Toxicity and Laboratory Performance of Insecticides to Field-Collected German Cockroaches (Dictyoptera: Blattellidae)

by

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Abstract

The toxicity and resistance levels of five technical grade insecticides (permethrin, chlorpyrifos, propoxur, imidacloprid, and fipronil) were determined for adult males of seven strains of the German cockroach, *Blattella germanica* (L.). One laboratory reared susceptible strain combined with six field-collected strains. Using topical application methods, fipronil was the most toxic insecticide to all seven strains. The LD_{50} values of fipronil to the susceptible strain and the field-collected strains B, D, E, G, H, and I were 1.33, 2.62, 11.53, 5.07, 7.66, 5.15, and 10.15 ng/insect, respectively. The field-collected strains were most resistant to permethrin among the five insecticides, except for strain H. The resistance ratios of strains B, D, E, G, and I to permethrin were 31.8, 37.3, 51.9, 34.9, and 37.5, respectively. With a resistance ratio of 6.4, the field-collected strain H was most resistant to chlorpyrifos. The field-collected strains had nearly no resistance to propoxur. Strains B, H, and I were not significantly resistant to imidacloprid when compared to the susceptible strain. Based on the different resistance ratios for each insecticide, we conclude that the field-collected strains had different treatment histories.

The repellency and performance of five selected insecticide formulations (each of which included one of the above insecticides) were determined using Ebeling choice boxes against male adult German cockroaches. Permethrin was the most repellent insecticide to the susceptible strain and field-collected strains B, E, G, and I with mean repellency of 55.97%, 66.89%, 85.92%, 27.16%, and 53.66%, respectively. Fipronil was the most repellent to strain D with repellency of 41.72%, and the five insecticides were similar in their repellency against strain H.

Fipronil reached PI values of 100 during the 14 d experiment and $t_{PImax/2} < 2$ d. Chlorpyrifos reached $PI_{max} \approx 100$ for the susceptible strain and field-collected strains D and H during the 14 d experiment as well. Permethrin, propoxur, and imidacloprid did not reach PI values of ≈ 100 during the 14 d experiment. According to the repellency and performance index, fipronil may provide effective control in field, while other insecticides may be incapable of providing good field performance.

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Table of Contents

List of Tables

List of Figures

Introduction

German cockroach

Cockroaches are one of the most ancient insect lineages and have remained unchanged in appearance for about 350 million years. Although there are more than 4000 cockroach species, only a few of them are associated with humans and fewer are well known as pests. The German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae), is one cockroach species that is considered as a major pest worldwide. It is a ubiquitous domiciliary pest which is almost always associated with the indoor environment. It may occur in kitchens, bathrooms, bedrooms, as well as restaurants, hospitals, and animal care and breeding facilities (Phillips et al. 2010).

Biology

The German cockroach is hemimetabolous, and its life cycle includes three life stages: egg, nymph, and adult. Given suitable conditions, the German cockroach can complete one life cycle in about 100 days (Gould and Deay 1940). The fertilization of the egg indicates the start of the life cycle of German cockroach. The eggs are aligned into two rows and are contained with an outer covering, i.e., an egg case or ootheca. The ootheca is small, yellowish brown and rectangular capsule. On average, the size of an egg case is about 8×3×2 mm (Tanaka 1976). It takes about 15 h to complete the process of the oothecal protrusion. Generally, egg cases will be rotate about 90 °to the right after protrusion (Roth 1970, Tanaka 1976), which probably allows females to crawl into narrow crevices while carrying them, and prevents accidental or premature dislodgement (Roth 1970). An egg case can be formed if the eggs are either fertilized or unfertilized. If a virgin female is unable to mate within about 14 d after emergence, an unfertilized ootheca will be produced (Roth 1970). The anterior end of an ootheca is permeable so that movement of water from the female to the developing eggs is possible (Ross and Mullins 1995). Tanaka (1976) stated that an egg case would display thirteen externally identifiable stages of embryonic development. The female will carry the ootheca until hatch or will drop it shortly before hatch (Barson and Renn 1983). After about 30 d from when it was produced, the egg case will be fully developed and ready to hatch (Ross and Mullins 1995). However, an ootheca does not always hatch. Fertilization (Roth 1970), temperature (Tsuji and Mizuno 1973), contact with insecticides (Barson and Renn 1983), infection of fungus (Archbold et al. 1987), and other factors influence ootheca hatch.

The egg stage comes to an end prior to hatch of the ootheca; then the nymphal stage begins. The German cockroach will go through a series of molts and generally 6 to 10 nymphal instars. The number of molts varies with sex and also depends on body size, temperature, injury, diet, and other factors (Ross and Mullins 1995). The nymphal stage lasts approximately 50 to 60 when the cockroaches are provided normal room temperature. Lower temperature will delay development while higher temperature will accelerate development. Density is another factor that influences nymphal development of the German cockroach. Isolation resulted in slower nymphal development even at favorable room temperature (Ross and Mullins 1995). However, if the density of the colony is too high, the duration of nymphal development will increase (Komiyama and Ogata 1977). Aggregation will accelerate nymphal development (Ross and Mullins 1995). Aggregation behavior is a result of the secretion of aggregation pheromone contained in the feces, which is a blend of substances (Ross and Mullins 1995). When nymphs are fully developed, the final molt takes place and the nymphs eclose into adults.

German cockroaches develop from egg to adult in about 90 d. The adults are fully winged and can use the wings to glide. Unlike some other *Blattella* spp., German cockroaches do not fly (Ross and Mullins 1995). Males and females can be easily distinguished in that males have a slender, tapering abdomen (Ross and Mullins 1995). The males have long narrow abdomens and styli on their assymetrical subgenital plates, while the females have wider abdomens and no styli on their subgenital plates.

Usually the German cockroaches are yellowish-brown in color and have two black longitudinal bands on their pronotum. Females attract males by secreting volatile and nonvolatile sex pheromones. Males recognize females through chemosensors in their antennae. Once a male contacts a female, it will raise its wings, turn 180° and expose its tergal glands on the dorsal side of its abdomen. The female will then mount the male and feed on the tergal gland secretions. The male will connect with the female with his hooked left phallomere. During mating, the male and the female face in opposite directions. The spermatophore is transferred from the male to the female and sperm moves from the spermatophore into the female's spermathecal gland. After about 12 h, the empty spermatophore will be expelled. One mating usually provides enough sperm for a female's entire reproductive life. About 6 d after emergence, adult males will be ready to mate (Ross and Mullins 1995). Virgin females have to mate within 14 d after emergence, or else they will produce unfertilized egg cases (Roth 1970). Not all egg cases are viable because ootheca of longer-lived females often fail to hatch. In general, all mated females will produce viable first and second egg cases; however, from the third egg case on, a decreasing number of ootheca will hatch (Ross and Mullins 1995).

The development of the German cockroach can be affected by air, food, water, and access to harborage (Appel 1997). Thus, to control the German cockroach, it is necessary to keep a clean and tidy indoor environment.

Economic and medical importance of the German cockroach

The German cockroach is considered a major domestic pest worldwide. With chewing mouthparts, like other cockroach species, the German cockroach is able to consume wide range of materials that human produce, consume, discard, or excrete. They can feed on decaying food or scraps, and are often associated with unsanitary areas. Their presence is thought to be a threat to human welfare (Brenner 1995). The German cockroach was one of the most important household pest, and accounted for a large percentage of the economic losses caused by insects (Guillebeau et al. 2001).

The German cockroach is considered a pest for several reasons. With chewing mouthparts, German cockroaches can bite. The bite of the German cockroach can lead to inflammation of skin cells, and later infection and necrosis of the tissue may occur as the result (Brenner 1995). Humans often find the German cockroach disgusting; another reason why they are considereds pests. They are often associated with unsanitary environment, so infestation will cause psychological stress to people who are living with these creatures (Brenner 1995). When the aversion to cockroaches becomes too strong, some people might behave irrationally. In an example of delusory parasitosis case reported by Schrut and Waldron (1963), a family moved to another home because family members believed that there was a “serious” infestation, while there was actually only a low level of cockroach infestation. Delusory parasitosis is a term used to refer to an emotional disorder in which the patient has an unwarranted belief that live organisms, such as mites or insects, are present on or in his body (Ebeling 1975). The term delusory cleptoparasitosis is used to describe the situation where people imagine infestations of insects or mites in the dwellings when there were no infestations in actual (Grace and Wood 1987). Although the German cockroach plays a minor role in such delusions, the health implications for people who experience the delusions are real (Brenner 1995).

Besides biting and causing psychological problems, the German cockroaches can also producers allergic reactions in sensitive people. Allergy is also known as hypersensitivity. It occurs because the immune system sometimes recognizes harmless antigens as harmful and then overreacts to them (Brenner 1995). Allergy is one of the most widespread and common chronic concerns. In the worst situations, it may put people's lives at risk. The German cockroaches carry multiple allergens, some of which are specific to the German cockroach while others are cross-reactive with allergens from other cockroach species (O'Connor and Gold 1999). Frequent contact with cockroaches will lead to higher rate of allergy. Higher contact rate with cockroaches is often associated with low socioeconomic status (O'Connor and Gold 1999). Efforts to reduce exposure to cockroach allergens should be included in the management of cockroach-allergic asthma (O'Connor and Gold 1999). A combination of IPM methods, chemical controls, and respiratory protection may achieve this purpose (O'Connor and Gold 1999). In addition to asthma, other allergic reactions attributed to contact with cockroach body parts, secretions, dry feces, and exuviae include coughing and skin rash.

The German cockroach can not only cause problems itself, but it can vector different types of pathogenic microorganisms, such as viruses, bacteria, fungi, etc. (Brenner 1995). Several Poliomyelitis viruses, which can result in polio, were isolated from the German cockroaches (Brenner 1995). Fecal-oral route and contaminated food or drink are possible ways of poliomyelitis virus transmission from German cockroaches to humans (Roth and Willis 1957). More pathogenic bacteria species were found associated with cockroaches than all other pathogenic microorganisms together (Roth and Willis 1957). About 40 species of pathogenic bacteria have been found naturally adhered to cockroaches, and 20 other species can be introduced to cockroaches experimentally (Roth and Willis 1957). *Salmonella* infection, which is related to human salmonellosis, has been found associated with cockroaches and cockroaches may play a role in transmitting pathogenic organism (Roth and Willis 1957). For instance, *Salmonella typhimurium* (Loeffler) Castellani and Chalmers is a species of bacteria that can cause food poisoning and can be transmitted by cockroaches (Roth and Willis 1957). *Mycobacterium leprae* (Armauer-Hansen) Lehmann and Neumann, a bacterial species that causes leprosy, has been reported to occur in the German cockroaches (Roth and Willis 1957). Two fungi, *Aspergillus fumigatus* and *A. niger*, which may cause pathological conditions, can naturally adhere to cockroaches (Roth and Willis 1957). A protozoan, *Entamoeba hystolytica*, a causative agent of amoebic dysentery, has been isolated from the German cockroaches (Brenner 1995). German cockroaches can also harbor large number of helminths. Some of the helminths may be primary parasites of humans and other vertebrates (Roth and Willis 1957). Helminths such as *Ancylostoma duodenale*, *Acaris lumbricoides*, other *Acaris* spp., and *Enterobius vermicularis* have been isolated from field-collected German cockroaches. These helminths are all pathogenic to humans (Brenner 1995).

The German cockroach is thought to be major pest worldwide not only because of the reasons listed above, but also because of their rapid population growth. Large populations make it difficult to control this insect. A female German cockroach can produce 4-10 egg cases during their lifetime; each contains about 30-40 eggs. The German cockroach has short life span (200 d), meaning that they reproduce in a very short time period. The short generation time of the German cockroach increases its chance of developing resistance to the insecticides used to manage its population. Thus, to reduce the chance of resistance in the German cockroach population, it is important to use chemical rotation and different products and strategies (Phillips et al. 2010).

