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Impacts of Bioassay Type on Insecticide Resistance Assessment in the German Cockroach (Blattodea: Ectobiidae)

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Abstract

The German cockroach, *Blattella germanica* (L.), is one of the most critical urban pests globally due to the health risks it imposes on people, such as asthma. Insecticides are known to manage large cockroach population sizes, but the rapid rate at which they develop resistance is a continuing problem. Dealing with insecticide resistance 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 insecticide susceptibilities, so resistance profiles must be considered. This study addressed the above issue in a controlled laboratory setting. Cockroach strains from Indianapolis, Indiana, Danville, Illinois, and Baltimore, Maryland, USA were used. Four insecticide active ingredients (AIs) most used by consumers and PMPs were selected for testing in vial bioassays to establish resistance profiles. Next, 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. The results indicate that emamectin benzoate (Optigard) was the most effective AI in causing the highest mortality in all strains in vial and no-choice bioassays; whereas, the other AIs and products were more impacted by resistance. The results acquired from these studies can help develop rapid tests for use by PMPs based on the no-choice feeding assay while also adding more information supporting current resistance and cross-resistance evolution theories.

Key words: emamectin benzoate, fipronil, indoxacarb, abamectin

The German cockroach, *Blattella germanica*, is an urban pest commonly found in urban structures worldwide (Nasirian 2017b, Vargo 2021). They are often considered a health pest due to their association with respiratory, fungal, and bacterial diseases through their depositing of feces, body parts, and cast exoskeletons in human dwellings (Celmeli et al. 2016; Nasirian 2017a, 2019). 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, even though they are not biological vectors, there may be an association between German cockroaches and viral respiratory diseases like COVID-19 (Nekoei et al. 2022). 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 target German cockroach infestations with bait insecticides. Bait insecticides are an effective control measure, particularly within integrated pest management (IPM) programs (Nasirian and Salehzadeh 2019, Gondhalekar et al. 2021). Baits have been recognized as an effective control method for over 150 years after Cowan (1865) first documented them for use as a 'Phosphor Paste' for cockroaches, black-beetles, rats, mice, etc. in 1865 and eventually leading to the first modern commercial baits containing hydramethylnon in the

1980s. 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. As a species, German cockroaches are known to be resistant to nearly all insecticides used against them. 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.

Physiological and behavioral mechanisms drive insecticide resistance in German cockroaches, and different populations demonstrate different resistance profiles depending on their insecticide selection history (Scharf et al. 1997, 2022). Studying physiological mechanisms and the pattern in how insecticides are used is essential to understanding insecticide resistance in the German cockroach (Gondhalekar and Scharf 2013, Scharf and Gondhalekar 2021, Zhu et al. 2016). The size of an insect population may also be linked to insecticide resistance. For example, researchers demonstrated that there is evidence of correlation between 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). In contrast, Rust and Reierson (1991) found no relationship between population size and resistance to a residual organophosphate insecticide (chlorpyrifos) in German cockroach populations from restaurants (Rust and Reierson 1991). However, there is little evidence in German cockroaches of how population size may impact bait insecticide resistance in urban housing, i.e., are populations large because they exhibit high insecticide resistance? One aspect of the current study considered how physiological resistance may correspond to population size in German cockroaches.

Glass vial bioassays are an excellent tool for studying insecticide resistance, and German cockroaches are well-suited 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 variable resistance profiles. Due to these significant prior results, DCs with vial assays were 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 useful assessment tools of insecticide resistance in field strains of German cockroaches. They are easy to conduct and cost-effective depending on available resources (Fardisi et al. 2017, Lee et al. 2022).

The primary objective of this study was to compare cockroach strains having a range of susceptibility levels in different bioassays for the purpose of understanding which assays are better for resistance monitoring. A secondary objective was to investigate possible links between three 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 using four AI-DCs against one susceptible and three resistant cockroach strains in vial assays, and 3) to compare choice and no-choice bait feeding assays with formulated products 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.

Materials and Methods

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 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 (Supp Table S1 [online only]). The B-MD strain was collected by Dr. Godfrey Nalyanya of Rentokil Inc. from a public market 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 cm/15.14 L; S.C. Johnson Inc., Racine, WI) with screened lids and held in a controlled environmental chamber at 26 ± 1°C and a photoperiod of 12:12 h (Light: Dark). 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.

Insecticides

Technical insecticide 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%), fipronil (98.3%), and emamectin benzoate (98.3%). The AIs were selected because they are currently registered for cockroach control. Four cockroach baits, Maxforce FC Magnum (fipronil 0.05%; Bayer, Research Triangle Park, NC), Vendetta Cockroach Gel Bait (abamectin B1 0.05%; McLaughlin Gormley King Co., Minneapolis, MN), Optigard Cockroach Gel Bait (emamectin benzoate 0.1%; Syngenta, Greensboro, NC) and Advion Cockroach Gel Bait (indoxacarb 0.6%; Syngenta, Greensboro, NC) were purchased from Univar (Indianapolis, IN) for testing in no-choice and choice feeding bioassays.

