physiology drive insecticide resistance, and studying physiological resistance is essential to understanding insecticide resistance in the German cockroach (Gondhalekar and Scharf 2013, 2021; Zhu et al. 2016). The size of the insect population may also drive physiological insecticide resistance. Researchers demonstrated that there is evidence of insecticide resistance with population size in the pink bollworm (*Pectinophora gossypiella*) and genetically modified *Bacillus thuringiensis* (Bt) corn (Caprio and Tabashnik 1992; Sisterton et al. 2004). However, there is little evidence in German cockroaches of how the size of the population impacts insecticide resistance, specifically if population sizes are large because they exhibit high insecticide resistance. The reported research examined how physiological resistance may correspond to population size in German cockroaches.

Glass vial bioassays are an excellent resource for studying insecticide resistance. German cockroaches are great for studying insecticide resistance in vial bioassays because the vials enable cockroach tarsal contact with insecticide residues and ingestion via tarsal grooming (Scharf et al. 1995). Insecticide residues can be tested with diagnostic concentrations (DCs) because they are less labor-intensive and require fewer insects than the conventional resistance ratio method, which initially requires the generation of concentration- or dose-mortality data for LC or LD estimation (Gondhalekar et al. 2013). Fardisi et al. (2017) established 14 insecticide AI DCs in susceptible laboratory strains and later tested the DCs on two different field strains with variance in resistance profiles. Due to their significant prior results, DCs with the vial assays are utilized in the current study and compared against other bioassay methods.

Additionally, insecticide bait feeding assays in choice or no choice formats can help confirm AI resistance (Fardisi et al. 2019). By utilizing the behavior of the German cockroach, the assays can be conducted to mimic field conditions because the cockroach does or does not have an option to choose between an insecticide bait (Ebeling et al. 1966; Wang et al. 2004). Overall, vial and bait feeding assays can be excellent assessment tools of insecticide resistance in field strains of German cockroaches. They are easy and cost-effective depending on available resources (Fardisi et al. 2017; Lee et al. 2022).

The objectives of this study were to compare cockroach strains having a wide range of susceptibility levels in different bioassays for (1) the purpose of understanding which assays are better for resistance monitoring purposes and (2) to investigate possible links between founding population sizes and cross-resistance levels. Study-specific objectives were as follows:

1. To identify a vial assay diagnostic concentration (DC) for the new insecticide AI emamectin benzoate.
2. To evaluate resistance to emamectin benzoate and fourteen other AI-DCs against one susceptible and three resistant cockroach strains.
3. Compare choice and no choice bait feeding assays against vial assays to determine which assay format might be optimal for resistance monitoring by PMPs.

This research can help PMPs make more informed management choices based on available resources and help researchers study resistance evolution in German cockroaches more effectively.

2.2 Materials and methods

2.2.1 Cockroach strains

Four German cockroach strains were used. The Johnson Wax strain (JWax-S) was used as a standard susceptible strain due to their lack of prior chemical exposure. Three field strains from housing sites in Danville, IL (D-IL strain), Indianapolis, IN (I-IN strain), and Baltimore, MD (B-MD strain) were collected during December 2014, March 2015, and September 2020. The field strains (except B-MD) were collected from multiple apartments across each site and pooled to establish laboratory “meta” populations. The B-MD strain was collected by Dr. Godfrey Nalyanya of Rentokil Inc. from a public housing site in Baltimore, MD, in September 2020 and then shipped to Purdue University. They were collected after control failures with multiple bait products, including a commercial indoxacarb formulation. The populations were maintained in laboratory culture without insecticide pressure. Colonies were reared in Ziploc plastic containers (44.3 by 30 by 17 centimeter^3/15.14 liter; S.C. Johnson Inc., Racine, WI, USA) with screened lids and held in a controlled environmental chamber at 26 ± 1˚C and a photoperiod of 12:12 (Light: Dark) hours. Cardboard for shelter, rodent diet (number 8604; Harlan Teklad, Madison, WI), and water were provided to the rearing boxes as necessary. All bioassays were conducted with adult males.

2.2.2 Insecticides

Technical grade gel bait and spray product AIs used in vial bioassays were purchased from ChemService (West Chester, PA), Fisher Scientific (Pittsburgh, PA), or Sigma-Aldrich (St. Louis, MO). These AIs included indoxacarb (99.1% purity), abamectin (98.3%), boric acid (99.9%), beta-cyfluthrin (99.5%), bifenthrin (99%), lambda-cyhalothrin (99.5%), fipronil (98.3%), dinotefuran (98.4%), imidacloprid (99.4%), acetamiprid (99.5%), clothianidin (99.5%), thiamethoxam (99.5%), chlorfenapyr (99.1%), hydramethylnon (99.5%), and emamectin benzoate (98.3%). The AIs were selected because they are currently registered by the EPA for cockroach control. Four cockroach baits, Maxforce FC Magnum (fipronil 0.5%; Bayer, Research Triangle Park, NC, USA), Vendetta Cockroach Gel Bait (abamectin B1 0.05%; McLaughlin Gormley King Co., Minneapolis, MN, USA), Optigard Cockroach Gel Bait (emamectin benzoate 0.1%; Syngenta, Greensboro, NC, USA) and Advion Cockroach Gel Bait (indoxacarb 0.6%; Syngenta, Greensboro, NC, USA) were purchased from Univar (Indianapolis, IN) for testing in no choice and choice feeding bioassays.

Fardisi et al. (2017) predetermined lethal concentrations (LCs) and DCs for all AIs listed above except emamectin benzoate in all strains with JWax-S as a positive control (as shown in Table 2.1). Bioassays were conducted in 30-mililiter Shell vials (25 by 95 millimeters; Kimble Chase, Vineland, NJ). The internal surfaces of the vials (71.67 centimeter^2) were treated with 0.5-mililiter insecticide dilutions. About 1 centimeter of the top of the vial was left untreated, as a cotton plug would cover it. Insecticide dilutions were made in acetone apart from boric acid, dissolved in methanol, and used immediately after preparation. Insecticide solutions were mixed thoroughly before being applied to each vial. After adding insecticide solutions, vials were rotated manually for 1 minute and then on a non-heating hotdog roller (Nostalgia Products LLC, Green Bay, WI) placed in a fume hood. Complete evaporation of acetone or methanol required about 30 minutes. Vials treated with acetone or methanol-only were used as controls.

Adult male cockroaches were anesthetized in plastic cups on ice before transferring to individual vials in groups of 10. Glass vials were plugged with cotton balls to prevent escape. Insecticide and control vials were kept vertically in controlled-environmental chambers with atmospheric conditions similar to rearing. In order to determine the LC and DC of emamectin benzoate, Fardisi et al. (2017) generated concentration-mortality data for the JWax-S strain by testing 8-18 concentrations for abamectin AI. Ten replicates for each AI and strain were performed, and mortality was recorded every 24 hours up to 72 hours. Due to the slower action speed of boric acid and hydramethylnon, mortality was scored up to 96 hours. The insects were considered dead if the AI knocked them down on their backs and the insects could not recover on their feet or walk.

For comparing mortality variation between the four strains, percentage mortality from diagnostic bioassays with individual AIs were arcsine transformed and analyzed by two-way factorial ANOVA in R statistical platform (RStudio Team 2022) followed by a post hoc Tukey's HSD test.