Insecticides

Insecticides are chemicals that are used to kill insects; most are synthetic. They can provide relatively rapid and efficient ways to reduce insect populations of insects (Wickham 1995). Many insecticides interfere with normal functions of the nervous system of insects. Some insecticides enter the gut of insects and are absorbed, so that they may act systemically to affect the nervous system or affect other physiological process (Wickham 1995).

Classification of insecticides may vary depending on our perspective. Depending on the ways insecticides enter insects' bodies, we can divide insecticides into 3 groups: stomach poisons, contact poisons, and fumigant poisons. Depending on whether they have residual activity, insecticides can be classified as systemic or contact. Insecticides can also be classified by their chemistry and origin, such as synthetic organic chemicals (chlordane, DDT, propoxur, etc.), organic chemicals of plant or animal origin (pyrethrin), and inorganic chemicals (boric acid, borax, sodium fluoride, etc.) (Wickham 1995).

There are different procedures for insecticide application. Spraying or dusting of surfaces, which takes advantage of the crawling habit and cockroach preference for contact with surfaces, were the principal means to control the German cockroaches (Wickham 1995), however baits have become the most commonly used insecticidal formulation since 1990 (Appel and Tanley

2000, Appel 2003, 2004). Space treatments such as fumigation are useful only under specialized circumstances for German cockroach control (Wickham 1995). Insecticides may be introduced to the market in varied formulations. There are formulations like aerosols (A), baits (B), dusts (D), emulsifiable concentrates (EC), granules (G), microencapsulates (M), pellets (P), ready-to-use (RTU), soluble powders (SP), wettable powders (WP), etc. (Wickham 1995).

Insecticides are not a new invention by modern people. They have been used for pest control, the German cockroach of course included, for a long time. In ancient times, people burned sulfur to control insect pests in fields (Delaplane 2000). Insecticides and other pesticides have become the most important method for pest management, especially after World War II. DDT, aldrin, dieldrin, and other kinds of pesticides were introduced during this period. These new chemicals were inexpensive, effective, and enormously popular (Delaplane 2000). Use of organic or inorganic insecticides is the most frequently used means to control the German cockroach (Rust 1995).

There are many factors that will affect the efficacy of insecticides. If an insecticide has residual activity, it may kill the insects for up to several months after application (Rust 1995). Temperature of the surrounding environment may affect the diffusion or volatilization of insecticides, making it easier or harder for German cockroaches to pick them up (Rust 1995). Relative humidity and moisture may change the concentration, and then affect the efficacy of insecticides (Rust 1995).

Insecticide resistance

Although the German cockroaches have been controlled by chemical insecticides for a relatively long time (at least from the early part of last century), questions about insecticide resistance did not arise until the 1940s, when chlorinated hydrocarbon insecticides were first introduced. The cyclodiene chlordane, which is in the chlorinated hydrocarbon class of insecticides, was applied for German cockroach control and became the insecticide of choice (Cochran 1995).

Insecticide resistance is the decrease in susceptibility of one pest population to an insecticide which was effective for controlling it relative to other populations. Practically, if a population of insects can no longer be killed or controlled by a certain concentration of an insecticide that was effective, then resistance exists in this population (Cochran 1995). Insecticide resistance will lead to pest control failures and is a concern worldwide. Failures in obtaining satisfactory control of German cockroach with chlordane were reported from Texas by 1951. High-level resistance to chlordane was found in field-collected strains in subsequent studies. Resistance to chlordane became widespread in the USA, which resulted in it being abandoned for cockroach control (Cochran 1995). DDT is a highly toxic pesticide which was not used commercially for German cockroach control, but after selection in laboratory studies, the German cockroach developed high-level resistance to it (Cochran 1995). Since the early 1950's, resistance to pyrethrins, which have been used for many years in controlling the German cockroach, became wide spread (Cochran 1995). Resistance to malathion, diazinon, and other insecticides was also found in many studies (Cochran 1995). It is obvious that German cockroaches are able to develop resistance to different classes of chemical insecticides, and the ability to develop resistance will certainly reduce the effectiveness of a variety of insecticides.

When an insect population develops resistance to a given insecticide, it will often become resistant to some other insecticides as well, which is termed cross-resistance (Cochran 1995). For example, DDT-resistant strains were highly resistant to DDT and to its analogue, such as methoxychlor, but not resistant to other insecticides (Clarke and Cochran 1959). Also, the chlordane-resistant strains were highly resistant to chlordane and its analogues, such as dieldrin, but was susceptible to the other insecticides tested (Clarke and Cochran 1959). Cross-resistance can usually be explained by similar modes of action of the insecticides. An altered enzyme may be able to degrade insecticides with similar chemistry (Cochran 1995). Resistance to insecticides may be caused by different mechanisms, and more than one mechanism may exist in single strain (Cochran 1995).

Metabolic resistance is considered as the most important mechanism of insecticide resistance (Georghiou 1972). Physiological mechanisms, especially physiological processes that lead to the reduction of penetration or transport of the insecticide to target site, may also cause high level resistance (Georghiou 1972).

The first mechanism of resistance to consider is different rates of insecticide penetration (Cochran 1995). In some specific cases, decrease of penetration was the only mechanism that caused low-level resistance (Georghiou 1972). An *in vivo* penetration study with [^{14}C] cypermethrin indicated that a decrease in cypermethrin penetration through the insect cuticle may contribute to the resistance to this insecticide in a strain of German cockroach (Valles et al. 2000). In some cockroach strains, this mechanism does not play a role in resistance to certain kinds of insecticides. Decreased rate of penetration was not a factor in resistance of three field-collected strains to pyrethroid insecticides (Pridgeon et al. 2002). Reduced rate of penetration, as mechanism for insecticide resistance, has not received much attention in cockroaches (Cochran 1995).

Metabolic resistance is the ability of insects to metabolize or deactivate insecticides quickly enough to avoid death (Cochran 1995). Usually these kinds of mechanisms can be traced to an increased amount of enzyme or enzyme system in a resistant population. Isolating an enzyme and testing its activity and levels in both susceptible and resistant strains can help detect metabolic mechanism (Cochran 1995). Cytochrome P450-dependent monooxygenase activity is often found to be responsible for insecticide resistance in the German cockroach. For example, it was found to contribute to resistance in a chlorpyrifos resistant strain, Dursban-R, which displayed a 20-fold level of resistance when compared with a susceptible strain (Siegfried et al. 1990). P450 monooxygenases are also involved in resistance to pyrethroid insecticides in some field-collected strains (Wei et al. 2001). Hydrolases refer to a type of enzyme that are involved in another type metabolic resistance mechanism and is also often detected in German cockroaches. Siegfried *et. al.* (1990) also found an enhanced ability to hydrolyze insecticide played an important role in resistance to chlorpyrifos. Wei *et. al.* (2001) found that in addition to P450 monooxygenases, hydrolases contribute to pyrethroid resistance in the field-collected German cockroach strain Apyr-R (Wei et al. 2001). The effects of cytochrome P450 monooxygenases and hydrolases can be suppressed or eliminated by some synergists. These synergists inhibit the specific metabolizing enzymes from functioning. PBO (piperonyl butoxide) and DEF (S,S,S,-tributyl phosphorotrithioate) are the two synergists that are most commonly used. PBO is used to suppress the activity of P450. If the use of PBO can enhance the efficacy of an insecticide, or can reduce the level of resistance to an insecticide in a particular strain, then P450 monooxygenases are probably involved in resistance (Cox 2002). Similarly, DEF is often used to inhibit the activity of hydrolases. If exposure to DEF reduces the level of insecticide resistance or helps the insecticide in control the insects, then activity of hydrolases may play a role in insecticide resistance in those insects. Sometimes both P450 monooxygenases and hydrolases are involved in insecticide resistance in one strain (Siegfried et al. 1990, Wei et al. 2001), while sometimes neither of them contributes to resistance (Wei et al. 2001).

Target-site insensitivity is another type of physiological resistance mechanism (Cochran 1995). The target site is a specific molecule (most commonly proteins: receptor, channel, enzyme, transcription factor), or when unknown the physiological component or pathway, that is directly affected by an insecticide. If the target site has any change (chemical or physical), the insecticide may no longer be effective in pest control. Target-site insensitivity may function in several ways (Cochran 1995); one of which is an altered acetylcholine esterase (AChE) molecule. Insecticides that were effective fail to inhibit the altered acetylcholine esterase, which leads to resistance. High level of insecticide resistance resulting from insensitive AChE has emerged in mosquitoes (Weill et al. 2004). Knockdown resistance or "kdr" factor, another altered target-site mechanism also occurs in German cockroaches (Cochran 1995). A *para* (*kdr*) mutation, which resides in a sodium channel gene fragment, was present in a German cockroach strain Apyr-R, indicating that this *para* (*kdr*) mutation has an important effect on pyrethroid resistance in this strain (Pridgeon et al. 2002). This "*kdr*" mechanism confers resistance to certain pyrethroids and DDT. Commonly used synergists do not influence knockdown resistance (Cochran 1995). Cyclodiene resistance in German cockroaches may also arise from decreased target-site sensitivity (Cochran 1995).

In addition to the three possible mechanisms mentioned above, behavioral resistance or changes in behavior also plays a role in insecticide resistance (Cochran 1995). If the target insects do not get a toxic dose, they may develop the ability to detect insecticide and learn to avoid it. This is the most obvious mechanism for behavioral resistance (Cochran 1995). Insects that are already resistant to a particular insecticide may not learn avoidance behavior because the resistance mechanisms already protect them from intoxication by those insecticides (Cochran 1995).

Insecticide resistance is genetic and is transmitted from generation to generation; transmission is an autosomal trait, without sex linkage (Cochran 1995). In most cases each kind of resistance is controlled by one specific gene, and in a few instances, more than one gene will control resistance (Cochran 1995). When an insect population is exposed to an insecticide, the most susceptible individuals will be killed first and individuals with resistance genes will survive. Before exposure, a population is generally homozygous susceptible but with a very low number of partially resistant individuals; a low concentration of insecticide would be enough for control. After being exposed to this insecticide for generations, the susceptible individuals will be eliminated, and the gene frequency of the resistance gene will increase in the population. At this time, the population becomes heterozygote, and the lethal dose becomes greater. When the gene frequency reaches a certain point (1.0), this gene will get fixed in the population, which means every individual has the resistance gene and the population would be homozygous; the lethal dose would need to be much greater. The insecticide may then fail in controlling this insect population (Cochran 1995).

The stability of resistance in German cockroaches depends on many factors. If it is a closed population and the resistance gene has fixed (i.e., is homozygous in all individuals) in this population, then resistance will persist. If it is an open population, gene frequency and competitiveness of different genotypes in the absence of insecticide selection will affect the stability of resistance (Cochran 1995). Generally, resistance to insecticides also confers some deleterious effects such as smaller body size, lower fecundity, etc. If a heterozygous population is not selected with insecticides, it would become more susceptible (Cochran 1995).

There are several techniques that can be used to detect or measure resistance to insecticides. Control failure may indicate that the insect population has developed resistance. However, control failure may result from many unrelated factors such as poor insecticide application, incorrect formulation, etc. Thus, more precise techniques are needed. Usually field-collected strains are compared with a susceptible strain that is cultured in laboratory (Cochran 1995).

The dosage-mortality response relationship is the most important principle for resistance measurement. When the dose of an insecticide is increased, the mortality of the tested population increases as well. By some specific mathematical transformations (probit mortality and log dose), it is possible to get a straight line relating dosage and mortality. This line can help determine the dose that is expected to kill a certain proportion of the insect population; LD_{50}, LD_{90}, and LD_{95} values are most commonly used (Cochran 1995).