Vial Bioassays

Fardisi et al. (2017) predetermined lethal concentrations (LCs) and DCs for all AIs listed above except emamectin benzoate in the JWax-S strain (Table 1). In order to determine the DC of emamectin benzoate, prior methods for abamectin were followed in which serial dilutions of emamectin benzoate were tested against the JWax-S strain to identify a candidate DC (Fardisi et al. 2017). Bioassays were conducted in 37-mL Shell vials (25 by 95 mL; Kimble Chase, Vineland, NJ). The internal surfaces of the vials (71.67 cm^2) were treated with 0.5-ml insecticide dilutions. About 1 cm of the top of the vial was left untreated, as a cotton plug would cover it. Insecticide dilutions were made in acetone and used

Table 1. Four active ingredient diagnostic concentrations for the JWax-S strain

Insecticide class	Insecticide active ingredients	DC per vial
Avermectin	Abamectin	2 µg
	Emamectin benzoate	0.125 µg
Oxadiazine	Indoxacarb	30 µg
Phenylpyrazole	Fipronil	0.1 µg

All concentrations shown are at LC_{99}. Diagnostic concentrations were determined by Fardisi et al. (2017) except for emamectin benzoate, which was determined in the current study.

immediately after preparation. Insecticide solutions were mixed thoroughly before being applied to each vial. After adding insecticide solutions, vials were rotated manually for 1 min and then on a non-heating hotdog roller (Nostalgia Products LLC, Green Bay, WI) placed in a fume hood. Complete evaporation of acetone required about 30 min. Vials treated with acetone-only were used as controls.

Adult male cockroaches were anesthetized in plastic cups on ice before transferring to individual vials in groups of ten. 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. Ten replicates for each AI and strain were performed, and mortality was recorded every 24 h up to 72 h. 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.

No-choice Assay Methods

Four gel bait products were screened against the JWax-S, I-IN, D-IL, and B-MD strains, as seen in AI-DC assays. Tested gel baits included Maxforce (fipronil), Vendetta (abamectin), Optigard (emamectin benzoate), and Advion (indoxacarb) (Supp Table S2 [online only]). 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 6 cm/0.739 L; 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-g gel bait and a water cup, and cardboard shelters were provided in each container. For controls, the gel bait was replaced with a 0.5-g 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-cm diameter). Ten replications for each strain–treatment combination were conducted. Mortality was checked every 24 h until 100% mortality was achieved in all strains. All assay boxes were kept 72 h after 100% mortality was achieved to ensure no recovery occurred.

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 15.24 cm high × 22.9 cm wide plastic boxes (27.3 × 4.9 cm), one painted black, were connected near the top by a 2.5-cm length of 0.64 cm tubing. One box (dark side) received 0.5 g 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 h (Light: Dark). Ten male cockroaches were released in the light side for acclimation one day before the experiment, and mortality was scored every 2 h and daily after 12 h up to 15 d. Container walls were lightly greased 2.5 cm from the top and closed tightly with lids to prevent escape. Only the lid for the light side contained a central meshed opening (3-cm diameter). Ten replications were done for choice assays on all strains with JWax-S as a positive control strain.

Data Analyses

For comparing variation among strains, vial 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. The impact of the baits on survivorship was analyzed with Kaplan-Meier analysis, and survivorship curves were compared with that of the JWax-S strain using log-rank tests in SPSS Statistics Version 28.0 (IBM Corporation, Armonk, NY). See Supplementary Materials for more information.

Results

Vial Bioassays

Diagnostic concentrations for use in vial bioassays, except for emamectin benzoate, were reported previously (Fardisi et al. 2017). The candidate emamectin benzoate diagnostic concentration (DC) was developed in the current research for the JWax-S strain. The DC for emamectin benzoate was determined from serial dilutions and was patterned after prior work done with abamectin (Fardisi et al. 2017). Due to acquiring high mortality in all strains at all test concentrations, the emamectin benzoate diagnostic concentration was estimated at 0.125 µg/vial. This concentration of emamectin benzoate is 16-fold lower compared to the abamectin diagnostic concentration reported previously (Fardisi et al. 2017) and effectively causes 100% mortality in the JWax-S strain by 72 h.

Figure 1 shows the percentage mortality comparisons for the four strains and four AIs at their respective DCs. Exposure to abamectin DC resulted in >70% mortality of all field strains. Highest mortality (93%) was achieved in the JWax-S strain when exposed to the abamectin DC; mortality in the I-IN, B-MD, and D-IL strains was lower at 83, 75, and 70%, but was not significantly different. Total mortality was achieved in all strains when exposed to the emamectin benzoate DC.