2.2.3 No choice assay methods

Four gel bait products were screened against the JWax-S, I-IN, D-IL, and B-MD strains to determine if similar mortality levels could be achieved, as seen in AI-DC assays. As shown in Table 2.2, the gel baits included Maxforce (fipronil), Vendetta (abamectin), Optigard (emamectin benzoate), and Advion (indoxacarb). Procedures described previously in other studies were used with minor modifications (Wang et al. 2004; Gondhalekar et al. 2011; Fardisi et al. 2017). Plastic containers were used (17.8 by 17.8 by six centimeter^3/0.739 liter; Glad boxes Clorox Co., Oakland, CA). The bioassays were conducted in a no choice format in which no competing food was provided. Polystyrene weighing dishes (Fisher Scientific, Pittsburgh, PA) were filled with 0.5-gram gel bait and a water cup, and cardboard shelters were provided in each container. For controls, the gel bait was replaced with an 0.5-gram piece of rodent diet. Adult males were starved for one day before assaying. To prevent the cockroaches from escaping, the container walls were lightly greased with petroleum jelly and mineral oil (2:3), and containers were closed tightly with lids containing a central meshed opening (3-centimeter diameter). Ten replications for each strain–treatment combination were conducted. Mortality was checked every 24 hours until 100% mortality was achieved in all strains. All assay boxes were kept 72 hours after 100% mortality was achieved to ensure no recovery occurred. For comparing variation among strains, mortality data were analyzed by two-way factorial ANOVA in R statistical platform (RStudio Team 2022), followed by univariate tests of significance for each day. To determine LT_{50}, LC_{90}, and LT_{99} estimates, time–mortality data were analyzed using a Probit Analysis Spreadsheet in Microsoft Excel (Mekapogu 2022). Control mortality was accounted for in Probit analysis by the method of Abbott (Abbott 1925).

2.2.4 Choice assay methods

Choice bioassays were performed as described previously with slight modifications. Modeled after Ebeling et al. (1966), disposable Tupperware™ plastic boxes were used. Two 6 inch high x 9 inch wide plastic boxes (27.3 x 4.9 centimeter), one painted black, were connected near the top by a 1-inch length of ¼ inch tubing. One box (dark side) was treated with the received 0.5 gram of gel bait. The other side (light side) contained only food and water and was not painted. The choice bioassays were held under ambient laboratory conditions (~25°C) with a photoperiod of 24:0 hours (Light: Dark). Ten male cockroaches were released in the light side for acclimation one day before the experiment, and mortality was scored every 2 hours and daily after 12 hours up to 15 days. Container walls were lightly greased 1 inch from the top and closed tightly with lids to prevent escape. Only the lid for the light side contained a central meshed opening (3-centimeter diameter). Ten replications were done for choice assays on all strains with JWax-S as a positive control.

2.3 Results

2.3.1 Vial bioassays

Diagnostic concentrations for use in vial bioassays, except for emamectin benzoate, were reported previously (Fardisi et al. 2017). The emamectin benzoate diagnostic concentration was developed in the current research for the JWax-S strain (Figure 2.1). Due to acquiring high mortality in all strains at a low concentration, the emamectin benzoate LC_{99} diagnostic concentration was estimated at 0.125 µg/vial. This diagnostic concentration for emamectin benzoate represents a 16-fold serial dilution of the abamectin diagnostic concentration reported by Fardisi et al. (2017) and effectively causes 100% mortality in the JWax-S strain by 72 hours (Figure 2.1).

Figure 2.2 shows the percentage mortality comparisons for the three strains for 15 AIs at their respective DCs. Exposure to abamectin and emamectin benzoate DCs resulted in >70% mortality of all field strains, and total mortality was achieved in all strains when exposed to the emamectin benzoate DC. Additionally, near 100% mortality was achieved as expected in JWax-S strain (93.0%) when exposed to the abamectin DC; however, mortality in the I-IN (83.0%), B-MD (75.0%) and D-IL strains (70.0%) was lower (Figure 2.2). Average mortality for the I-IN strain was 90–96% on the clothianidin, indoxacarb, or thiamethoxam DCs. The D-IL strain achieved lower mortality (66–89%) and the B-MD strain had lower mortality in the range of 1-24% when exposed to the same DCs as above (Figure 2.2). When tested against DCs of clothianidin, dinotefuran, indoxacarb, and lambda-cyhalothrin, there were significant mortality differences among strains, as well as 100% mortality in the JWax-S strain as expected.

Acetamiprid, beta-cyfluthrin, bifenthrin, indoxacarb, and lambda-cyhalothrin DC assays resulted in 1–90% mortality in the field strains, while JWax-S achieved 87–100% mortality. When exposed to acetamiprid at its DC, the I-IN, D-IL, and B-MD strains achieved only 58%, 51%, and 8% mortality. When exposed to beta-cyfluthrin at its DC, the I-IN, D-IL, and B-MD strains only achieved 57%, 43%, and 6% mortality. Only 90%, 66%, and 1% mortality were achieved in I-IN, D-IL, and B-MD strains when exposed to indoxacarb at its DC. Only 69%, 31%, and 2% mortality were achieved in I-IN, D-IL, and B-MD strains when exposed to lambda-cyhalothrin at its DC. For bifenthrin, I-IN, D-IL, and B-MD field strains had significantly lower mortality than JWax-S (41%, 42%, and 3% mortality; Figure 2.2).

Finally, I-IN strain mortality when exposed to thiamethoxam was 93%, but it was around 80% for fipronil, dinotefuran, and chlorfenapyr (89%, 84%, and 83% mortality). Mortality in the D-IL stain was <95% in all instances with fipronil, thiamethoxam, dinotefuran, and chlorfenapyr DCs (92%, 89%, 88%, and 71% mortality). B-MD mortality was <35% in all instances with dinotefuran, fipronil, thiamethoxam, and chlorfenapyr (32%, 29%, 24%, 13%; Figure 2.2).

The diagnostic concentration determinations for boric acid, hydramethylnon, and imidacloprid were done with slight modifications from that detailed above. Boric acid and hydramethylnon concentration–mortality results for all strains were scored after 96 hours (Figure 2.2). For imidacloprid, 72-hour mortality results were used. However, neither boric acid, hydramethylnon, or imidacloprid resulted in 100% mortality in any strain, which is consistent with earlier findings by Fardisi et al. (2017, 2019).

Imidacloprid killed the highest field strain proportions among all insecticides at its DC. There were no significant differences among lab and field strain mortality levels with boric acid and hydramethylnon; however, there was a statistical difference between field strains with imidacloprid, specifically in B-MD (JWax-S: 44%, I-IN: 33%, D-IL: 16%, and B-MD: 1%). Mortality was comparatively lower for all four strains with hydramethylnon (JWax-S: 27%, I-IN: 17%, D-IL: 15%, and B-MD: 4%) and boric acid (JWax-S: 18%, I-IN: 13%, D-IL: 12%, and B-

MD: 3%) at their DCs (Figure 2.2). When assayed with either boric acid, imidacloprid, or hydramethylnon, there were no statistically significant differences among the four strains based on Tukey's HSD tests.

2.3.2 No choice bioassays

Percent mortality up to 6 days is shown for all four strains when provided commercially-formulated gel baits containing fipronil, abamectin, emamectin benzoate, and indoxacarb (Figure 2.3). LT_{50} values for different gel baits determined through probit analysis ranged between 0.06 to 8.0 days for the JWax-S, I-IN, D-IL, and B-MD strains (Table 2.4). All strains achieved 100% mortality after ten days except the highly resistant B-MD strain. With Maxforce (fipronil) gel bait, all strains except I-IN and B-MD achieved >90% mortality within one day after starting assays. The rank of strains in this assay from least to most tolerant was JWax-S, I-IN, D-IL, and B-MD.