The slope of the log dose-probit straight line can be informative as well. If an insect population has a large (steep) slope, this population is homogeneous in its response to the insecticide. If the population has a small (shallow) slope, it indicates that this insect population is heterogeneous and individuals in this population can response to the insecticide quite differently (Cochran 1995). Resistance levels can be obtained by comparing LD data from a resistance to a susceptible strain. The resistance ratio is calculated by the formula RR=LD_{50} of resistant strain/LD_{50} of susceptible strain (Cochran 1995).

LD_{50}, LC_{50}, and LT_{50} methods are often used to test different materials and application methods. In LD_{50} method test, a certain number (e.g., 10) of insects are exposed to one of a series of insecticide dosages (µg/insect) that give 10 or 20% to 80 or 90% mortality and replicated several times. The medial lethal dose is referred to as LD_{50} (Cochran 1995), or the dose that kills 50% of a given population after a period of time. In LC_{50} test, tested insects are exposed to a series of concentrations of insecticides (µl/ml) in groups for a certain period of time and subsequently the insects are placed in untreated containers. Mortality is recorded at a fixed time point (e.g., 24 hours later) after treatment. The medial lethal concentration obtained by this method is called LC_{50} or the concentration that kills 50% of the population after a period of time. LT_{50} method uses time as the variable. A standard concentration of an insecticide is used for the test. The insects are all exposed to a treated surface or bait with a single concentration of insecticide. Knockdown could be recorded periodically, until all the insects are killed or just mortality could be recorded at set periods (e.g., hourly, daily, etc.). The medial lethal value here is called LT_{50} or the time it takes to kill 50% of the population (Cochran 1995).

Although German cockroaches have developed resistance to many insecticides, it does not mean that all German cockroach populations are resistant to all insecticides. Some populations that are resistance to some insecticides may be susceptible to other types of insecticides. There are several possible ways to forestall or overcome resistance. Moderating control practices by not exposing a cockroach strain to the same insecticide every generation is one effective approach (Cochran 1995). Increasing the concentration of insecticide used is another approach that can eliminate resistance genes by killing even a homozygous resistant population (Cochran 1995). Mixing two or more insecticides with different modes of action and rotation of insecticides with different modes of action may work as well (Cochran 1995). In recent years, the concept "Integrated Pest Management (IPM)" has gained attention in urban pest management. The IPM concept was first developed by agricultural scientists in the 1960s and seeks to keep pests at economically insignificant levels (Delaplane 2000). It is a pest control approach that focuses on pest prevention by eliminating the root causes of pest problems. IPM includes crop production methods that discourage pests (in agricultural system), encourages use of beneficial predators or parasites that attack pests, and timing of pesticide applications to coincide with the most susceptible period of the pest's life cycle (Delaplane 2000). When applying IPM approaches, it is assumed that a certain low level of pests is tolerable and elimination of pests is not a necessary goal. Elimination of pests may result in a decrease in beneficial predators or parasites (Delaplane 2000). IPM is sometimes a substitute of pesticides, but it is more often applied to improve effectiveness or to reduce overall use of pesticides (Delaplane 2000). It has been pointed out that the IPM methods for German cockroach control, including cleaning, removing harborage areas, increasing air flow, using spot sprays, as well as sticky traps and insecticidal baits, are more sustainable and may provide better control in the long term at a similar cost compared with only bait treatment (Wang and Bennett 2006).

Literature review

Although insecticide resistance has occurred and has been the cause of control failures, insecticides are still the most frequently and widely used method for pest control. Most insecticides are synthetic compounds that disrupt specific biological systems associated with insects (Wickham 1995). Based on their modes of action and their chemical structure, insecticides can be classified into pyrethroid, organophosphophate, carbamate, neonicotinoid, phenylpyrazole, as well as other chemical classes (IRAC 2012).

Pyrethorids are a group of synthetic insecticides that were derived from long-term studies on structural requirements for the activity of pyrethrins. These chemicals are different from others in that they have either rapid flushing and knockdown action and/or toxicity to insects at very low doses with great safety to humans and most animals (Wickham 1995). Pyrethroid compounds have gained great popularity in control of agricultural pests as well as household

(Wickham 1995). Permethrin [3-(phenoxyphenyl) methyl (+,-)-cis, trans-3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropanecarboxylate] is one of the most commonly used pyrethroid compounds for control of the German cockroach. It was first registered and tolerances established in the USA in 1979 for use on cotton (EPA 2009). It is yellow-brown to brown liquid and at room temperature, technical grade permethrin may tend to partly crystallize. Pure permethrin melts at 34-39 ℃ and is odorless while technical grade permethrin has sweet odor (Extension Toxicity Network 1985). As an insecticide, permethrin is available in most formulations including sprays, dusts, etc., but it is principally used as WP or MC at 0.125-0.25% or EC at 0.5-1.0% and as a powder at 0.5% (Wickham 1995). Permethrin is a broad-spectrum neurotoxin and kills many kinds of insect pests. Its mode of action is to prolong sodium channel activation on the neuron membrane. It can quickly paralyze insects' nervous systems and produce a quick "knockdown" effect on insect pest populations. When it is ingested, permethrin can also act as a stomach poison. When permethrin is contacted directly by target pests, it has contact effects. It can kill adults, eggs, and larvae, and has a repellent effect on insects. Permethrin has up to 12 weeks insecticidal activity after application (Extension Toxicity Network 1993a). Generally, permethrin has low mammalian toxicity and it is poorly absorbed by skin (Extension Toxicity Network 1993a). However, it can do harm to cats by inducing muscle tremors, seizures and other symptoms (Dymond and Swift 2008); it is also highly toxic to fish and some other aquatic animals (Jolly et al. 1978).

Organophosphates (OP) are the general name for esters of phosphoric acid. OPs are the basis of many pesticides. Chlorpyrifos, [O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate], is one of the organophosphorous compounds that has been used for control of the German cockroach (Wickham 1995). It is a crystalline organophosphate insecticide, acaracide, and miticide at room temperature; its melting point is 41.5-43.5 ℃. Chlorpyrifos was introduced in 1965 by the Dow Chemical Company and is known by trade names Dursban or Lorsban. It is a broad-spectrum insecticide that is able to kill a wide range of pests, including cutworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, lice, etc. (Extension Toxicity Network 1993b). Chlorpyrifos is a neurotoxin which is active by contact, ingestion, and vapor action. It causes nearly irreversibly phosphorylation of acetylcholinesterase enzymes of tissues, which results in accumulation of acetylcholine at its target sites and eventually leads to the death of insects (Extension Toxicity Network 1984). Chlorpyrifos is available in many formulations, such as EC, D, spray, granular, and WP. It primarily acts on insects as a contact poison (Extension Toxicity Network 1993b), but has moderate toxicity to humans. Its may affect the central nervous system, the cardiovascular system, and the respiratory system; it can also irritate skin and eyes (Extension Toxicity Network 1993b). Because of its toxicity to humans, homeowner use of chlorpyrifos was eliminated except for ant and roach baits in child resistant packaging and fire ant mound treatments in June, 2000 (EPA 2016). A proposal to revoke all chlorpyrifos food residue tolerance has been issued as well (EPA 2016).

Carbamate compounds are another group of insecticides that work by blocking action of acetylcolinesterases to increase transmission of nervous impulses (Wickham 1995). There are three compounds in this group that are significant in German cockroach control, but propoxur is the most common (Wickham 1995). Propoxur [2-isopropoxyphenyl methylcarbamate], also known as Baygon, has been applied worldwide for many years as an insecticide for German cockroach control (Wickham 1995). It was first registered as an insecticide in 1963.Technical propoxur is a white to cream-colored crystalline solid. It may be used in combination with azinphosmethyl, chlorpyrifos, cyfluthrin, dichlorvos, disulfoton, or methiocarb. Propoxur is effective against a variety of insect pests such as ants, cockroaches, crickets, flies, and mosquitoes (Extension Toxicity Network 1996a). Propoxur is one of the chemicals that has, to a large extent, replaced DDT in the control of black flies and mosquitoes (Extension Toxicity Network 1993c). Propoxur has contact and stomach action. As a contact poison, it has long lasting activity for direct contact with target pests (Extension Toxicity Network 1996a). Propoxur is available in several types of formulations and products, including EC, WP, baits, aerosols, fumigants, granules, and oilsprays (Extension Toxicity Network 1996a). Propoxur works by suppressing the funtion of acetylcolinesterases, in a similar manner as other carbamate and organophosphate insecticides. Propoxur has relatively high toxicity and fast knockdown activity (Wickham 1995). Propoxur is classified to be highly toxic to humans and can be absorbed by breathing, eating, and through direct skin contact (Extension Toxicity Network 1993c). Its chronic (long-term) inhalation exposure can lead to depressed cholinesterase levels, headaches, vomiting, and nausea in humans (EPA 2000). It was removed from home use in 1990s because of its toxicity to humans (Chumley 2010). In recent years, some people suggested reregistering propoxur for bedbug control, but this request was denied by the EPA (Chumley 2010).

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine, 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine] is one of neonicotinoid insecticides that are used for German cockroach control. The neonicotinoids are a relatively new class of insecticides, which includes acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam. Neonicotinoids generally have low toxicity to mammals (acute and chronic), birds, and fish (Tomizawa and Casida 2005). The low affinity of neonicotinoids for vertebrate nicotinic receptors relative to insects leads to their favorable toxicological profile

(Tomizawa and Casida 2005). Imidacloprid was used in products sold in the United States in 1994 and is colorless crystal with melting point of 136.4-143.8 ℃ (Extension Toxicity Network 1996b). As one of the neonicotinoids, it is used increasingly worldwide as an insecticide. Imidacloprid interferes with nicotinic acetylcholine receptors (nAChRs) and has selective toxicity for insects over vertebrates (Matsuda et al. 2001). Specifically, it blocks the nicotinergic neuronal pathway that is more abundant in insects than in warm-blooded animals, which makes the chemical selectively more toxic to insects than warm-blooded animals. The neurotransimitter, acetylcholine, will accumulate because of the blockage, resulting in the insect's paralysis, and eventually death. Imidacloprid has both of contact and stomach effects (Extension Toxicity Network 1996b). Imidacloprid is available in formulations like dustable powder, granular, seed dressing (flowable slurry concentrate), soluble concentrate, suspension concentrate, and wettable powder (Extension Toxicity Network 1996b).

Another relatively new and broad-spectrum insecticide is fipronil [(RS)-5-Amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)pyrazole-3-carbonitrile] which belongs to the phenylpyrazole class. The proposed target site for phenylpyrazole insecticides is the GABA-gated chloride channel (Cole et al. 1993). Fipronil provides outstanding protection of crops with great selective toxicity for insects over mammals (Hainzl and Casida 1996). Fipronil, registered in the United States in 1996, is one of the newest insecticides. It is low-acting and a highly effective, broad-spectrum insecticide that is an excellent choice for the control of many crop, public hygiene, and veterinary pests. It is effective at a low to very low rates (Nasirian et al. 2006) and works by blocking the transmission of signals by the inhibitory neurotransmitter gama-aminobutyric acid (Hainzl and Casida 1996). It is highly toxic to the German cockroach and other pests of public and medical importance. Topical and oral toxicity of fipronil to German cockroaches is as great or greater than the toxicity of chlorpyrifos (Kristensen et al. 2005). Fipronil's higher toxicity in insects than in mammals is a result of the greater sensitivity of GABA receptors in insects compared with mammals. In addition to GABA receptors, the glutamate-activated chloride channels (GluCls), which are present in insects, but not in mammals, are highly sensitive to fipronil and play a crucial role in selectivity of fipronil to insects over mammals. There are two types of glutamate-induced chloride currents that have been observed: desensitizing and non-desensitizing. GluCls are the basis for development of selective and safe insecticides (Narahashi et al. 2010). Fipronil may have potential risk to fish, honey bees, birds, and marine and aquatic invertebrates (NPTN 1996).