Indoxacarb DC assays resulted in different mortalities in the field strains, while 100% mortality was achieved in JWax-S. Conversely, 90%, 66%, and 1% mortality were achieved in I-IN, D-IL, and B-MD strains when exposed to indoxacarb at its DC.

Finally, I-IN strain mortality when exposed to fipronil was 89%. Mortality in the D-IL strain was 92% with fipronil. B-MD mortality was 29% with fipronil, while in the JWax-S strain mortality was 100%.

No-choice Bioassays

Survival probability in no-choice assays up to 6 d is shown for all four strains when provided commercially-formulated gel baits containing the AIs fipronil, abamectin, emamectin benzoate, and indoxacarb (Supp. Fig. S1 [online only]). Kaplan-Meier survival values for different gel baits ranged from 1 to 6 d for the JWax-S, I-IN, D-IL, and B-MD strains (Fig. 2). All strains achieved 100% mortality by ten days except the highly resistant B-MD strain. With Maxforce (fipronil) gel bait, all

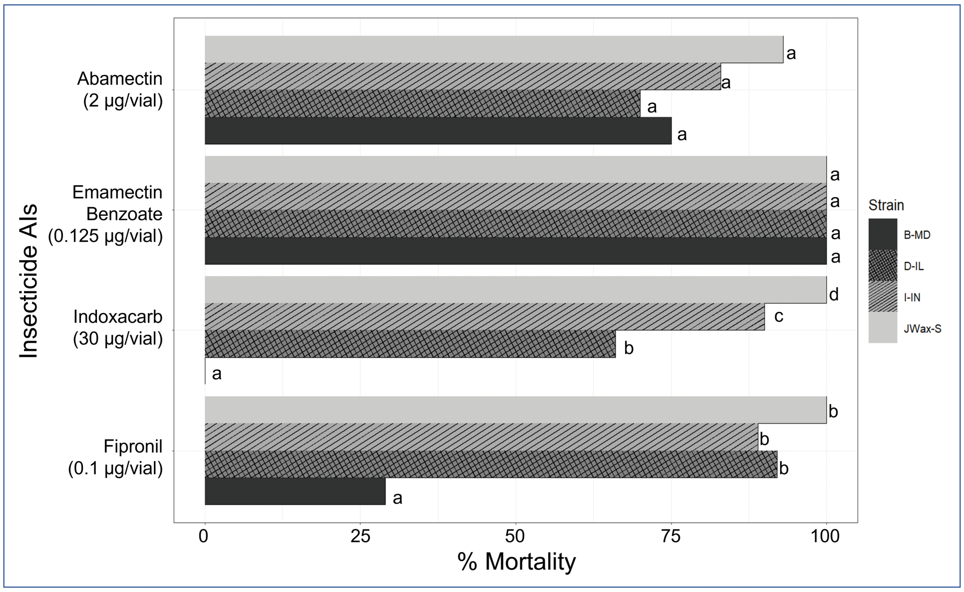

Fig. 1. Diagnostic vial assay results showing average mortality in the JWax-S, I-IN, D-IL, and B-MD strains of German cockroaches after 72 h. Bars for each insecticide AI with the same letter are not significantly different by two-way factorial ANOVA and univariate analyses (*P* < 0.05).