2.3.3 Choice bioassays

Percent mortality up to 6 days is shown for all four strains when provided gel baits containing fipronil, abamectin, emamectin benzoate, and indoxacarb (Figure 2.4). LT_{50} values for different gel baits determined through probit analysis ranged between 0.04 to 1.89 days for the JWax-S, I-IN, D-IL, and B-MD strains (Table 2.5). >90% mortality was achieved after 13 days for all strains. With Optigard (emamectin benzoate) gel bait, all strains achieved >70% mortality within two days after starting assays. The rank of strains in this assay from least to most tolerant was B-MD, I-IN, JWax-S, and D-IL, which is different from the no choice assays.

2.4 Discussion

Four different German cockroach strains were selected for this study. The JWax-S strain was selected as the susceptible strain due to its limited insecticide exposure. Its population size was not relevant due to being reared in a laboratory for decades. The I-IN strain was selected as the small population strain, and the D-IL strain for the large population strain based on their documented sticky trap catches from prior field studies (Table 2.3; Fardisi et al. 2019). The B-MD strain was included due to its unknown population size and difficulty of control with insecticides.

This study tested currently registered insecticide AIs and commercial bait products against cockroach strains with different population starting sizes and resistance levels. More importantly, the study further suggests rapid tests that have apparent utility to test currently registered AIs on field strains with unknown population sizes. Vial, choice, and no choice feeding assays were conducted on four different strains of German cockroaches with differences in insecticide resistance profiles and founding population sizes. Emamectin benzoate showed the highest mortality in vial assays, and boric acid showed the least mortality in all strains. This is likely related to borate chemistry in general in the vial assay format (Fardisi et al. 2017). No choice bait assays confirmed vial results significantly more strongly than choice assays in all strains (see Table 2.6). The B-MD strain demonstrates the highest insecticide resistance out of all the strains. All indications are that it had a large field population size (Dr. Godfrey Nalyanya *pers comm*).

2.4.1 Resistance mechanisms in German cockroaches

Each strain was tested from established DCs from Fardisi et al. (2017) to establish resistance profiles for each AI. Vial bioassays were selected to assess physiological insecticide resistance because physiological resistance is the most common category in German cockroaches and includes metabolism by detoxification enzymes, target site modifications, and penetration barriers (Scharf et al. 1998; Wang et al. 2004; Gondhalekar et al. 2013). The two main implications of physiological resistance are cross- and multiple-resistance. Cross-resistance is when resistance to two insecticides from different classes is due to a single mechanism, and multiple-resistance is when resistance to multiple insecticides occurs due to multiple mechanisms (Scharf and Gondhalekar 2021). Vial assays are also relatively quick to use for resistance monitoring in the laboratory setting (Gondhalekar et al. 2011). Fardisi et al. (2017, 2019) determined that vial assays are reliable for efficiently and accurately determining AI resistance for field strains to improve pest management decision-making. Additionally, vial assays can effectively predict resistance to bait AIs because test cockroaches readily groom and ingest insecticides off their tarsi (Scharf and Gondhalekar 2021). Vial assays can thus be an excellent resource for resistance monitoring if a laboratory is available.

2.4.2 Implications behind boric acid, imidacloprid, and hydramethylnon

However, there were still issues encountered with boric acid, imidacloprid, and hydramethylnon in all strains, including the JWax-S strain. Fardisi et al. (2017) demonstrated similar results. Imidacloprid has a unique uptake compared to its related neonicotinoids, which display agonism at the nicotinic acetylcholine receptor, low contact toxicity, and knock-down recovery (Kaakeh et al. 1997; Tan et al. 2007). Hydramethylnon and boric acid suggest similar issues. Hydramethylnon can work phenomenally well as a bait insecticide, but it has weak topical toxicity due to its limited cuticular penetration (Ko et al. 2016). Boric acid is known to achieve high mortality when formulated as a bait or dust (Zurek et al. 2003; Gore and Schal 2004). There is little evidence to support boric acid has topical toxicity (Sierras et al. 2018); however, because vial assays enable at least some ingestion, the lack of efficacy for boric acid in vial assays is perplexing and should be further investigated.

2.4.3 Variation in mortality success among different insecticide classes

Emamectin benzoate had overall high mortality across all strains. It is a derivative of the avermectin family that possesses much higher activity at lower doses than other traditional insecticides (Guo et al. 2015). The EPA approved emamectin benzoate within the last decade to control emerald ash borer (*Agrilus planipennis*) (Poland et al. 2010). Also, Liang et al. (2020) recently demonstrated that emamectin benzoate is an excellent AI to control diamondback moths (*Plutella xylostella*). Along with Lee et al. (2022), the results from the present study suggest that emamectin benzoate could be an excellent AI for the control of resistant German cockroach populations regardless of population size, cross-resistance profiles, or prior insecticide exposure history.

Neonicotinoid insecticides exhibited high mortality in the D-IL and I-IN strains except for acetamiprid. Fardisi et al. (2017) acquired similar results in both strains, although D-IL has lost some resistance over time. Acetamiprid may possess a similar uptake mechanism to its close relative imidacloprid. Tan et al. (2007) demonstrated that imidacloprid and acetamiprid achieved only 0 and 20% mortality in German cockroaches. The neonicotinoid class of insecticides was previously grouped due to their relative maximum levels of acetylcholine-meditated current production in German cockroaches (Tan et al. 2007). However, more research is needed to assess neonicotinoid resistance mechanisms in German cockroaches.

Pyrethroid insecticides and indoxacarb were not effective against all field strains, also as previously demonstrated in Fardisi et al. (2017). Pyrethroid insecticides have been used extensively since the 1980s and have nearly ubiquitous high-level resistance in cockroaches (Valles and Yu 1996; Scharf et al. 1997; Wei et al. 2001; Limoee et al. 2006; Chai and Lee 2010). Indoxacarb appears to follow the pyrethroid trend (*Present Study* and Scharf et al. 2022). Curl (2011) confirms that German cockroaches likely had significant prior selection pressure due to high market sales for indoxacarb products. Overall, German cockroaches typically exhibit widespread insecticide resistance to easily accessible AIs extensively used by consumers and pest management professionals (DeVries et al. 2019).

2.4.4 Resistance profiles in field collected strains

The German cockroaches from Baltimore exhibited the highest frequency of insecticide resistance to all AIs (except emamectin benzoate). The B-MD strain is thus classified as a highly resistant cockroach strain with a low variation of resistance to different AIs (Wu and Appel 2017). In this regard, Wu and Appel (2017) determined that German cockroach strains that exhibited high insecticide resistance did so due to heavy selection pressure towards genetic homogeneity. The B-MD strain exhibits similar characteristics. Since emamectin benzoate is a newer AI than the other AIs tested and is part of a newer class of insecticides, resistance may not have been selected in any of these strains yet (Scharf and Gondhalekar 2021). The B-MD strain may also exhibit a large field population size due to possibly having a variety of mutations that give resistance to a range of insecticides (Messer and Petrov 2013). However, emamectin benzoate appears unique enough not to be affected by cross-resistance with other AIs. More experiments should be conducted to examine specific population characteristics and resistance mechanisms in the B-MD strain or the Danville "single bait" strain collected after surviving five months of treatments with abamectin gel bait (Fardisi et al. 2019).