Objectives

The toxicity of five techinical grade insecticides will be determined for six field-collected German cockroach strains and an insecticide susceptible laboratory strain. LD_{50} values and resistance ratios for these insecticides against six strains will be determined by topical application. The toxicity (LT_{50}), repellency, and laboratory performance of five formulated insecticides to the field-collected and laboratory German cockroach strains will be determined using the Ebeling choice box assay.

Expected Outcome

The null hypothesis for the resistance tests is that the field-collected strains have not developed resistance to the five insecticides, and the alternative hypothesis is that those field-collected strains have developed significant resistance to the insecticides tested. For the choice box experiments, the null hypothesis is that the insecticides have no repellency to the field-collected German cockroach strains, while the alternative hypothesis is that the insecticides tested are repellent to the German cockroach.

Resistance to insecticides by field-collected German cockroach strains (Dictyoptera: Blattellidae)

The German cockroach *Blattella germanica* (L.), is an economically important pest because it contaminates food, and may cause allergic reactions and asthma in sensitive humans with its feces and exuviae (Brenner 1995, O'Connor and Gold 1999). German cockroaches can also vector many pathogenic microorganisms, including viruses, bacteria, protozoa, and helminthes (Roth and Willis 1957, Brenner 1995). Some people exposed to German cockroach even suffer from delusory cleptoparasitosis, a psychological disease (Brenner 1995). There have been numerous studies on the control of German cockroaches, using a variety of synthetic insecticides, and essential oils (Appel 1992, Nasirian et al. 2006, Phillips et al. 2010). The continuous use of insecticides, the most commonly used method to control the German cockroach, has resulted in this species developing insecticidal resistance, making it even harder to control this annoying insect species. The German cockroach has a relatively short generation time and high reproductive rate, making it difficult to control (Phillips et al. 2010). Populations of the German cockroach have developed resistance to the organochlorine, organophosphate, carbamate, and pyrethroid classes of insecticide (Scott et al. 1990), and resistance has resulted in control failures (Cochran 1995).

A survey that aimed to investigate insecticide practices used for the control of the German cockroach and to assess the level of residents' awareness and knowledge of integrated pest management was conducted in rural North Carolina (Dingha et al. 2013). The majority (71%) of the residents admitted that they or their family member would apply pesticides to deal with the domestic pest problem in their home, while only 16% worked with a professional contractor (Dingha et al. 2013). The main method used for pest control in homes was pesticides. About 65% of residents said they apply pesticides routinely regardless of need. It was reported that most of residents were unfamiliar with integrated pest management (IPM) and related measures of control or prevention (Dingha et al. 2013).

Sprays were the most commonly used formulation by the residents. In the homes that worked with a professional contractor to cope with pests, in only a few cases the contractors would offer residents pest control options other than sprays. Few of the contractors would provide residents with the names of the chemicals, and in fewer cases the contractors left copies of the pesticide labels. Few residents kept written records of pesticides applied. More than half of the residents surveyed stored pesticides in their homes, and 61% reported that their practices in pest control was not effective (Dingha et al. 2013).

Six German cockroach strains were collected from Franklin County, North Carolina, after the survey. As is described above, the pesticides applied (both type and frequency) in the respondents' homes were unclear. It is also not clear whether or not the six strain of the German cockroach developed resistance to the insecticides that were popular in German cockroach control. The toxicity of five insecticides (permethrin, chlorpyrifos, propoxur, imidacloprid, and fipronil) that gained popularity in German cockroach control were tested with topical application methods. This study aims to investigate the resistance and toxicity profiles of the six field-collected strains five insecticides. The results may give us indications about the control history for the German cockroaches in the Franklin County, NC.

Permethrin, an insecticide which blocks sodium channels, has been used for German cockroach control since it was marketed in 1979 (EPA 2009). Chlorpyrifos and propoxur, that blocks acetylcholinesterase, have also been applied for this purpose since introduced in 1965 (Extension Toxicity Network 1993b) and 1959 (Wickham 1995), repectively. Imidacloprid and fipronil are relatively new insecticides, but have gained popularity in German cockroach control (Cox 2001, Nasirian et al. 2006). Because of their popularity in the control of German cockroach, it is possible that these insecticides have been applied by the residents or contractors to cope with German cockroach populations in Franklin County, North Carolina, which could result in the resistance of German cockroaches to these five insecticides or other insecticides that work by similar mode of action.

Materials and Methods

Cockroach strains Seven strains of German cockroach were used in this study, including a susceptible strain. The susceptible strain that was used in this project has been reared in the laboratory without exposure to any insecticides for >40 years. The six field-collected strains: B, D, E, G, H, and I were collected in Franklin County, North Carolina in 2011-2012 (Table 1). The treatment history of these field-collected strains was unknown. After collection, the cockroaches were immediately sent to Auburn University, Auburn, AL by overnight shipping.

The susceptible strain was kept in extra-large trash cans at 28 ± 2 ℃, with 40-55% RH, and a photoperiod of 12:12 (L:D). The six field-collected strains were reared in 3.8 L glass jars at 28 ± 2 ℃, 40-55% RH, and a photoperiod of 12:12 (L:D). All seven strains were provided cockroach harborage (corrugated cardboard), dry dog food (Purina Dog Chow, Raulston Purina, St. Louis, MO), and clean water. The German cockroach colonies were fed and watered once a week.

Only males were used in this study because of their relatively homogenous physiological milieu and their body mass is more uniform when compared to females (Abd-Elghafar et al. 1990). Also, the females were needed to reproduce and increase the size of the colonies. All the cockroaches used were briefly anesthetized with CO_2 to facilitate handling during the experiments.

Body mass. Mean body masses of the strains were determined by weighing 20 randomly selected male adult cockroaches of each strain. The balance used was analytical lab balance GH-200 from A&D. Capacity of the balance was 220g, and the minimum display was 0.0001g according to instruction manual. The cockroaches were briefly anesthetized before experiment.

Chemicals and insecticides. Five technical grade insecticides: permethrin (94.34%, provided by FMC Corp., Princeton, NJ), chlorpyrifos (99%, Dow, Midland, MI), propoxur (>95%, Ultra Scientific, N. Kingstwon, RI), imidacloprid (97.7%, Bayer Corp., Kansas, MO), and fipronil (≥97%, Chem-impex Int'l Inc., Wood Dale, IL) were used in this study. Stock solutions were prepared by dissolving these insecticides into analytical grade acetone (Fisher Scientific, Fair Lawn, NJ).

Topical application. Male cockroaches were selected from their original colonies and maintained in smaller glass jars at least one day before topical applications. Harborage, dog food, and clean water were provided. The cockroaches were lightly anesthetized by CO_2 during the topical applications. Topical application was performed by using a 25-μl micro-applicator (Hamilton Co., Reno, NV). Two 0.5 μl drops (1 μl total) of insecticide solution (in acetone) were delivered to one insect on the intersegmental membrane between first and second ventral thoracic segments. A series of 4 to 7 doses of each insecticide that resulted in >0 and <100%

mortality was applied. Groups of 10 German cockroaches were used for each treatment. In the control treatment, the cockroaches received 1 μl acetone alone.

After each treatment, groups of 10 German cockroaches were placed a 162.5-ml (5.5-oz) plastic cup (Georgia-Pacific, Atlanta, GA) with 2 cm piece of dental wick saturated with water. Mortality was recorded 24 or 72 (fipronil) hours after the topical applications. All topical applications were conducted under laboratory conditions and each treatment was replicated 3 times on different days for a total at least 30 males per concentration. A cockroach was scored dead if it was paralyzed on its back and unable to move.

Data analysis. Mean masses of German cockroach strains were analyzed with one-way analysis of variance (proc glm) and tukey's mean separation test in SAS (SAS Institute 2004). The LD_{50} values (toxicity) of each insecticide to every German cockroach strain were estimated by probit analysis with PoloPlus (Robertson et al. 2007). The LD_{50} values of fipronil was estimated by SAS (proc probit) (SAS Institute 2004) because SAS provides additional calculation options. Probit analysis allows quantitative analysis of the dose-response relationship in chemicals (Sakuma 1998). Correlations of resistance ratios between insecticides and correlations of body mass between resistance were analyzed with Pearson correlation by SAS (proc corr) (SAS Institute 2004).

Results

Body mass. There was a significant difference in body mass among strains ($P < 0.05$). Mean body mass ranged from 0.0472 to 0.0570 g for stains S and D, respectively. Strain H and susceptible strain were not significantly different in body mass. Strain I, E, and G had significantly greater body mass than Strain H and the susceptible strain. Strain B and D were significantly heavier than the other five strains (Table 2).

Topical application. There was no mortality in the control treatment at 24 or 72 h. The susceptible strain had an LD_{50} value at 0.239 μg/insect for permethrin (Table 3). The LD_{50} values of the field-collected strains for permethrin ranged from 1.32 μg/insect for strain H to 12.403 μg/insect for strain E (Table 3). The resistance ratios of the field-collected strains for permethrin ranged from 5.5 to 51.9-fold for strains H and E, respectively (Table 3). Five out of six field-collected strains showed very high resistance levels, all >30-fold. Only strain H did not have high level resistance to permethrin. For the homogeneity of response (slope of the log-dose probit relationship), the susceptible strain had the greatest slope value, 5.922. Among the field-collected strains, strain I and strain H had the greatest, 5.397, and the lowest, 2.243, slope value, respectively. The slope values ranged from 3.218 to 4.738 for the other 4 field-collected strains (Table 3).

The LD_{50} value of the susceptible strain for chlorpyrifos was 0.243 μg/insect (Table 4), and ranged from 1.265 μg/insect to 2.250 μg/insect for field-collected strains E and I, respectively (Table 4). Among the six field-collected strains, strain I had the greatest resistance to chlorpyrifos, with a resistance ratio of 9.3 (Table 4); and strain E had the lowest resistance ratio (5.2) of the field-collected strains (Table 4). With a resistance ratio of 8.9, strain G also showed a high level of resistance against chlorpyrifos. As is shown in Table 2, all field-collected strains had resistance ratios >5. For the homogeneity response (slopes), the susceptible strain of course had the greatest slope, 7.489 (Table 4), indicating high homogeneity of the population. Strain B had the lowest slope (3.673) whereas the strain D had relatively high homogeneity with a slope of 6.071 (Table 4).

The susceptible strain had LD_{50} value for propoxur of 0.974 μg/insect (Table 5). LD_{50} values ranged from 0.896 μg/insect for strain D to 1.489 μg/insect for strain H (Table 5). None of the field-collected German cockroach strains had high resistance ratios to propoxur compared with the susceptible strain. The highest resistance ratio was 1.5-fold in strain H (Table 5). Strains D, G, and I had resistance ratios lower than 1, but had overlapping 95% confidence interval about the LD_{50} values, indicating that these three strains were as susceptible to propoxur as the susceptible strain. For the homogeneity response (slope), the field-collected strain B had the highest slope value, 3.968 (Table 5), which is different from the results of permethrin and chlorpyrifos. The susceptile strain and the strain D and G had similar response in homogeneity. Strain E, H, and I showed similar response in homogeneity, slightly lower than that of susceptible strain (Table 5).

The LD_{50} value of the susceptible strain for imidacloprid was 0.168 μg/insect and ranged from 0.199 μg/insect to 0.563 μg/insect for field-collected strains I and D, respectively. Strains D, E, and G had resistance ratios >2.5, while strains B, H, and I had resistance ratios of ≤1.5 (indicating that these three strains are as susceptible to imidacloprid as the susceptible strain). None of the field-collected strains had a resistance ratio >3.4 (Table 6). For the homogeneity response (slope), field-collected strain D had the greatest slope (3.663), and the susceptible strain had the second largest slope (2.651) (Table 6). The strain H had a slope of 1.862, which was the lowest among the tested strains (Table 6). Other four field-collected strains had similar slopes from 2.024 to 2.336 (Table 6).