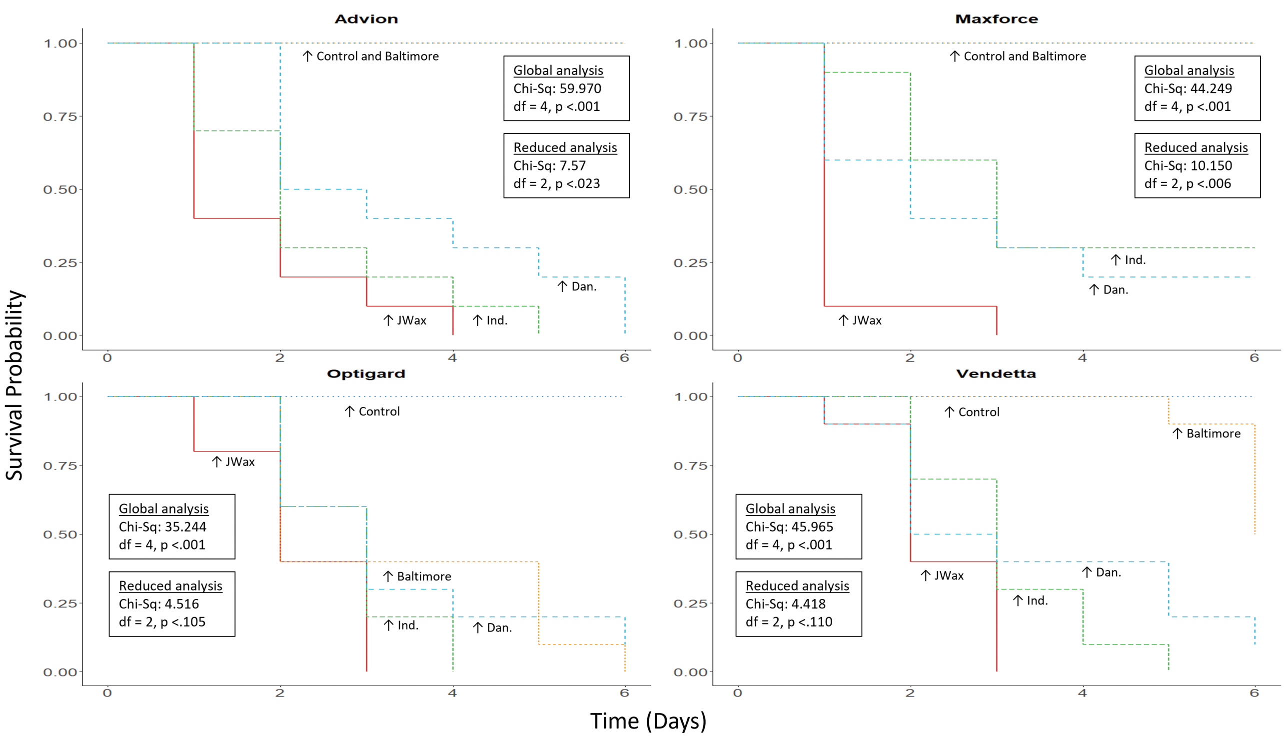

Fig. 2. No-choice bait assay survivorship of the JWax-S, I-IN, D-IL, and B-MD cockroach strains over time. Log-rank tests were performed to determine the differences amongst strains. *P*-values indicate differences between strains. Global analysis (df = 4) indicates that all strains were compared while reduced analysis (df = 2) indicates that only JWax-S, I-IN, and D-IL strains were compared.

strains except I-IN and B-MD achieved >90% mortality within one day after starting assays. The mean mortality for untreated controls in all strains combined was <5%. The rank of strains in no-choice assays from least to most tolerant was JWax-S, I-IN, D-IL, and B-MD

Choice Bioassays

Survival probability in choice assays up to 6 d is shown for all four strains when provided commercially-formulated gel baits (Supp. Fig. S2 [online only]). Kaplan-Meier survival analysis values for different gel baits ranged between 1 to 15 days for the JWax-S, I-IN, D-IL, and B-MD strains (Fig. 3). Greater than 90% mortality was achieved after 13 d for all strains. With Optigard (emamectin benzoate) gel bait, all strains achieved >70% mortality within two days after starting assays. The mean mortality for untreated controls in all strains combined was <10%. The rank of strains in choice assays from least to most tolerant was B-MD, I-IN, JWax-S, and D-IL, which is notably different from the no-choice assays.