No choice bait feeding assays with commercial fipronil, abamectin, emamectin benzoate, and indoxacarb gel baits confirmed the vial assay results with similar mortality rates (Table 2.6). No choice bait assays exhibited faster mortality rates than choice bait assays and had more homogeneous results across susceptible and resistant strains. For this reason, no choice assays appear better suited for PMP-based resistance monitoring. Choice assays, a.k.a. choice boxes, were created to mimic field experiments in the laboratory, specifically to take advantage of German cockroach locomotor circadian rhythms. Male German cockroaches conduct most of their locomotion activity in darkness, typical for nocturnal insects (Dreisig and Nielsen 1971). From prior experiments, Ebeling et al. (1966) observed that German cockroaches quickly moved towards darkness even if insecticidal dusts bordered dark areas. The I-IN and D-IL strains were collected from low-income housing in Indiana and Illinois, USA. It is known that European residents in low-income housing will live in darkness year-round in order to save money due to living in energy and fuel poverty, which occurs when "a household is not able to satisfy socially and materially the required levels of its energy services" (Kolokotsa and Santamouris 2015). New field experiments could focus on human behaviors and attitudes in different pest management scenarios to test for potential synchrony between human and cockroach circadian patterns.

2.4.5 Summary and conclusions

Overall, vial and bait feeding assays can be excellent assessment tools of insecticide resistance in field strains of German cockroaches. They are both easy to conduct and cost-effective, depending on the resources available (Fardisi et al. 2017; Lee et al. 2022). The B-MD German cockroach strain from Baltimore, Maryland, was the most resistant strain and has been reported to come from a large field population (Dr. Godfrey Nalyanya *pers comm*). Management in the field is possible with field strains possessing similar resistance traits as the B-MD strain with bait rotation of currently registered AI baits (Fardisi et al. 2019). Additionally, emamectin benzoate appears to offer further hope for managing especially-resistant strains like B-MD.

In summary, one objective of this study was to test cockroach strains from two known source-population sizes and compare their insecticide resistance profiles. Once established, an unknown population size (e.g., B-MD) may be estimated based on acquired results; however, more studies should be conducted to estimate population sizes based on insecticide susceptibility levels in German cockroaches. The study also aimed to test newly registered AIs on field strains with differing insecticide susceptibilities. In general, newly registered AIs were the most effective overall against all strains, while older AIs were the worst. Specifically, emamectin benzoate (Optigard bait) was the most effective AI to manage all strains. The results acquired from this study can help develop a newer and rapid test to use in the field based on the no choice feeding assay while also adding more information supporting current resistance and cross-resistance evolution theories.

Table 2.1. Fifteen active ingredient diagnostic concentrations for the JWax-S strain. All concentrations shown are at LC_{99}, except imidacloprid, hydramethylnon, and boric acid which are LC_{90} diagnostic concentrations. Diagnostic concentrations were determined by Fardisi et al. (2017) except for emamectin benzoate, which was determined in the current study.

Insecticide Class	Insecticide Active Ingredients	DC Per Vial
Avermectin	Abamectin	2 µg
	Emamectin Benzoate	0.125 µg
Pyrethroid	Bifenthrin	2 µg
	Beta-Cyfluthrin	1 µg
	Lambda-Cyhalothrin	1 µg
Nicotinoid	Acetamiprid	1 mg
	Clothianidin	200 µg
	Dinotefuran	20 µg
	Imidacloprid	7 mg
	Thiamethoxam	30 µg
Oxadiazine	Indoxacarb	30 µg
Phenylpyrazole	Fipronil	0.1 µg
Respiratory Inhibitors	Boric Acid	60 mg
	Chlorfenapyr	14 µg
	Hydramethylnon	16 µg

Table 2.2. Different bait or insecticide treatments for no choice or choice feeding bioassays.

Bait/Insecticide Treatment	Active Ingredient (%)
Maxforce FC Select Roach Bait Gel	Fipronil (0.01%)
Advion Cockroach Gel Bait	Indoxacarb (0.6%)
Vendetta Cockroach Gel Bait	Abamectin (0.05%)
Optigard Cockroach Gel Bait	Emamectin benzoate (0.1%)
Rodent Diet	No insecticide included

Table 2.3. Number of German cockroaches sampled at the times the I-IN and D-IL populations were initially collected from the field. Cockroaches were with sticky traps in two different apartment complexes in Danville, IL, and Indianapolis, IN. Data from Fardisi et al. (2019).

	Danville (Fair Oaks)	Indianapolis (Laurelwood)
Total Apartments sampled	52	52
Apartments with German Cockroaches	32 (61.54%)	14 (26.92%)
Highest Cockroaches per Apartment	284	31
Lowest Cockroaches per Apartment	1	3
Mean	67.91	14.64
Median	38.50	11.00
Standard Deviation	79.26	11.54
Standard Error	14.01	2.04

Table 2.4. Calculated LT_{50} and LT_{99} values in **no choice** bait assays in the JWax-S, I-IN, D-IL, and B-MD strains of German cockroaches. Values shown in brackets are the lower and upper 95% confidence limits.

Strain	Bait Treatment*	*n*	Slope	LT_{50} in days (95% FL)	LT_{50}RR**	LT_{99} in days (95% FL)	LT_{99}RR**	X^2
JWax-S	Control	70	1.234	>50	--	>50	--	0.946
I-IN	Control	70	1.126	>50	--	>50	--	0.992
D-IL	Control	70	1.104	>50	--	>50	--	0.971
B-MD	Control	90	--	>50	--	>50	--	--
JWax-S	Maxforce	100	--	<0.05	--	<0.05	--	--
I-IN	Maxforce	100	1.172	0.117 (0.042, 0.329)	2.3	11.822 (4.221, 33.110)	236.4	1.000
D-IL	Maxforce	100	1.119	0.064 (0.022, 0.187)	1.3	7.750 (2.674, 22.465)	155	0.989
B-MD	Maxforce	100	1.458	1.284 (0.866, 1.903)	25.7	>50	1056.7	0.999
JWax-S	Advion	100	1.423	0.162 (0.058, 0.450)	--	6.970 (2.505, 19.391)	--	--
I-IN	Advion	100	2.056	0.499 (0.280, 0.891)	3.1	7.116 (3.986, 12.702)	1	0.797
D-IL	Advion	100	2.193	0.676 (0.416, 1.097)	4.2	7.831 (4.826, 12.707)	1.1	0.998
B-MD	Advion	100	1.637	7.988 (6.828, 9.345)	49.3	>50	43.8	0.011
JWax-S	Optigard	100	3.137	0.533 (0.317, 0.896)	--	3.052 (1.817, 5.127)	--	--
I-IN	Optigard	100	3.066	0.764 (0.501, 1.165)	1.4	4.383 (2.873, 6.685)	1.4	--
D-IL	Optigard	100	3.008	0.824 (0.557, 1.219)	1.5	4.902 (3.314, 7.251)	1.6	0.924
B-MD	Optigard	100	1.957	0.548 (0.316, 0.951)	1	8.443 (4.868, 14.646)	2.8	0.973

Table 2.4 continued

JWax-S	Vendetta	100	3.977	0.726 (0.488, 1.079)	--	2.791 (1.877, 4.150)	--	--
I-IN	Vendetta	100	2.940	0.893 (0.605, 1.318)	1.2	5.580 (3.781, 8.234)	2	0.796
D-IL	Vendetta	100	2.614	0.929 (0.631, 1.367)	1.3	7.284 (4.950, 10.718)	2.6	0.970
B-MD	Vendetta	100	3.312	2.515 (2.001, 3.160)	3.5	12.843 (10.221, 16.139)	4.6	0.985

* Active ingredients for baits: Advion (indoxacarb), Maxforce (fipronil), Optigard (emamectin benzoate), Vendetta (abamectin).
**Resistance ratios at the LT_{50} and LT_{99} levels obtained by dividing field strain values by the JWax-S values.