Fipronil killed all of the strains at very low rates (Table 7). The LD_{50} value of the susceptible strain against fipronil was only 1.33 ng/insect (Table 7). The LD_{50} values for the field-collected strains ranged from 2.62 ng/insect for strain B to 11.53 ng/insect for strain D

(Table 7). Strain D was the most resistant strain to fipronil among these six field-collected strain with a resistance ratio of 8.7 (Table 7); strain I had the second largest resistance ratio of 7.6 (Table 7). Field-collected strain B had the lowest resistance ratio to fipronil, 2.0. For the homogeneity response (slopes), the susceptible strain had the greatest homogeneity with slope of 4.804 (Table 7). Strain H had the lowest slope value, which was 1.502 (Table 7). Other 5 field-collected strains had similar slope values, from 2.030 to 2.641 (Table 7).

Cross-resistance. The correlations of resistance ratios between the selected insecticides were analyzed with Pearson correlation. There was no significant correlation between the resistance of the insecticides (Table 8).

Correlation between body mass and resistance. The relationship between body mass and resistance were also analyzed by Pearson correlation. No significant correlation was found between resistance and body mass of the field-collected German cockroach populaitons (Table 9).

Discussion

Fipronil has the greatest toxicity to all seven strains we tested in this study. It can kill the German cockroach strains in nanogram quantities (Table 7), which is consistent with the results by Wei et al. (2001), who reported that fipronil had LD_{50} values of 3.9 ng/insect and 8.9 ng/insect for the susceptible strain ACY and the field-collected strain Apyr-R, respectively. In a study conducted in Iran, the susceptible strain had LD_{50} value of 0.96 ng/insect for fipronil (Nasirian et al. 2006). With an LD_{50} value of 0.974 μg/insect (Table 5), propoxur was the least toxic insecticide to the susceptible strain. Imidacloprid was the second most toxic insecticide to the susceptible strain. Chlorpyrifos and permethrin showed similar toxicity to the susceptible strain.

As a broad-spectrum pesticide, permethrin has gained popularity since it was introduce for use on cotton in the US (EPA 2009). Its fast knockdown effect and the various formulations of this chemical made permethrin widely spread and used over the world for control of many different pests. The popularity of permethrin has gradually led to insecticidal resistance of the pests to this chemical. In previous studies, field-collected strains were found to be highly resistant to permethrin. The resistance ratios of those strains were nearly or higher than 100 or even 200 (Wei et al. 2001, Pridgeon et al. 2002). Other insects such as house fly, which have also been treated with permethrin, were found to have a resistance ratio of as great as 260-fold (Liu and Xin 2000).

In this study, five of the six field-collected strains (B, D, E, G, and I) had relatively high LD_{50} values to permethrin, indicating the lower toxicity of permethrin to these strains. When compared with the susceptible strain, these five strains had high resistance ratios. Strain E had the greatest resistance level (RR=51.9) to permethrin, and strain B, D, G, and I had resistance ratios >30 (Table 3).

There were different results, however, for the susceptibility of strain H to permethrin. The LD_{50} value for this strain was 1.323 μg/insect, and the resistance ratio was only 5.5 (Table 3). The results for this strain indicate that permethrin may not have been the primary insecticide used for pest control in the area where strain H was collected. Permethrin was reported to induce skin sensations and paresthesia in exposed workers; numbness, itching, tingling, and burning were side effects of permethrin as well (WHO 1989). It is possible that some people had skin problems after using insecticides containing permethrin to control strain H, so they stopped applying insecticide formulations with permethrin, which may be the reason for low resistance level of strain H.

Chlorpyrifos controls many types of pests like ants, flies, and cockroaches (Extension Toxicity Network 1993b). This chemical was made into many types of formulations as well, and was often used for German cockroach control (Wickham 1995). Because of its toxicity to humans, chlorpyrifos was eliminated except for ant and roach baits in child resistant packaging and fire ant mound treatments in June, 2000 (EPA 2016). In our study, all field-collected strains tested showed resistance ratios to chlorpyrifos >5 (Table 4). Strain E was the least resistant strain with resistance ratio of 5.2; strain G and strain I had greatest resistance ratios. It is possible that chlorpyrifos was used more often in the areas where strains G and I were collected.

Chlorpyrifos had been eliminated for home use for about 10 years prior to when these strains were collected. These field-collected strains still have resistance levels from 5.2 to 9.3 to chlorpyrifos, indicating that the resistance to this OP chemical is persistent in German cockroach populations. Cross-resistance arising from the use of other insecticides that have similar mode of action to chlorpyrifos such as Dichlorvos, which was proven to have cross-resistance to chlorpyrifos in *Laodelphax striatellus* (Fallén) (Wang et al. 2010), might be another reason for the resistance of these strains to chlorpyrifos.

Propoxur also works on the acetylcholinesterase, similar to chorpyrifos, but it showed different toxicity and resistance in this study. Although propoxur was the least toxic insecticide to the susceptible strain, it was more effective against the field-collected strains (Table 5). None of the field-collected strains showed high level of resistance. More than half of the field-collected strains were as susceptible to propoxur as the laboratory susceptible strain (Table 5).

Propoxur was withdrawn from indoor use in 1990s because of its toxicity to humans (The New York Timess 2009), much earlier than chlorpyrifos, and home use products containing propoxur were no longer on the market. When the strains were collected, it had been nearly 20 years since propoxur was withdrawn. Lack of use and selection pressure may be a reason for the susceptibility of these field-collected strains to this chemical.

Chlorpyrifos and propoxur have similar modes of action (Brenner 1995, Extension Toxicity Network 1996a, EPA 2016). However, the results for chlorpyrifos and propoxur in these tested strains were quite different, and showed no evidence of cross-resistance between chlorpyrifos and propoxur (Table 8). This is consistent with a previous study that no significant relationship between resistance to these two insecticides (Valles 1998).

Imidacloprid is a relatively new insecticide and has selective toxicity for insects over mammals (Matsuda et al. 2001). This chemical has a rather quick knockdown effect on the German cockroach strains tested. However, the knockdown did not last for long, and many of the insects recovered after a few hours. Propoxur was similar with imidacloprid in this way. Three strains, B, H, and I, had resistance ratios no higher than 1.6 (Table 6). Strain I had resistance ratio of 1.2 (Table 6), which indicates that this strain had nearly no resistance against imidacloprid. It is possible that these three strains were not treated with imidacloprid, which may lead to their low or lack of resistance.

The three strains, D, E, and G, had resistance ratios to imidacloprid of >2.5 (Table 6). None of these field-collected strains showed resistance ratios higher than 3.4, indicating that these German cockroach strains are not highly resistance to this insecticide. It may be because imidacloprid is relatively new and has not been used for German cockroach control for very long. These German cockroach strains had similar response in homogeneity (slope of the log-dose probit relationship), and the responses were similar to that of the susceptible strain. Imidacloprid not being used in these areas can be a reason for the similarity shown in homogeneity response. Some German cockroach strains were reported to have cross-resistance to imidacloprid with propoxur and pyrethroid (Wen and Scott 1997, Wei et al. 2001). However, the resistance to imidacloprid in these field-collected German cockroach strains seems had no relationship with their resistance to permethrin (Table 8). Imidacloprid was the second most toxic insecticide tested in this study not only to the susceptible strain, but also to all the field-collected strains.

Mortality of fipronil treated German cockroaches was recorded 72 h after treatment because it is a slow-acting insecticide and the results at 24 and 48 h were not stable. It is the most toxic among the tested insecticides. The LD_{50} for the susceptible strain was only 1.33 ng per insect (Table 7). Fipronil is also effective against the other strains in nanogram quantities (Table 7). The resistance ratios of the six field-collected strains vary from 2.0 to 8.7 (Table 7). Strain B was the most susceptible to fipronil among them. The strain G had largest slope of the log-dose probit relationship, meaning that the individuals in this strain are more homozygous in tolerance to fipronil. The resistance ratios of strain E and H were similar, but these two strains had different responses in homogeneity (Table 7). This may arise from different control history for the two strains.

Fipronil, like imidacloprid, is a relatively new insecticide. It was made available in bait formulations for control of the German cockroach (Holbrook et al. 2003), and is effective. However, it has been reported that some German cockroach strains that were not treated with fipronil had high levels of resistance to fipronil. The reason for this phenomenon may be that the mechanism of cyclodiene resistance increased tolerance to fipronil or more than one cross-resistance mechanism could be involved (Holbrook et al. 2003). Although fipronil was the most toxic among the insecticides tested, the resistance ratios of the field-collected strains for fipronil were also higher than that of propoxur and imidacloprid. This may be due to the widespread use of fipronil because of its high efficacy.

Insecticides with selective toxicity to insects over mammals such as imidacloprid and fipronil could be used for German cockroach control. However, as tolerance has increased because of cross-resistance (Holbrook et al. 2003), fipronil should be used with caution, or German cockroaches could ultimately develop resistance to fipronil. Insecticides like chlorpyrifos and propoxur may not be suitable for indoor use as they do not have selective toxicity, which may do harm to humans by direct contact (Extension Toxicity Network 1993b) (EPA 2000). Permethrin will not provide good control of German cockroaches in Franklin County, North Carolina because of the high levels of resistance. There may be several possible mechanisms in resistance to these insecticides. It was reported that animals with larger body masses may be less susceptible to insecticides (Gish and Chura 1970, Phillips et al. 2010). However, our results indicated that resistance of these field-collected German cockroach populations had no relationship with body mass, which means that the resistance was attributed to other mechanisms (Table 9). Pridgeon et al. (2002) found P450 monooxygenases and hydrolases, as well as *para* mutation plays a role in pyrethroid resistance, depending on strains. Wen and Scott (1997) suggested that resistance of a susceptible strain to imidacloprid is a result of P450 monooxygenase-mediated detoxification. It was suggested that both cytochrome P450 monooxygenases and hydrolytic enzymes were involved in resistance to chlorpyrifos in German cockroaches (Siegfried et al. 1990). Mechanisms responsible for propoxur resistance included penetration, cytochrome P450-dependent mono-oxygenases and hydrolytic enzymes (Siegfried and Scott 1990). Wei et al. (2001) found that resistance to fipronil was not decreased by PBO, indicating that P450 monooxygenases were not the mechanism for resistance to fipronil. GABA receptor insensitivity, which is the mechanism for cyclodiene resistance (Bloomquist 1993), may undermine the efficacy of fipronil in cockroach control as cross-resistance to fipronil has been detected (Holbrook et al. 2003). Piperonyl butoxide (PBO), S,S,S-tributyl phosphorotrithioate (DEF), and injection application can be used to investigate the mechanisms in insecticide resistance of these field-collected German cockroaches.

Overall, because of its high resistance, permethrin would not be efficient for German cockroach control in the Franklin County, NC. Imidacloprid and fipronil, with high toxicity, may perform well in control of the German cockroach.

Table 1. List of participants.

No.	Code	Type of residence	Approx. Age of home (Years)	No. of bath rooms	No. of toilets	No. of sinks in Kitchen	No. in household	Chemicals used previously for roach control	Use contractor for pest management
1	I	-	64	1	1	1	4	Nothing	Nothing
2	H	Mobile	-	1	1	1	3	Roach and ant spray	No/self management of pest
3	G	Mobile	9	1	1	1	6	Raid, unknown get bait	No/self management of pest
4	E	Mobile	20	1	1	1	6	Raid	No/self management of pest
5	D	Mobile	16	1	1	1	3	Raid, Max, Combat	No/self management of pest
6	B	Mobile	15	1	1	1	4	Raid, Combat	No/self management of pest

Table 2. Mean masses of German cockroach strains measured in grams

Strain	*n*	Mean ± SD[a]
S	20	0.0472 ± 0.0044a
H	20	0.0481 ± 0.0055a
I	20	0.0493 ± 0.0053ab
E	20	0.0502 ± 0.0060ab
G	20	0.0536 ± 0.0047bc
B	20	0.0558 ± 0.0025c
D	20	0.0570 ± 0.0031c

[a] Means within a column followed by the same letter are not significantly different (P <0.05).