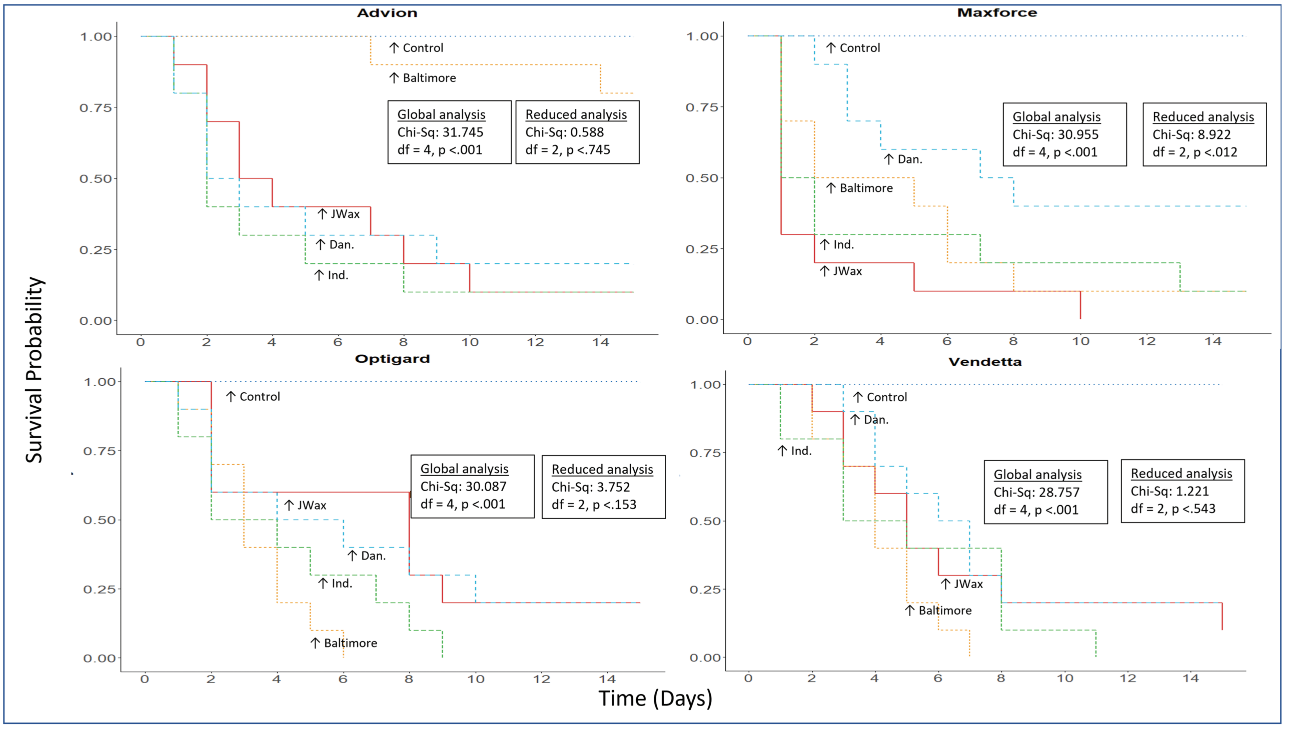

Fig. 3. Choice bait assay survivorship of the JWax-S, I-IN, D-IL, and B-MD cockroach strains over time. Log-rank tests were performed to determine the differences amongst strains. *P*-values indicate differences between all strains. Global analysis (df = 4) indicates that all strains were compared while reduced analysis (df = 2) indicates that only JWax-S, I-IN, and D-IL strains were compared.

Discussion

Four different German cockroach strains were used in this study. The JWax-S strain was chosen 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 chosen as a small population strain, and the D-IL strain for a large population strain based on their documented glue trap catches from prior field studies (Supp Table S1 [online only]). Trap catch is relevant for estimating the size of a population or infestation in the context of community-wide pest management (Fardisi et al. 2019, Miller and Smith 2020). The B-MD strain was included as a recently-collected field strain and due to repeated control failures immediately prior to its collection.

This study tested currently registered commercial bait products and their insecticide AIs against the above cockroach strains having different population starting sizes and resistance levels. More importantly, the study identified candidate rapid tests that have apparent utility for testing currently registered AIs on field strains with unknown resistance status. Vial, choice, and no-choice feeding assays were conducted on four different cockroach strains with differences in insecticide resistance profiles. Emamectin benzoate caused the highest mortality in vial assays, and indoxacarb showed the least mortality in all strains. No-choice bait assays confirmed vial results more strongly than choice assays in all strains. The B-MD strain demonstrated the highest insecticide resistance out of all the strains and all indications are that the B-MD strain had a large field population size (Dr. Godfrey Nalyanya, personal communication).

Resistance Mechanisms in German Cockroaches

Each strain was tested using 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, Scharf and Gondhalekar 2021). 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 tool for resistance monitoring if a laboratory is available.

Variation in Mortality among Different Insecticide Classes

Emamectin benzoate caused 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), which agrees with our findings of high activity at low concentrations. 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 effective 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 otherwise-resistant German cockroach populations regardless of population size, cross-resistance profiles, or prior insecticide exposure history.

Indoxacarb was variably ineffective against all field strains, also as previously demonstrated in Fardisi et al. (2017). Indoxacarb has been used extensively since the 2000s and has shown high-level resistance in many populations (*Present Study*; Davari et al. 2018, Salehzadeh et al. 2020, Scharf et al. 2022). Curl's (2011) market analysis identified high market sales for indoxacarb products, which supports the existence of high indoxacarb selection pressure on a wide geographic scale. Overall, German cockroaches typically exhibit widespread insecticide resistance to easily accessible AIs extensively used by consumers and pest management professionals (DeVries et al. 2019).

Resistance Profiles in Field Collected Strains

The Baltimore (B-MD) strain exhibited the highest resistance frequencies across AIs with the exception of 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 tested strains yet (Scharf and Gondhalekar 2021). Although we do not have field population information as reported for the I-IN and D-IL strains, the B-MD field population experienced multiple indoxacarb control failures and exhibited a large field population size that would have a greater probability of possessing a variety of resistance mutations (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). Additionally, the agreement in results for vial and gel bait feeding assays suggests the candidate vial DC for emamectin benzoate (0.125 µg/vial) is reasonable for resistance monitoring purposes.