Table 2.5. Calculated LT_{50} and LT_{99} values in **choice** bait assays in the JWax-S, I-IN, D-IL, and B-MD strains of German cockroaches. Values shown in brackets are the lower and upper 95% confidence limits.

Strain	Bait Treatment*	*n*	Slope	LT_{50} in days (95% FL)	LT_{50}RR**	LT_{99} in days (95% FL)	LT_{99}RR**	X^2
JWax-S	Control	100	0.651	>50	--	>50	--	1
I-IN	Control	70	0.754	>50	--	>50	--	1
D-IL	Control	100	1.196	>50	--	>50	--	1
B-MD	Control	100	0.065	>50	--	>50	--	1
JWax-S	Maxforce	100	0.93	0.063 (0.024, 0.167)	--	18.898 (7.171, 49.802)	--	1
I-IN	Maxforce	100	0.707	0.040 (0.014, 0.112)	0.6	>50	4.2	1
D-IL	Maxforce	100	1.065	0.672 (0.393, 1.147)	10.7	>50	5.9	1
B-MD	Maxforce	100	1.146	0.155 (0.072, 0.332)	2.46	16.344 (7.614, 35.082)	0.865	1
JWax-S	Advion	100	1.467	0.675 (0.424, 1.075)	--	26.040 (16.353, 41.467)	--	1
I-IN	Advion	100	1.037	0.189 (0.092, 0.389)	0.28	31.982 (15.507, 65.963)	1.23	1
D-IL	Advion	100	0.985	0.365 (0.195, 0.684)	0.54	>50	3.13	1
B-MD	Advion	100	1.003	0.974 (0.574, 1.653)	1.44	>50	--	1
JWax-S	Optigard	100	1.599	1.041 (0.701, 1.547)	--	29.882 (20.117, 44.388)	--	1
I-IN	Optigard	100	1.555	0.646 (0.406, 1.028)	0.6	20.236 (12.725, 32.182)	0.7	1
D-IL	Optigard	100	1.437	0.727 (0.452, 1.167)	0.7	42.722 (26.591, 68.637)	1.4	1
B-MD	Optigard	100	2.555	0.745 (0.492, 1.130)	0.716	6.066 (4.001, 9.197)	0.203	0.974

Table 2.5 continued

JWax-S	Vendetta	100	2.097	1.546 (1.133, 2.109)	--	20.082 (14.720, 27.398)	--	1
I-IN	Vendetta	100	1.707	0.772 (0.471, 1.106)	0.5	17.630 (11.498, 27.032)	0.9	0.999
D-IL	Vendetta	100	2.178	1.839 (1.379, 2.453)	1.2	21.615 (16.204, 28.834)	1.08	1
B-MD	Vendetta	100	2.668	1.384 (1.025, 1.868)	0.895	10.309 (7.637, 13.915)	0.513	0.939

* Active ingredients for baits: Advion (indoxacarb), Maxforce (fipronil), Optigard (emamectin benzoate), Vendetta (abamectin).
**Resistance ratios at the LT_{50} and LT_{99} levels obtained by dividing field strain values by the JWax-S values.

Table 2.6. Pearson's *r* correlation between bioassays in the JWax-S, I-IN, D-IL, and B-MD strains of German cockroaches.

AI	Correlation Type	*t*	*df*	*p*-value	95% C.I.	Sample Estimates
Combined	Vial x No choice LT_{50}	-5.0906	14	0.0001645	(-0.9300036, -0.5162578)	-0.8057597
	Vial x No choice LT_{99}	-8.9259	14	3.74E-07	(-0.9730918, -0.7857675)	-0.9222486
	Vial x Choice LT_{50}	-0.17605	14	0.8628	(-0.5303485, 0.4594095)	-0.046999
	Vial x Choice LT_{99}	-0.85343	14	0.4078	(-0.6467852, 0.3071907)	-0.2223773
Fipronil	Vial x No choice LT_{50}	-13.606	2	0.005359	(-0.9998934, -0.7615037)	-0.9946413
	Vial x No choice LT_{99}	-17.816	2	0.003136	(-0.9999377, -0.8533257)	-0.9968642
	Vial x Choice LT_{50}	0.20053	2	0.8596	(-0.9487025, 0.9705269)	0.1403924
	Vial x Choice LT_{99}	0.8304	2	0.4937	(-0.8858183, 0.9870785)	0.5063439
Indoxacarb	Vial x No choice LT_{50}	-5.0048	2	0.03768	(-0.99923825, -0.01639951)	-0.9623191
	Vial x No choice LT_{99}	-4.4332	2	0.0473	(-0.99903915, 0.09941835)	-0.9526988
	Vial x Choice LT_{50}	-1.3523	2	0.3089	(-0.9927772, 0.8040290)	-0.6911001
	Vial x Choice LT_{99}	-1.8985	2	0.198	(-0.9956478, 0.6941408)	-0.801953
Abamectin	Vial x No choice LT_{50}	-0.68584	2	0.5636	(-0.9845474, 0.9037370)	-0.4363545
	Vial x No choice LT_{99}	-1.4399	2	0.2866	(-0.9933850, 0.7878752)	-0.7134423
	Vial x Choice LT_{50}	-0.47038	2	0.6844	(-0.9795663, 0.9265165)	-0.3156064
	Vial x Choice LT_{99}	0.27533	2	0.8089	(-0.9432233, 0.9734072)	0.1910966
Emamectin Benzoate	Vial x No choice LT_{50}	--	2	--	--	--
	Vial x No choice LT_{99}	--	2	--	--	--
	Vial x Choice LT_{50}	--	2	--	--	--
	Vial x Choice LT_{99}	--	2	--	--	--