Table 3. Toxicity of permethrin topically applied to the German cockroaches

Strain	n	LD_{50}(95% CI)(μg/insect)	Slope(SE)	χ^2	df	RR[a]
S	210	0.239(0.200-0.279)	5.922(0.725)	9.867	5	-
B	150	7.608(4.612-11.489)	3.218(0.434)	5.226	3	31.8
D	150	8.905(7.751-10.099)	4.290(0.625)	1.156	3	37.3
E	150	12.403(8.308-17.089)	3.454(0.531)	4.164	3	51.9
G	150	8.341(7.293-9.382)	4.738(0.671)	2.192	3	34.9
H	120	1.323(0.990-1.729)	2.243(0.371)	0.014	2	5.5
I	150	8.966(8.002-9.986)	5.397(0.741)	1.805	3	37.5

[a] RR, LD_{50} of field-collected strains/LD_{50} of susceptible strain.

Table 4. Toxicity of chlorpyrifos topically applied to the German cockroaches

Strain	n	LD_{50}(95% CI)(μg/insect)	Slope(SE)	χ^2	df	RR[a]
S	210	0.243(0.226-0.260)	7.489(0.895)	3.292	5	-
B	210	1.843(1.596-2.105)	3.673(0.431)	1.648	5	7.6
D	180	1.686(1.429-1.916)	6.071(0.789)	4.053	4	6.9
E	180	1.265(1.095-1.427)	4.451(0.570)	1.447	4	5.2
G	180	2.164(1.937-2.403)	5.108(0.602)	0.99	4	8.9
H	150	1.544(1.329-1.780)	4.12(0.570)	1.608	3	6.4
I	180	2.250(1.957-2.551)	4.115(0.504)	0.972	4	9.3

[a] RR, LD_{50} of field-collected strains/LD_{50} of susceptible strain.

Table 5. Toxicity of propoxur topically applied to the German cockroaches

Strain	n	LD_{50}(95% CI)(μg/insect)	Slope(SE)	χ^2	df	RR[a]
S	180	0.974(0.813-1.148)	3.438(0.415)	1.609	4	-
B	120	1.100(0.932-1.280)	3.968(0.663)	1.389	2	1.1
D	150	0.896(0.608-1.209)	3.692(0.578)	4.005	3	0.9
E	120	1.276(1.025-1.578)	2.972(0.459)	0.394	2	1.3
G	150	0.769(0.651-0.906)	3.483(0.519)	1.009	3	0.8
H	90	1.489(1.186-2.106)	2.979(0.666)	0.222	1	1.5
I	120	0.920(0.700-1.149)	2.774(0.462)	1.261	2	0.9

[a] RR, LD_{50} of field-collected strains/LD_{50} of susceptible strain.

Table 6. Toxicity of imidacloprid topically applied to the German cockroaches

Strain	n	LD_{50}(95% CI)(μg/insect)	Slope(SE)	χ^2	df	RR[a]
S	180	0.168(0.106-0.276)	2.651(0.326)	7.399	4	-
B	120	0.268(0.205-0.346)	2.336(0.409)	0.06	2	1.6
D	150	0.563(0.460-0.669)	3.663(0.479)	1.033	3	3.4
E	180	0.439(0.348-0.543)	2.320(0.302)	2.183	4	2.6
G	150	0.502(0.393-0.651)	2.312(0.308)	1.066	3	3.0
H	180	0.251(0.191-0.329)	1.862(0.245)	2.912	4	1.5
I	180	0.199(0.151-0.259)	2.024(0.258)	2.599	4	1.2

[a] RR, LD_{50} of field-collected strains/LD_{50} of susceptible strain.

Table 7. Toxicity of fipronil topically applied to the German cockroaches

Strain	n	LD_{50}(95% CI)(ng/insect)	Slope(SE)	χ^2	df	RR[a]
S	270	1.33(1.22-1.45)	4.804(0.537)	80.14	2	-
B	160	2.62(1.94-3.32)	2.089(0.352)	35.24	3	2.0
D	130	11.53(8.75-15.57)	2.119(0.341)	38.72	3	8.7
E	200	5.07(1.21-38.02)	2.241(0.627)	12.77	3	3.8
G	200	7.66(6.31-9.20)	2.641(0.321)	67.65	3	5.8
H	130	5.15(2.50-7.57)	1.503(0.346)	18.85	3	3.9
I	150	10.15(7.86-14.30)	2.030(0.357)	32.4	2	7.6

[a] RR, LD_{50} of field-collected strains/LD_{50} of susceptible strain.

Table 8. Relationship between resistance ratios of selected insecticides [a]

	Chlorpyrifos	Propoxur	Imidacloprid	Fipronil
Permethrin	-0.04292 0.9357	-0.46186 0.3565	0.43174 0.3926	0.20738 0.6934
Chlorpyrifos		-0.74793 0.0873	-0.22830 0.6635	0.37969 0.4578
Propoxur			-0.38980 0.4449	-0.62430 0.1852
Imidacloprid				0.39569 0.4374

[a] For each pair of insecticides, top number is the correlation coefficient and bottom number is the p-value.

Table 9. Correlation between body mass and resistance [a]

	Permethrin	Chlorpyrifos	Propoxur	Imidacloprid	Fipronil
Weight	-0.43150 0.3929	0.44657 0.3747	0.05097 0.9236	-0.48512 0.3294	0.28769 0.5804

[a] For each combination of weight and insecticide, top number is correlation coefficient and bottom number is p-value.

Repellency and performance of selected insecticides to field-collected German cockroaches (Dictyoptera: Blattellidae)

The German cockroach, *Blattella germanica* (L.), is an economically important pest worldwide. Its feces and exuviae may cause allergic and asthmatic reactions in sensitive humans and it also contaminates food (Brenner 1995, O'Connor and Gold 1999). German cockroaches can be vectors of many pathogenic microorganisms, including viruses, bacteria, protozoa, and helminthes (Roth and Willis 1957, Brenner 1995). Some people even suffer from delusory cleptoparasitosis, a psychological disease, because of exposure to German cockroaches (Brenner 1995). Their relatively short generation time and high reproductive rate make the German cockroach difficult to control (Phillips et al. 2010).

Insecticides are the most commonly used method for pest control in homes, apartments, and commercial kitchens (Wang and Bennett 2006). The performance of these insecticides can be affected by many factors. Addition of attractants to insecticides, insecticidal formulation, concentration, type of surface to which the insecticide is applied, as well as other factors can influence the effectiveness of insecticides (Ebeling et al. 1966). Repellency is one critical factor that affects field performance of insecticides. Repellency, or at least long term repellency, is a result of an associated learning process of individuals exposed to sublethal concentrations of a repellent (Ebeling et al. 1966, Metzger 1995). When German cockroaches are exposed to negative stimuli such as repellent insecticides, if it survives from the toxic effect of insecticide, it will learn to avoid the treated area and remain in insecticide free areas because it has associated the negative stimuli with the treated area. If the cockroaches receive a sublethal dose, which only make them sick, once the cockroaches recover from the effect, they will learn to avoid the insecticide, making the insecticide repellent (Ebeling et al. 1966).

Repellent insecticides can be used for prevention (i.e., protection of specific locations) and inspection (Oswalt et al. 1997) if they are used properly. For some sensitive equipment, important documents, or food, which cannot be treated with insecticides, repellent insecticides or other repellents can be used around these items and protect them from becoming infested (Ngoh et al. 1998). However, if insecticides that are designed to kill pests have repellency, their repellency would decrease their effectiveness for German cockroach control (Ebeling et al. 1966). Cockroaches repelled by insecticides would spread an infestation to different parts of home or building rather than reducing the pest population (Ebeling et al. 1966). Some insecticidal baits may be contaminated and lose their effectiveness if they are exposed to repellent insecticides (Appel 2004).

Six German cockroach strains were collected in rural Franklin County, North Carolina, after a survey that aimed to investigate insecticide practices used for the control of the German cockroach and to assess the level of residents' awareness and knowledge of integrated pest management (Dingha et al. 2013). The exact pesticides applied (both type and frequency) in the respondents' homes were unclear.

The potential efficacy of several selected insecticides for control of field populations of the German cockroach was investigated. We selected insecticides that are or were commonly used for German cockroach management. In addition, we have previously (Wu 2016) determined the toxicity and resistance ratios of these insecticides to these field-collected strains using topical application of technical insecticides. The objective of this study was to determine the repellency, toxicity, and to estimate the potential for field effectiveness of several insecticides using the Ebeling choice-box methodology. The choice-box test is a simulation of field performance of insecticides. In general, Ebeling choice boxes measure both efficacy and repellency of an insecticide and are a useful laboratory method to evaluate insecticides (Rust and Reierson 1993). If an insecticide performs well in choice-box test, it has the potential to perform well in field use (Rust and Reierson 1978, 1993). Some insecticides that have high toxicity in topical application studies may perform poorly in field tests because of their repellency to the insects (Rust and Reierson 1978).

Materials and methods

Cockroach strains. Seven strains of German cockroaches were used in this study, including a susceptible strain. The susceptible strain has been reared in the laboratory without exposure to any insecticides for >40 years. The six field-collected strains: B, D, E, G, H, and I were collected in Franklin County, North Carolina in 2011-2012 (Dingha et al. 2013). The complete treatment histories of these field-collected strains was unknown. After collecting, the cockroaches were immediately sent to Auburn University, Auburn, AL by overnight shipping.

The susceptible strain was kept in extra-large trash cans at 28 ± 2 ℃, with 40-55% RH, and a photoperiod of 12:12 (L:D). The six field-collected strains were reared in 3.8L glass jars at 28 ± 2 ℃, 40-55% RH, and a photoperiod of 12:12 (L:D). All seven strains were provided cockroach harborage, dry dog food (Purina Dog Chow, Raulston Purina, St. Louis, MO), and clean water. The German cockroaches were fed and supplied clean water weekly.

Only males were used in this study because of their relatively homogenous physiological milieu and their body mass is more uniform when compared to those of females (Abd-Elghafar et al. 1990). Besides, the females were needed to reproduce and increase the size of the colonies. All the cockroaches used were briefly anesthetized with CO_2 to facilitate handling during the experiments.

Chemicals and insecticides. The chemicals used in this study were formulated chlorpyrifos (12.6%, Spectracide Termite & Ant Control Concentrate, Division of United Industries Corp., St. Louis, MO), imidacloprid (21.4%, Dominion 2L Termiticide/Insecticide, Control Solutions Inc., Pasadena, TX), fipronil (9.1%, Termidor SC Termiticide/Insecticide, BASF Corp., NC), permethrin (2.5%, Blackflag Flea Killer Concentrate, Division of United Industries Corp., St. Louis, MO), and propoxur (70%, Baygon, Mobay Chemical Corp., Kansas City, MO). As directed on the product labels, fipronil was diluted to 0.06% solution and the other insecticides were diluted to 0.05% solutions with spring water.