No-choice bait feeding assays with commercial fipronil, abamectin, emamectin benzoate, and indoxacarb gel baits confirmed the vial assay results with similar mortality rates. No-choice bait assays had faster mortality rates than choice 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 conditions in the laboratory, and further take advantage of German cockroach locomotor circadian rhythms. Male German cockroaches conduct most of their locomotion activity in darkness, which is 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. New field experiments could focus on how cockroach circadian rhythms might affect resistance status and assessment.

Summary and Conclusions

In summary, one objective of this study was to test cockroach strains from two known source-population sizes (I-IN, D-IL) and compare their insecticide resistance profiles. The study also aimed to test a newly registered AI, emamectin benzoate, on field strains with differing insecticide susceptibilities. In general, more newly-registered AIs were the most effective overall against all strains, while older AIs were least effective, supporting the idea that the longer amount of time a product is in the market, the greater the potential for widespread resistance problems. Specifically, emamectin benzoate (Optigard bait) was the most effective AI tested against all strains and we further identified an effective vial assay DC for emamectin benzoate of 0.125 µg/vial. Emamectin benzoate thus appears to offer promise for managing especially-resistant strains like B-MD.

Overall, results presented here suggest that vial and bait feeding assays are suitable tools to assess insecticide resistance in field strains of German cockroaches. They are both relatively uncomplicated 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 tested and has been reported to come from a large field population (Dr. Godfrey Nalyanya, personal communication). Based on field sampling data, the D-IL strain came from a larger field population than the I-IN strain and displayed generally broader cross-resistance profiles (Supp Table S1 [online only]; Fardisi et al. 2017, 2019). However, as would be expected, resistance levels in the D-IL and I-IN strains declined with several years of lab rearing, but nonetheless have persisted to a certain degree as seen in the present study. Management in the field is possible with field strains possessing similar resistance traits as the B-MD strain with rotation of currently registered AI baits (Fardisi et al. 2019, Miller and Smith 2020). The results acquired from this study can help develop a 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.

Acknowledgments

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Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

References Cited

Caprio, M. A., and B. E. Tabashnik. 1992. Gene flow accelerates local adaptation among finite populations: simulating the evolution of insecticide resistance. *J. Econ. Entomol*. 85: 611–620. doi:10.1093/jee/85.3.611

Celmeli, F., S. T. Yavuz, D. Turkkahraman, O. Simsek, A. Kılınc, and B. E. Sekerel. 2016. Cockroach (*Blattella germanica*) sensitization is associated with coexistence of asthma and allergic rhinitis in childhood. *Pediatr. Allergy Immunol*. 29: 38–43.

Cowan, F. 1865. *Curious facts in the history of insects*. J.B. Lippincott & Co., Philadelphia.

Curl, G. 2011. *A strategic analysis of the US structural pest control industry*. Specialty Product Consultants, LLC, Mendham, NJ.

Davari, B., S. Kashani, H. Nasirian, M. Nazari, and A. Salehzadeh. 2018. The efficacy of MaxForce and Avion gel baits containing fipronil, clothianidin and indoxacarb against the German cockroach (*Blattella germanica*). *Entomol. Res*. 48: 459–465. doi:10.1111/1748-5967.12282

DeVries, Z. C., R. G. Santangelo, J. Crissman, A. Suazo, M. L. Kakumanu, and C. Schal. 2019. Pervasive resistance to pyrethroids in German cockroaches (Blattodea: Ectobiidae) related to lack of efficacy of total release foggers. *J. Econ. Entomol*. 112: 2295–2301.

Do, D. C., Y. Zhao, and P. Gao. 2016. Cockroach allergen exposure and risk of asthma. *Allergy*. 71: 463–474. doi:10.1111/all.12827

Dreisig, H., and E. T. Nielsen. 1971. Circadian rhythm of locomotion and its temperature dependence in *Blattella germanica*. *J. Exp. Biol*. 54: 187–198. doi:10.1242/jeb.54.1.187

Ebeling, W., R. E. Wagner, and D. A. Reierson. 1966. Influence of repellency of the efficacy of blatticides: learned modification of behavior of the German cockroach. *J. Econ. Entomol*. 99: 1374–1388.

Fardisi, M., A. D. Gondhalekar, and M. E. Scharf. 2017. Development of diagnostic insecticide concentrations and assessment of insecticide susceptibility in German cockroach (Dictyoptera: Blattellidae) field strains collected from public housing. *J. Econ. Entomol*. 110: 1210–1217. doi:10.1093/jee/tox076

Fardisi, M., A. D. Gondhalekar, A. R. Ashbrook, and M. E. Scharf. 2019. Rapid evolutionary responses to insecticide resistance management interventions by the German cockroach (*Blattella germanica* L.). *Sci. Rep*. 9: 8292. doi:10.1038/s41598-019-44296-y

Finkas, L., L. Block, M. Lu, B. Yu, M. Lee, and C. Iribarren. **2022**. Retrospective analysis of COVID-19 incidence and health outcomes among patients with asthma in a large integrated health care delivery system. *J. Allergy. Clin. Immunol*. 149: AB57. doi:10.1016/j.jaci.2021.12.218

Gondhalekar, A. D., and M. E. Scharf. 2012. Mechanisms underlying fipronil resistance in a multiresistant field strain of the German cockroach. *J. Econ. Entomol*. 49: 122–131.