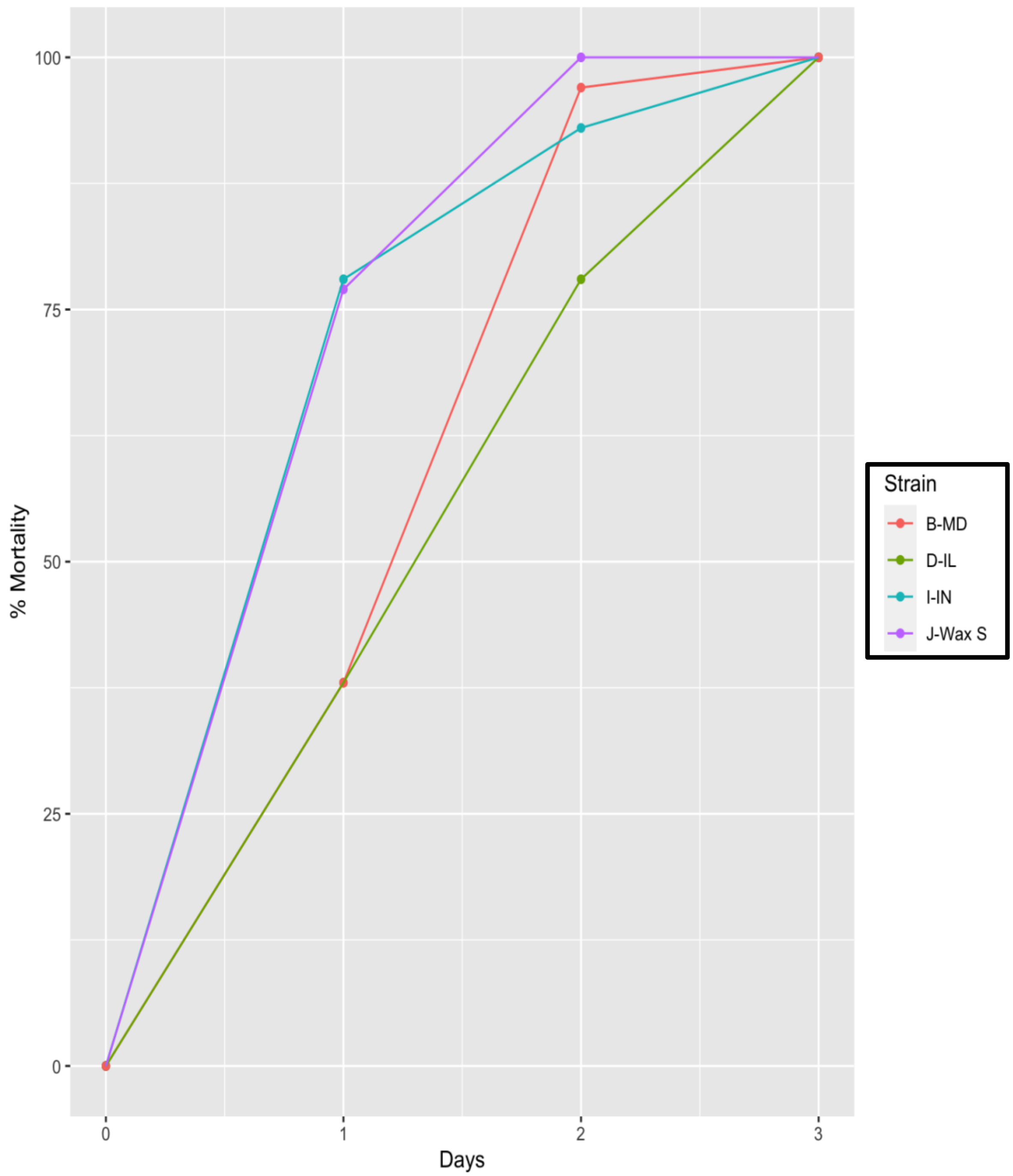

Figure 2.1.Vial assay results of the established emamectin benzoate DC mortality and time in the JWax-S, I-IN, D-IL, and B-MD strains of German cockroaches.

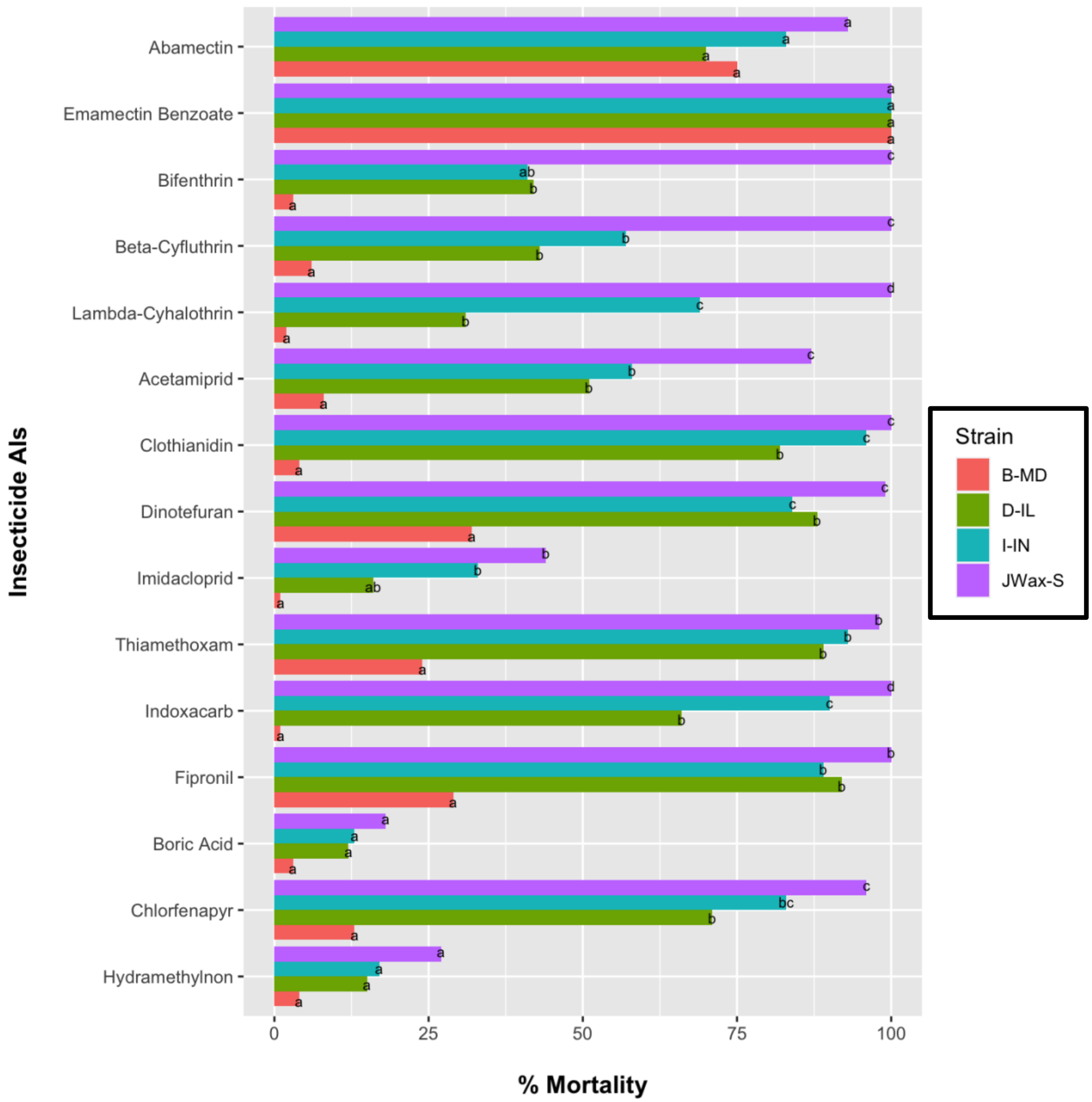

Figure 2.2. Vial assay results of average insecticide AI mortality in the JWax-S, I-IN, D-IL, and B-MD strains of German cockroaches.