Ebeling Choice-Box. The repellency of five formulated insecticides to German cockroaches was determined using Ebeling choice-boxes (Ebeling et al. 1966) (Fig. 1). The choice-box is a square box which is divided by a partition into two equal compartments. There is a hole near the top of the partition, allowing the cockroaches to move freely from one compartment to another. The two compartments are the light and the dark compartment. Food and water were provided in the light compartment. The light side is not treated with insecticides, which simulates places in home that are normally illuminated, such as a kitchen counter. The other compartment (dark side) was treated with insecticides and resembles dark voids where German cockroach harbor during the day. The hole in the partition was plugged by a cork from the dark side at first. Twenty adult male German cockroaches were placed into the untreated light compartment, and were allowed to acclimate for 2 h. After 2 h, the cork in the partition was removed, and the insects were allowed to move between the two compartments. The light side was covered with transparent Plexiglass, allowing data to be taken and preventing the cockroaches from escaping. The treated, dark compartment was also covered with transparent Plexiglass, and also covered with an opaque sheet. The dark side represents the dark places where the German cockroaches prefer and where the insecticides were applied. The choice-boxes were exposed to a photoperiod of 12:12 (L:D) h at 28 ℃. The number of live and dead cockroaches in both compartments was recorded about 4-5 hours into the photophase daily for 14 d. The percentage of live cockroaches found in the untreated, light compartment of each choice-box during the photophase was defined as the percentage of repellency.

Treatments consisted of 2 ml of 0.06% fipronil, 0.05% imidacloprid, 0.05% chlorpyrifos, 0.05% permethrin, and 0.05% propoxur solutions in spring water. Insecticide solutions were evenly applied onto 14.6 by 29.8 cm aluminum foil covered inserts that tightly fit the floor of the dark side. The treated foils were allowed to dry and the insects were placed into the light side of the choice box. Each treatment was replicated 4-6 times. Controls were treated with 2 ml of spring water alone.

Data analysis. Toxicity (LT_{50}) of the insecticides was analyzed using probit analysis with SAS (proc probit). Repellency was analyzed by repeated measures ANOVA with SAS (SAS Institute 2004). Least square means difference was used to separate mean repellency with SAS (SAS Institute 2004).

The performance index (PI) is calculated as an estimate of potential field performance.

$$\text{PI} = 1 - \left[\frac{\text{Number alive} + \text{Number alive in light side}}{\text{Number dead} + \text{Initial total number}} \right] \times 100$$

PI values range from -100, complete repellency and no mortality to +100, complete mortality and no repellency. Control, no repellency or mortality, has a PI value of 0 (Appel 2003).

The PI, which combines mortality and repellency (Rust and Reierson 1978, Appel 1992, 2003), was analyzed using a nonlinear regression model and SigmaPlot v13 software (Systat Software Inc. 2014). A rectangular hyperbola model was used for analysis of the performance index. The following function was fit to the daily PI values: $y = ax/(b + x)$, where y = PI, a = PI_{max}, $b = t_{PImax/2}$, and x = day.

Results

Toxicity in choice boxes. In the susceptible strain, the LT_{50} value of German cockroaches in control choice boxes was 24.95 d, significantly greater (based on non-overlap of the 95% CI) than all the insecticide treatments (Table 10). The LT_{50} values for the insecticide treatments ranged from 0.33 d for permethrin to 6.18 d for imidacloprid (Table 10). In strain B, the LT_{50} value of German cockroaches in control choice boxes was 27.86 d (Table 11). The LT_{50} values of insecticide treatments ranged from 0.98 d to 438.20 d for fipronil and permethrin, respectively (Table 11). Permethrin had the lowest toxicity to strain according to LT_{50} value. The LT_{50} value of imidacloprid was not significantly different than that of the control, based on non-overlap of the 95% CI (Table 11). In strain D, the LT_{50} value of control choice boxes was 161.19 d (Table 12). LT_{50} values of the insecticide treatments ranged from 0.51 d for chlorpyrifos to 192.35 d for permethrin (Table 12). The control, permethrin, and propoxur did not have significantly different LT_{50} values (Table 12). In strain E, the LT_{50} value of German cockroaches in control choice boxes was 59.76 d (Table 13). LT_{50} values of insecticide treatments ranged from 1.35 d for fipronil to 34.95 d for permethrin (Table 13). The LT_{50} values of permethrin and the control were not significantly different (Table 13). The LT_{50} value of strain G in control choice boxes was 184.66 d (Table 14). For the insecticide treatments, the LT_{50} values ranged from 1.51 d to 482.83 d for fipronil and permethrin, respectively (Table 14). Permethrin and imidacloprid had greater LT_{50} values than the control (Table 14). In strain H, the LT_{50} of German cockroaches in control choice boxes was 43.81 d (Table 15). LT_{50} values of the insecticide treatments ranged from 0.68 d to 34.67 d for chlorpyrifos and imidacloprid, respectively (Table 15). LT_{50} values of control and imidacloprid were not significantly different (Table 15). In strain I, the LT_{50} value of German cockroaches in the control choice boxes was 47.08 d (Table 16). LT_{50} values of insecticide treatment for strain I ranged from 1.57 d for fipronil to 59.09 d for permethrin (Table 16). LT_{50} value of permethrin was not significantly different from that of the control (Table 16). Among all the insecticides, chlorpyrifos and fipronil were most toxic to all the field-collected strains whereas permethrin was the least toxic to all field-collected strains except for strain H (Tables 11-16).

Repellency. In the susceptible strain, overall repellency (i.e., averaged over all 14 d) of the untreated control was 18.58 $\pm$ 3.32%, whereas repellency of the insecticides ranged from 21.18 $\pm$ 3.90% for imidacloprid to 55.97 $\pm$ 3.38% for permethrin (Table 17). Repellency of permethrin was significantly greater ($P<0.05$) than all other insecticides except chlorpyrifos ($P = 0.2420$). In strain B, repellency of the untreated control was 26.77 $\pm$ 1.95% (Table 18). Repellency of the insecticide treatments ranged from 18.37 $\pm$ 1.95% for chlorpyrifos to 66.89 $\pm$ 1.95% for permethrin (Table 18). Repellency of control, imidacloprid, and propoxur treatments were not significantly different ($P > 0.05$). The repellency of chlorpyrifos was not significantly different than imidacloprid ($P = 0.1422$). In strain D, repellency of the control was 15.77 $\pm$2.19%, less than all insecticide treatments except for propoxur (Table 19). Repellency of insecticide treatments ranged from 21.24 $\pm$2.19% to 41.72 $\pm$5.80% for propoxur and fipronil, respectively (Table 19). The repellency of chlorpyrifos was not significantly different from the other insecticide treatments ($P > 0.05$). There was no significant difference between imidacloprid and permethrin ($P = 0.2375$), and imidacloprid and propoxur ($P = 0.2562$). In strain E, repellency of the control was 15.55 $\pm$3.34% (Table 20). Repellency of insecticide treatments ranged from 21.54 $\pm$3.32% for propoxur to 84.92 $\pm$3.32% for permethrin (Table 20). Repellency of permethrin was significantly greater than all treatments (Table 20). Chlorpyrifos was not significantly different from fipronil ($P = 0.3523$) and the control was not significantly different from fipronil ($P = 0.1379$) or propoxur ($P = 0.0855$). Fipronil, imidacloprid, and propoxur were not significantly different from each other. In strain G, repellency of the control was 6.68 $\pm$ 1.77%, less than all insecticide treatments except fipronil (Table 21). Repellency of insecticide treatments ranged from 4.37 $\pm$4.29% for fipronil to 27.16 $\pm$1.77% for permethrin (Table 21). Repellency of permethrin was the greatest. Chlorpyrifos, imidacloprid, and propoxur were not significantly different from each other. In strain H, the control treatment had repellency of 34.76 $\pm$2.60% (Table 22). Repellency of insecticide treatments ranged from 20.96 $\pm$2.60% to 29.11 $\pm$ 3.27% for propoxur and chlorpyrifos (Table 22), respectively. Among the insecticide treatments, chlorpyrifos and propoxur had significantly different repellency ($P < 0.05$). Control repellency was significantly different from imidacloprid, permethrin, and propoxur. In strain I, repellency of the control was 17.13 $\pm$1.79% (Table 23). Repellency of the insecticide treatments ranged from

20.61 ± 1.78% for imidacloprid to 53.66 ± 1.78% for permethrin (Table 23). Permethrin was more repellent than all other treatments. Chlorpyrifos, fipronil, and propoxur were not significantly different. Control, fipronil, and imidacloprid treatments were not significantly different.

Performance index. The PI_{max} (maximum performance index value) value, calculated from the rectangular hyperbolic model, of the control in the susceptible strain was 44.71 ± 7.54 (Fig. 2). No PI_{max} value of control for the field-collected strains was >50 (Fig. 2). PI_{max} values of permethrin to the susceptible strain and field-collected strain H were 89.17 ± 6.69 and 57.11 ± 7.05, respectively (Fig. 3). PI_{max} values of permethrin for the other field-collected strains were <20 (Fig. 3), over all 14 d. PI values of control and permethrin increased linearly during the experiments (Figs. 2-3). The PI_{max} values of chlorpyrifos reached 100 for the susceptible strain (Fig. 4). For field-collected strains B, G, and I, the PI_{max} of chlorpyrifos was <85 (Fig. 4), while for strains D and H, the PI_{max} of chlorpyrifos was >95 (Fig. 4). Within 1 d, PI values of chlorpyrifos became positive and reached maximum values in 7 d in all strains (Fig. 4). PI_{max} values of propoxur in susceptible strain was 81.37 ± 4.67 (Fig. 5). PI_{max} values of propoxur for the field-collected strains were <60 (Fig. 5). PI_{max} value of imidacloprid in susceptible strain was 65.94 ± 13.48 (Fig. 6). For field-collected strains, PI_{max} values ranged from 18.53 ± 14.60 to 64.17 ± 13.48 (Fig. 6). PI values of propoxur and imidacloprid increased gradually during the 14 d experiment (Figs. 5-6). PI values of fipronil in all strains were positive in 2 d and reached 100 in 4 d (Fig. 7).

Rectangular hyperbola model was used for analysis of PI values because the PI increased exponentially at the beginning of the experiment and then reached a stable asymptotic thereafter; however, the PI values in experiments with control, permethrin, propoxur for strain I, and imidacloprid for strains B and H increased linearly with time, so they were analyzed with linear regression. All treatments produced significant results when analyzed using one of the above models ($P < 0.05$) except for permethrin in strain D ($P > 0.05$) (Tables 24-29).

Discussion

Permethrin was the least toxic insecticide to the field-collected German cockroaches based on the LT_{50} values except for strain H (Tables 10-16). Chlorpyrifos and fipronil were more toxic than the other insecticides tested (Tables 10-16). Imidacloprid was the least toxic insecticide to the susceptible strain (Table 10). In topical application experiments, the toxicity of imidacloprid and propoxur to field-collected was similar to the susceptible strain (Wu 2016); however, in Ebeling choice box tests, toxicity of propoxur and imidacloprid was greater in the field-collected strains than the susceptible strain (Tables 10-16).

Permethrin was significantly more repellent than other insecticides to both the susceptible strain and field-collected strains B, E, G, and I (Tables 17-18, 20-21, 23), which may partially explain the low toxicity (LT_{50}) of permethrin to these strains in Ebeling choice boxes. Permethrin was more toxic (Wu 2016) and less repellent to strain H (Table 22), leading to better performance of permethrin for this strain.

Fipronil was more repellent to strains B and D than other treatments (Tables 18 and 19), while it was similarly or less repellent to other strains than other treatments (Tables 17, 20-23). It was also pointed out that high repellency would result in poor performance in choice boxes (Ebeling et al. 1968, Rust and Reierson 1978). However, the performance of fipronil for all strains (strains B and D included) was the best among the insecticide combining all 14 d (Figs. 2-7). Fipronil is a highly effective insecticide for German cockroach control that can kill at a very low rate (Holbrook et al. 2003, Nasirian et al. 2006); however, it is a slow-acting insecticide (Wei et al. 2001). Individuals moved from the dark to light compartment for food and water after contact with fipronil, then the effect of fipronil inhibited them from returning to the dark side, which may account for its greater apparent repellency to strains B and D.