Gondhalekar, A. D., and M. E. Scharf. 2013. Preventing resistance to bait products. July: 42-46 (http://www.pctonline.com/pct0713-preventing-resistance-bait-products.aspx).

Gondhalekar, A. D., C. Song, and M. E. Scharf. 2011. Development of strategies for monitoring indoxacarb and gel bait susceptibility in the German cockroach. *Pest Manag. Sci*. 67: 262–270. doi:10.1002/ps.2057

Gondhalekar, A. D., W. Scherer, R. K. Saran, and M. E. Scharf. 2013. Implementation of an indoxacarb susceptibility monitoring program using field-collected German cockroach isolates from the United States. *J. Econ. Entomol*. 106: 945–953.

Gondhalekar, A. D., A. G. Appel, G. M. Thomas, and A. Romero. 2021. A review of alternative management tactics employed for the control of various cockroach species (Order: Blattodea) in the USA. *Insects*. 12: 550. doi:10.3390/insects12060550

Guo, M., W. Zhang, G. Ding, D. Guo, J. Zhu, B. Wang, D. Punyapitak, and Y. Cao. 2015. Preparation and characterization of enzyme-responsive emamectin benzoate microcapsules based on a copolymer matrix of silica–epichlorohydrin–carboxymethylcellulose. *R. Soc. Chem*. 5: 93170–93179.

Harada, K., E. Thanik, N. DeFelice, J. Bhatia, R. Lopez, S. Galvez, M. Bixby, E. Dayanov, D. Bush, and E. Garland. 2022. Housing conditions and access to care for children with asthma during COVID-19 pandemic in New York City. *J. Allergy Clin. Immunol*. 149: AB57. doi:10.1016/j.jaci.2021.12.219

Huang, C., Y. Wang, L. Xingwang, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, *et al*. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 395: 497–506.

Ko, A. E., D. N. Bieman, C. Schal, and J. Silverman. 2016. Insecticide resistance and diminished secondary kill performance of bait formulations against German cockroaches (Dictyoptera: Blattellidae). *Pest Manag. Sci*. 72: 1778–1784. doi:10.1002/ps.4211

Lee, S. H., D. H. Choe, M. K. Rust, and C. Y. Lee. 2022. Reduced susceptibility towards commercial bait Insecticides in field German cockroach (Blattodea: Ectobiidae) populations from California. *J. Econ. Entomol*. 115: 259–265. doi:10.1093/jee/toab244

Liang, Y., Y. Gao, W. Wang, H. Dong, R. Tang, J. Yang, J. Niu, Z. Zhou, N. Jiang, and Y. Cao. 2020. Fabrication of smart stimuli-responsive mesoporous organosilica nano-vehicles for targeted pesticide delivery. *J. Hazard. Mater*. 389: 122075. doi:10.1016/j.jhazmat.2020.122075

Messer, P. W., and D. A. Petrov. 2013. Population genomics of rapid adaption by soft selective sweeps. *Trends Ecol. Evol*. 28: 659–669. doi:10.1016/j.tree.2013.08.003

Miller, D. M., and E. P. Smith. 2020. Quantifying the efficacy of an Assessment-Based Pest Management (APM) Program for German Cockroach (L.) (Blattodea: Blattellidae) control in low-income public housing units. *J. Econ. Entomol*. 113: 375–384.

Nasirian, H. 2017a. Contamination of cockroaches (Insecta: Blattaria) to medically fungi: a systematic review and meta-analysis. *JMM*. 27: 427–448.

Nasirian, H. 2017b. Infestation of cockroaches (Insecta: Blattaria) in the human dwelling environments: a systematic review and meta-analysis. *Acta Trop*. 167: 86–98. doi:10.1016/j.actatropica.2016.12.019

Nasirian, H. 2019. Contamination of cockroaches (Insecta: Blattaria) by medically important bacteriae: a systematic review and meta-analysis. *J. Med. Entomol*. 56: 1534–1554. doi:10.1093/jme/tjz095

Nasirian, H., and A. Salehzadeh. 2019. Control of cockroaches (Blattaria) in sewers: a practical approach systematic review. *J. Med. Entomol*. 56: 181–191. doi:10.1093/jme/tjy205

Nekoei, S., F. Khamesipour, M. Benchimol, R. Bueno-Marí, and D. Ommi. 2022. SARS-CoV-2 transmission by arthopod vectors: a scoping review. *Biomed Res. Int*. 2022: 4329423. doi:10.1155/2022/4329423

Poland, T. M., D. G. McCullough, D. A. Herms, L. S. Baurer, J. R. Gould, and A. R. Tluzeck. 2010. Management tactics for emerald ash borer: chemical and biological control. GTR-NRS-P-75: 21st USDA Interagency Research Forum on Invasive Species; 2010 Jan 12-15; Annapolis, Maryland. Pennsylvania (USA): USDA Forest Service; 2010 June 22.