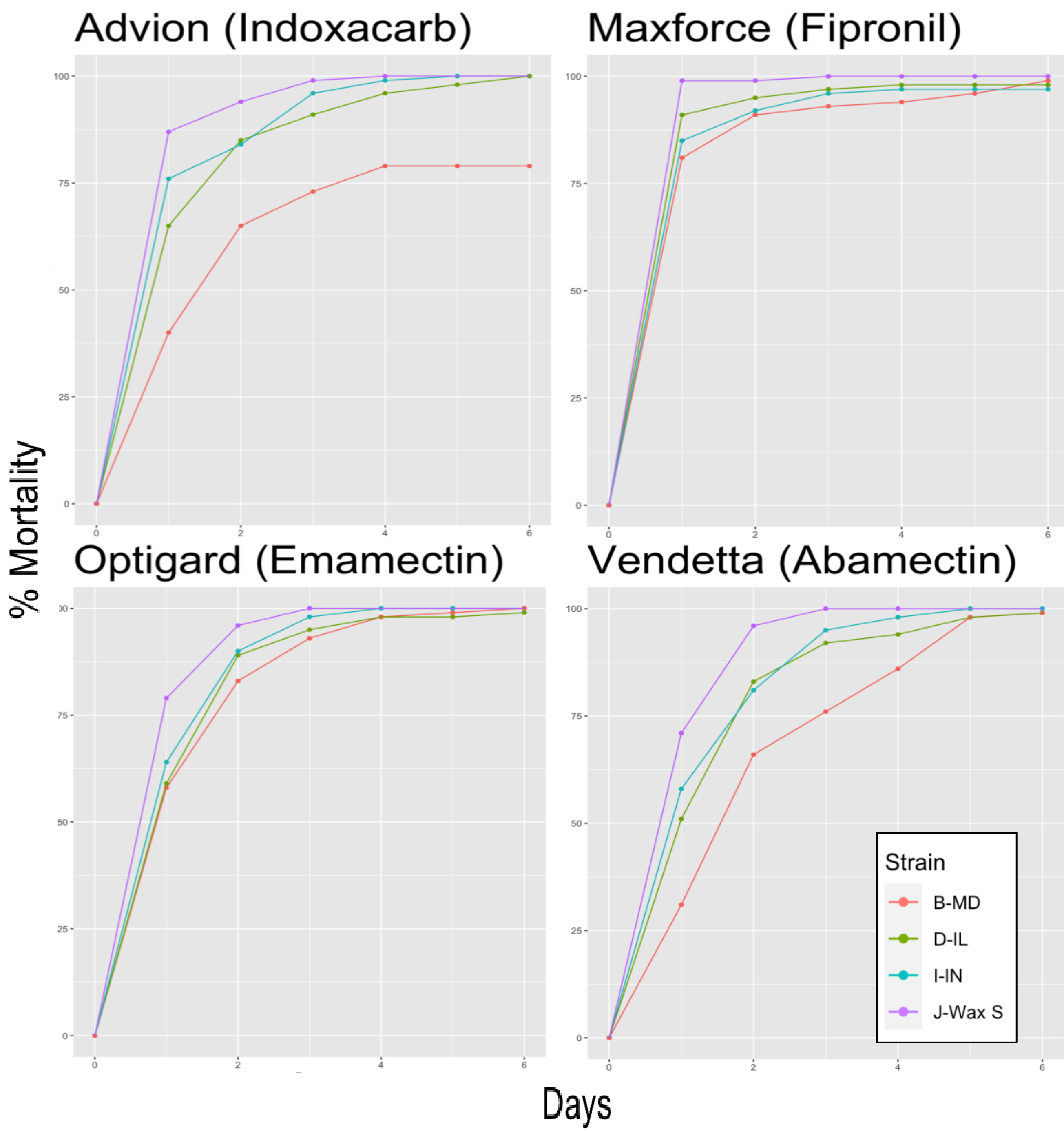

Figure 2.3. No choice bait assay results of average bait mortality in the JWax-S, I-IN, D-IL, and B-MD strains of German cockroaches.

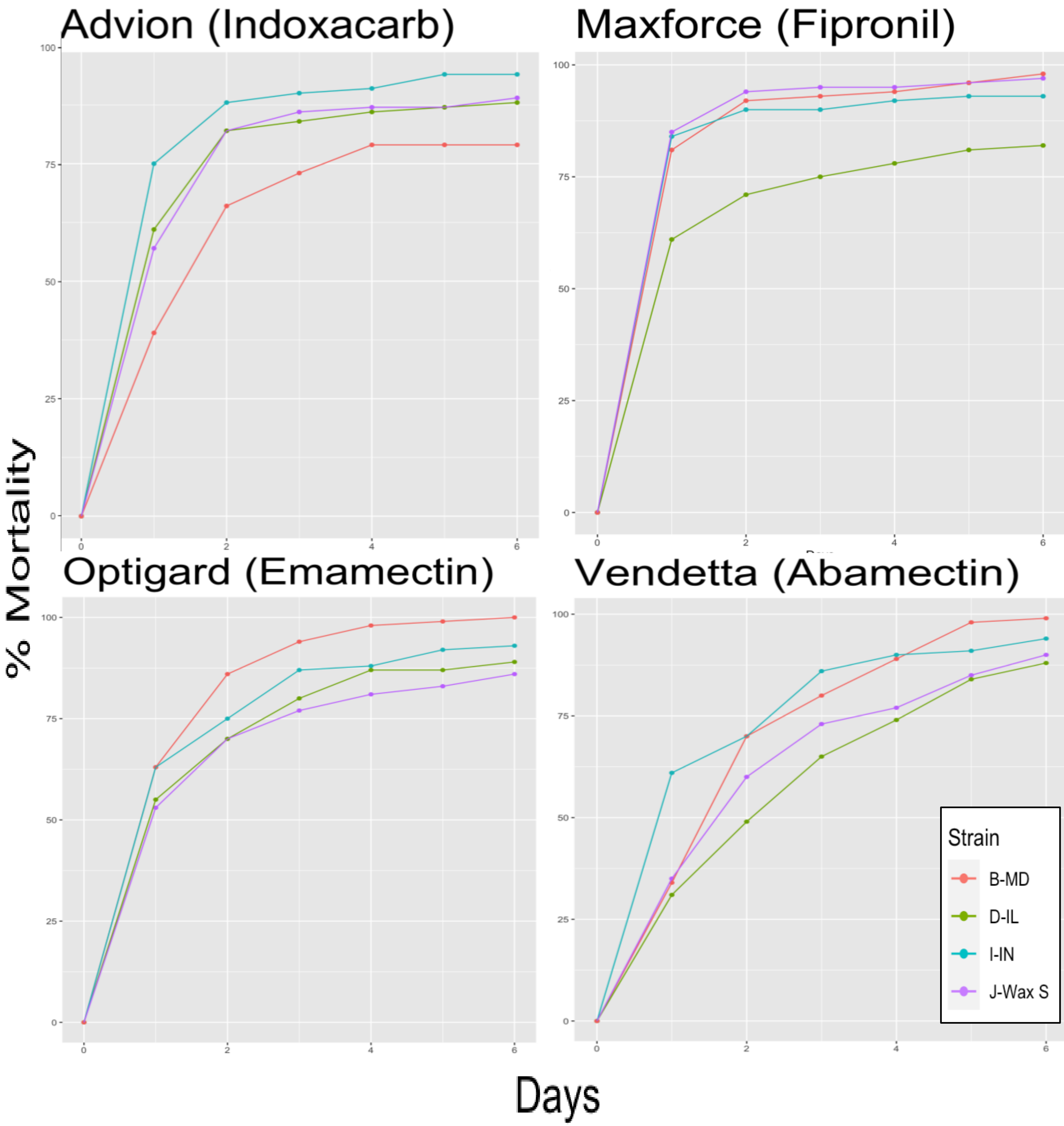

Figure 2.4. Choice bait assay results of average bait mortality in the JWax-S, I-IN, D-IL, and B-MD strains of German cockroaches.

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CHAPTER 3. SUMMARY AND CONCLUSION

The German cockroach (*Blattella germanica*) is one of the most critical pests in indoor urban environments. It causes a significant health risk for humans due to their large population sizes and grooming habits. Due to the potentially severe health risks of the cockroach, several control measures are widely used. Insecticides have dominated over other control measures due to their quick action and most promising results against the cockroach. However, the rate at which cockroaches develop resistance to insecticides is alarming to residents inhabiting urban structures and the professionals applying the insecticide treatments. This study designed an experiment to establish resistance profiles to various cockroach strains that each display unique population characteristics. This study's primary purpose was to understand better the relationship between *B. germanica* populations and insecticide resistance and to compare cockroach strains having a wide range of susceptibility levels in different bioassays for (1) the purpose of understanding which assays are better for resistance monitoring purposes, and (2) for investigating possible links between founding population sizes and cross-resistance levels. Fifteen AIs, specifically in the avermectin, pyrethroid, nicotinoid, oxadiazine, phenylpyrazole, and respiratory inhibitor insecticide classes, were registered at the time of the experiments were tested at single diagnostic concentrations (DCs) in vial bioassays. No choice and choice assays with four currently registered bait products were selected to confirm AI resistance through cockroach behavior, along with a new utility for PMPs to monitor resistance in the field. Finally, selected population characteristics were observed in virgin females and nymphs in each cockroach strain to determine if certain population growth traits contributed to insecticide resistance. The results indicate that emamectin benzoate is the most effective AI in causing the highest mortality in all strains in vial bioassays. No choice assays confirmed vial assay results, with Optigard (emamectin benzoate) being the most effective bait in all strains. In terms of growth characteristics, the time a female carries its egg case and takes for a nymph to become an adult varies across all strains.

For the first part of Chapter 2, resistance profiles were established among three different strains of cockroaches. The cockroach strains were from Indianapolis, Indiana (I-IN), Danville, Illinois (D-IL), and Baltimore, Maryland (B-MD), with fifteen AIs in DCs. Emamectin benzoate was the most effective AI, while boric acid and hydramethylnon were the least effective AIs in all strains. The B-MD strain was resistant to nearly every AI, while there were variations in each AI class in the other strains.

For the second part of Chapter 2, no choice and choice assays were conducted to confirm the established resistance profiles from the vial assays in four currently registered bait insecticides. No choice assays confirmed that the fipronil (Maxforce), indoxacarb (Advion), abamectin (Vendetta), and emamectin benzoate (Optigard) AIs from the vial assays are effective in all strains. The B-MD strain was the most tolerant of the insecticide baits, while the control strain was the least tolerant in the no choice assays. The reverse can be observed in the choice assays, with the D-IL strain as the most tolerant while the B-MD strain is the least tolerant of the insecticide baits.

In the last part of the study, specific population growth dynamics of all the cockroach strains were compared. The number of days from ootheca to nymph, days from nymph to adult, number of nymphs, number of adults, and number of each sex were compared in virgin females and their offspring. The days from ootheca to nymph were significantly different between the JWax-S and D-IL strains, and the days from nymph to adult were significantly different between the B-MD and the other strains. These results suggest that possible fitness tradeoffs in the B-MD strain are associated with their significant resistance status across multiple insecticide classes.

This study confirmed that cockroaches sampled from larger populations showed higher resistance and cross-resistance levels. Resistance was higher and more uniform across locations to AIs available over the counter (i.e., pyrethroid insecticides) that are extensively over-used. Finally, resistance was lower and less uniform to AIs available only for professional use (i.e., emamectin benzoate). Vial assays are an excellent assay to conduct on unknown field strains if laboratory equipment and time are available. However, no choice bait feeding assays may be better for professionals who do not have laboratory equipment and time.

APPENDIX A. GROWTH CHARACTERISTICS OF FIELD STRAINS

Summary:

In my second chapter, I have found that several insecticides are either effective or are not effective in impacting several field strains of German cockroaches. To determine if the resistance of each field strain is associated with each strain's unique population dynamics, I compared different growth characteristics in the JWax-S, I-IN, D-IL, and B-MD cockroach strains (see Table A.1). These strains were chosen for study due to their variety of resistance expressed towards different insecticide classes. Twenty virgin female German cockroaches were aged from their final nymph instar to adult and placed in a larger container with adult male German cockroaches. When ootheca first appeared, females were placed individually in their own container and observed until their egg case dropped. Females were later discarded, and new nymphs were observed until molting into adults.

Based on a one-way ANOVA analysis in R, there was a significant effect between the strains in both the days from ootheca to nymph emergence ($F(3,53) = 3.688$, $p = 0.0174$) and the days from nymph to adult emergence ($F(3,53) = 13.47$, $p = 0.00000119$). As shown in Figure A.1, Tukey's HSD Test for multiple comparisons in R found that the mean value of the days from ootheca to nymph was significantly different between the JWax-S and D-IL strains ($p = 3.688$, 95% C.I. = [26, 58]) and that the mean value of the days from nymph to adult was significantly different between the B-MD and the other strains ($p = 0.00000119$, 95% C.I. = [43, 82]). With regards to the other dependent variables, there were no other significant effects between the strains in a one-way ANOVA analysis. These findings suggest fitness differences among strains, particularly the B-MD strain which took nearly 1-week longer to pass through its immature instars relative to the other strains tested.

Table A.1. Observational characteristics of the JWax-S, I-IN, D-IL, and B-MD strains. See Chapter 2 for strain details.

Strain	*n* (Number of Replicates)	Average days from ootheca to nymph	Average days from nymph to adult	Average number of nymphs	Average number of adults	Average number of males	Average number of females
JWax-S	11	36	50	32	28	15	13
I-IN	21	33	55	32	29	15	14
D-IL	11	29	55	32	35	17	18
B-MD	14	34	63	31	28	13	15

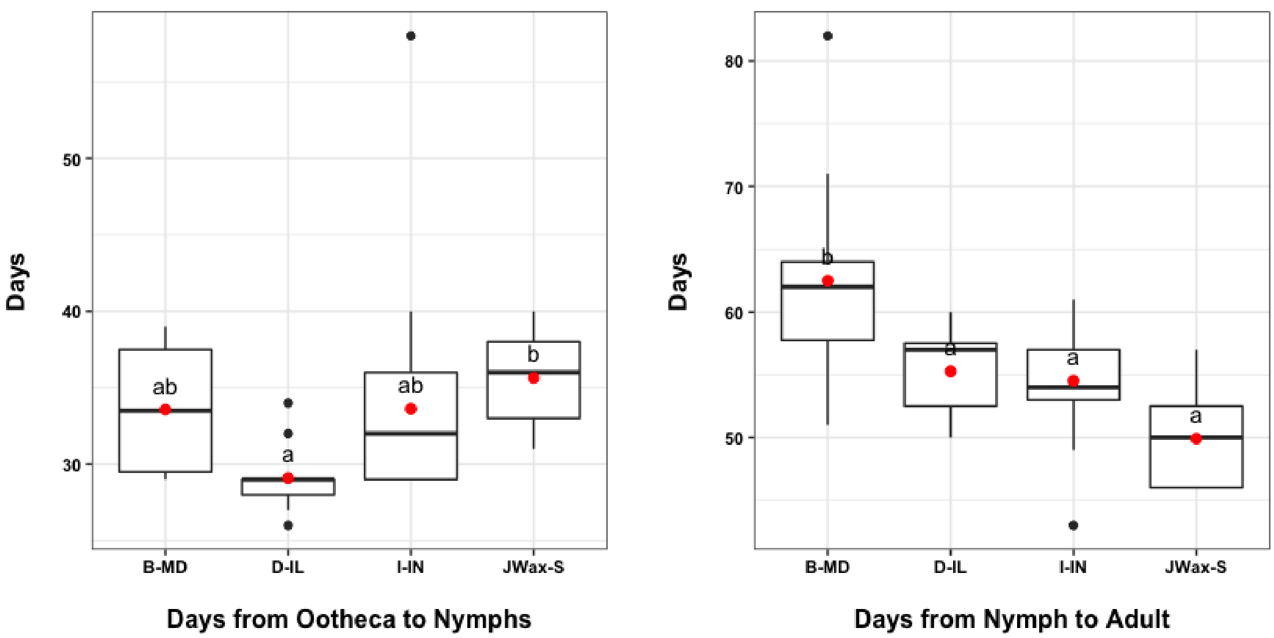

Figure A.1. Visual results of the different strains from a one-way ANOVA test and a Tukey's HSD Test for multiple comparisons.

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