RStudio Team. **2022**. *RStudio: integrated development for R*. RStudio, Inc., Boston (MA). Available at: http://www.rstudio.com/.

Rust, M. K., and D. A. Reierson. 1991. Chlorpyrifos resistance in German cockroaches (Dictyoptera: Blattellidae) from restaurants. *J. Econ. Entomol*. 84: 736–740. doi:10.1093/jee/84.3.736

Salehzadeh, A., Z. Darvish, B. Davari, and H. Nasirian. 2020. The efficacy of baits containing abamectin, dinotefuran, imidacloprid and pyriproxyfen + abamectin against *Blattella germanica* (L.) (Blattaria: Blattellidae), the German cockroach. *Afr. Entomol*. 28: 225–237.

Schal, C., and R. L. Hamilton. 1990. Integrated suppression of synanthropic cockroaches. *Annu. Rev. Entomol*. 35: 521–551. doi:10.1146/annurev.en.35.010190.002513

Scharf, M. E., and A. D. Gondhalekar. 2021. Insecticide resistance: perspectives on evolution, monitoring, mechanisms and management, pp. 231–267. *In* C. Wang, C. Y. Lee, M. K. Rust (eds), *Biology and management of the German cockroach*. CABI, Boston, MA.

Scharf, M. E., G. W. Bennett, B. L. Reid, and C. Qui. 1995. Comparisons of three insecticide resistance detection methods for the German cockroach. *J. Econ. Entomol*. 88: 536–542. doi:10.1093/jee/88.3.536

Scharf, M. E., W. Kaakeh, and G. W. Bennett. 1997. Changes in an insecticide-resistant field population of German cockroach after exposure to an insecticide mixture. *J. Econ. Entomol*. 90: 38–48. doi:10.1093/jee/90.1.38

Scharf, M. E., J. J. Neal, and G. W. Bennett. 1998. Changes of insecticide resistance levels and detoxication enzymes following insecticide selection in the German cockroach, *Blattella germanica* (L.). *Pest. Biochem. Physiol*. 59: 67–79.

Scharf, M. E., Z. M. Wolfe, K. R. Raje, M. Fardisi, J. Thimmapuram, K. Bhide, and A. D. Gondhalekar. 2022. Transcriptome responses to defined insecticide selection pressures in the German cockroach (*Blattella germanica* L.). *Front. Physiol*. 12: 816675.

Sisterton, M. S., L. Antilla, Y. Carriere, C. Ellers-Kirk, and B. E. Tabashnik. 2004. Effects of insect population size on evolution of resistance to transgenic crops. *J. Econ. Entomol*. 97: 1413–1424.

Vargo, E. L. 2021. Dispersal and population genetics, pp. 143–152. *In* C. Wang, C. Y. Lee, M. K. Rust (eds), *Biology and management of the German cockroach*. CABI, Boston, MA.

Wang, C., and G. W. Bennett. 2006. Comparative study of integrated pest management and baiting for German cockroach management in public housing. *J. Econ. Entomol*. 99: 879–885. doi:10.1603/0022-0493-99.3.879

Wang, C., M. E. Scharf, and G. W. Bennett. 2004. Behavioral and physiological resistance of the German cockroach to gel baits. *J. Econ. Entomol*. 97: 2067–2072. doi:10.1093/jee/97.6.2067

Wang, C., M. M. A. El-Nour, and G. W. Bennett. 2008. Survey of pest infestation, asthma, and allergy in low-income housing. *J. Commun. Health*. 33: 31–39.

Whalon, M. E., M. Mota-Sanchez, and R. M. Hollingworth. 2022. Arthropod resistant to pesticides database (ARPD). Available online: https://www.pesticideresistance.org/index.php (accessed 1 February 2022).

Wu, X., and A. G. Appel. 2017. Insecticide resistance of several field-collected German cockroach (Dictyoptera: Blattellidae) strains. *J. Econ. Entomol*. 110: 1203–1209. doi:10.1093/jee/tox072

Zhu, F., L. Lavine, S. O'Neal, M. Lavine, C. Foss, and D. Walsh. 2016. Insecticide resistance and management strategies in urban ecosystems. *Insects*.7: 2. doi:10.3390/insects7010002