The PI values for the untreated control boxes were higher than predicted (PI value of 0) (Fig. 2), but can be a result of natural mortality in some of the choice boxes (Appel 1992). The PI_{max} of the susceptible strain was the greatest for permethrin, and PI_{max} of strain H was the second (Fig. 3). In topical applications, strain H was the least resistant to permethrin (Wu 2016), which may account for better performance of permethrin for this strain. PI values of permethrin for field-collected strain B were negative during the entire 14 d, and the PI_{max} values of other four strains were <20 (Fig. 3), suggesting poor performance potential of permethrin to control these strains. The remaining cockroaches would be able to continue contaminating the environment and would be a nucleus for reinfestation of a treated home or apartment. The PI values of permethrin for strain D did not change significantly during the 14 d experiment (Fig. 3) and did not fit the linear model (P >0.05) (Table 25), which indicates that permethrin probably cannot provide good control for strain D in the field. The PI values for permethrin indicate that it would not be capable of providing effective control under field conditions, and it is not recommended for control of these field-collected German cockroaches.

Chlorpyrifos reached PI_{max} values of ≈100 for the susceptible strain and field-collected strains B, D, E, H, and I, and a PI_{max} of 94.81 ± 3.28 for strain E (Table 26). Some of the estimates of PI_{max} were >100, but PI_{max} values ± 2 SE overlap 100 or were very close to 100, which can be attribute to an artifact of the model. However, during the 14 d experiment, chlorpyrifos reached PI_{max} of 100 only for the susceptible strain, and PI_{max} values of ≈100 for field-collected strains D and H (Fig. 4). The estimated PI_{max} values mean that chlorpyrifos can provide control, however probably not in the 14 d, though. The live cockroaches can keep contaminating the environment and their reproduction may be faster than the control. A PI_{max} value of ≈100 indicates no repellency and complete mortality (Rust and Reierson 1978, Appel and Tanley 2000, Appel 2003). An insecticide that does not produce PI_{max} values ≈100 may perform poorly in field trials (Rust and Reierson 1978, Appel 1990, 1992), indicating that chlorpyrifos may not be effective enough for control of the field-collected strains except for strains D and H. Chlorpyrifos obtained $t_{PImax/2}$ values of <1 d for the susceptible strain and field-collected strains D and H, reflecting its rapid kill. With PI_{max} values of ≈100, chlorpyrifos would be expected to perform well to control of strains D and H.

PI values of propoxur did not exceed 60 for any strain (Fig. 5). Rust and Reierson (1993) found that low levels of resistance may result in poor control under field conditions, which could be the reason for the poor performance of propoxur in Ebeling choice boxes as propoxur exhibited very low resistance ratios in topical applications (Wu 2016). PI_{max} values estimated with the rectangular hyperbola model did not reach 100, indicating that propoxur probably cannot provide complete control of the field-collected strains.

Imidacloprid reached PI_{max} values of ≈100 for the susceptible strain and field-collected strain E estimated with the rectangular hyperbolic model (Table 28). However, during the 14 d experiment, imidacloprid did not reach PI_{max} values of ≈100 for any strain (Fig. 6), indicating that imidacloprid may perform poorly in control of these field-collected German cockroaches. Similarly with propoxur, imidacloprid had low resistance ratios for the field-collected strains in topical applications (Wu 2016), which may explain its poor performance.

Fipronil reached PI_{max} values of $\approx$100 and $t_{PImax/2}$ values of <2d for all strains with the rectangular hyperbolic model (Table 29). Similar to chlorpyrifos, some of the PI_{max} estimates were >100, but $PI_{max} \pm 2$ SE overlap 100 or were very close to 100, which can be attributed to an artifact of the model. During the 14 d experiment, fipronil reached PI_{max} values of 100 for all strains, and had positive PI values in 2 d (Fig. 7). With large PI values and fast control ($t_{PImax/2}$ values of <2 d), fipronil can be recommended as an effective insecticide for control of these field-collected German cockroaches. Ebeling et al. (1966, 1967) demonstrated that there was an inverse or approximately inverse relationship between insecticide efficacy when German cockroaches were confined to a treated surface (Petri dish continuous exposure) and their efficacy when the insects were not confined to an insecticide residue (Ebeling choice box). Fipronil had high toxicity in both topical application (low LD_{50} values) (Wu 2016) and Ebeling choice boxes (low LT_{50} values) (Tables 17-23) and was the most effective insecticide (PI_{max} values of 100). The good performance of fipronil in choice boxes may result from its feature of being slow-acting. After their first contact with the fipronil residue, the cockroaches would not be affected and would be able to move between the two compartments. Their instinct for darkness (Metzger 1995) made them stay in the dark compartment and receive a greater dose of fipronil until death.

In conclusion, permethrin, imidacloprid, and propoxur may perform poorly if they are used to control field-collected strains of the German cockroach. Chlorpyrifos may not be an ideal insecticide either because it did not reach PI_{max} values of 100, and the remaining individuals will continue contamination of the environment and to reproduce. The results of Ebeling choice box tests for fipronil indicates that fipronil can be an excellent insecticide for control of field strains of the German cockroach.

Topical application and Ebeling choice boxes indicated that field-collected German cockroaches have developed low to very high level of resistance to several insecticides and these insecticides may not perform well in the field. According to the results, fipronil can be excellent for German cockroach control, but it should be used with caution in that German cockroaches are able to develop resistance to many insecticides (Holbrook et al. 2003, Nasirian et al. 2006).

Table 10. Toxicity of insecticides against the susceptible strain of the German cockroach

Treatment	n	LT_{50}(95% CI)(Day)	Slope(SE)	χ^2	P
Control	80	24.95(18.75-40.76)	1.77(0.26)	47.95	0.0001
Permethrin	80	0.33(0.004-1.03)	0.65(0.20)	10.84	0.0010
Chlorpyrifos	80	0.48(0.12-0.70)	3.81(1.18)	10.44	0.0012
Propoxur	80	0.68(0.02-1.63)	0.73(0.22)	10.99	0.0009
Imidacloprid	80	6.81(4.72-10.37)	1.38(0.34)	16.84	0.0001
Fipronil	80	1.05(0.96-1.13)	8.00(1.33)	36.10	0.0001

Table 11. Toxicity of insecticides against the German cockroach strain B

Treatment	n	LT_{50}(95% CI)(Day)	Slope(SE)	χ^2	P
Control	80	27.86(21.19-42.99)	1.59 (0.20)	63.90	0.0001
Permethrin	80	438.20(96.89-69529)	0.55(0.15)	12.88	0.0003
Chlorpyrifos	80	2.47(1.35-3.45)	0.93(0.17)	30.16	0.0001
Propoxur	80	16.09(9.30-131.32)	0.62(0.21)	8.88	0.0029
Imidacloprid	80	23.21(17.53-37.04)	1.49(1.49)	52.01	0.0001
Fipronil	80	0.98(0.88-1.06)	7.29(1.32)	30.58	0.0001

Table 12. Toxicity of insecticides against the German cockroach strain D

Treatment	n	LT_{50}(95% CI)(Day)	Slope(SE)	χ^2	P
Control	80	161.19(63.44-1465)	0.86(0.18)	22.53	0.0001
Permethrin	80	192.35(63.54-4023)	0.63(0.15)	17.15	0.0001
Chlorpyrifos	80	0.51(0.27-0.77)	1.40(0.16)	80.38	0.0001
Propoxur	80	114.34(27.08-5.34E12)	0.47(0.21)	5.02	0.0250
Imidacloprid	80	39.56(20.14-276.41)	0.67(0.17)	15.44	0.0001
Fipronil	80	1.38(1.10-1.63)	6.15(1.13)	29.62	0.0001

Table 13. Toxicity of insecticides against the German cockroach strain E

Treatment	n	LT_{50}(95% CI)(Day)	Slope(SE)	χ^2	P
Control	80	59.76(28.81-480.40)	1.12(0.28)	16.33	0.0001
Permethrin	80	34.95(23.58-69.31)	1.04(0.15)	46.10	0.0001
Chlorpyrifos	80	1.70(1.05-2.31)	1.31(0.17)	57.13	0.0001
Propoxur	80	12.27(6.18-39100901)	0.64(0.30)	4.40	0.0359
Imidacloprid	80	14.86(12.32-19.35)	1.30(0.15)	79.70	0.0001
Fipronil	80	1.35(1.24-1.45)	7.05(0.69)	103.22	0.0001

Table 14. Toxicity of insecticides against the German cockroach strain G

Treatment	n	LT_{50}(95% CI)(Day)	Slope(SE)	χ^2	P
Control	80	184.66(58.54-7489)	0.90(0.24)	14.13	0.0002
Permethrin	80	482.83(104.84-76664)	0.58(0.16)	13.10	0.0003
Chlorpyrifos	80	2.37(0.72-3.77)	1.01(0.27)	14.02	0.0002
Propoxur	80	72.66(35.82-168.29)	0.27(0.20)	1.78	0.1824
Imidacloprid	80	295.26(82.25-12451)	0.64(0.16)	15.58	0.0001
Fipronil	80	1.51(1.39-1.63)	6.67(0.60)	124.76	0.0001

Table 15. Toxicity of insecticides against the German cockroach strain H

Treatment	n	LT_{50}(95% CI)(Day)	Slope(SE)	χ^2	P
Control	80	43.81(26.53-120.31)	1.15(0.20)	32.19	0.0001
Permethrin	80	19.51(11.88-70.69)	0.46(0.12)	14.98	0.0001
Chlorpyrifos	80	0.68(0.34-1.05)	1.33(0.17)	61.51	0.0001
Propoxur	80	12.42(9.32-20.08)	0.82(0.15)	31.67	0.0001
Imidacloprid	80	34.67(22.73-77.11)	1.19(0.19)	37.12	0.0001
Fipronil	80	1.13(1.02-1.24)	6.12(0.71)	73.75	0.0001

Table 16. Toxicity of insecticides against the German cockroach strain I

Treatment	n	LT_{50}(95% CI)(Day)	Slope(SE)	χ^2	P
Control	80	47.08(30.81-97.30)	1.16(0.16)	52.26	0.0001
Permethrin	80	59.09(35.53-144.67)	0.83(0.12)	46.58	0.0001
Chlorpyrifos	80	2.38(1.71-2.99)	1.14(0.13)	75.87	0.0001
Propoxur	80	12.59(7.38-92.47)	0.47(0.17)	7.83	0.0051
Imidacloprid	80	10.83(9.51-12.72)	1.25(0.11)	125.08	0.0001
Fipronil	80	1.57(1.47-1.66)	7.77(0.59)	172.35	0.0001

Table 17. Repellency of insecticides against the susceptible German cockroach strain determined in Ebeling choice boxes

Treatment	n	% Alive in light[a] Mean ± SE	DF	P
Control	80	18.58 ± 3.32a	53	<0.0001
Permethrin	80	55.97 ± 3.38d	53	<0.0001
Chlorpyrifos	80	42.47 ± 11.05bcd	53	0.0004
Propoxur	80	32.24 ± 3.32c	53	<0.0001
Imidacloprid	80	21.18 ± 3.90ab	53	<0.0001
Fipronil	80	32.47 ± 11.05ab	53	0.0054

[a] Mean over the entire test. Means followed by the same letters were not significantly different (P <0.05).

Table 18. Repellency of insecticides against the German cockroach strain B determined in Ebeling choice boxes

Treatment	n	% Alive in light[a] Mean ± SE	DF	P
Control	80	26.77 ± 1.95b	53	<0.0001
Permethrin	80	66.89 ± 1.95d	53	<0.0001
Chlorpyrifos	80	18.37 ± 1.95a	53	<0.0001
Propoxur	80	25.65 ± 1.95b	53	<0.0001
Imidacloprid	80	22.47 ± 1.95ab	53	<0.0001
Fipronil	80	44.84 ± 6.83c	53	<0.0001

[a] Mean over the entire test. Means followed by the same letters were not significantly different ($P < 0.05$).

Table 19. Repellency of insecticides against the German cockroach strain D determined in Ebeling choice boxes

Treatment	n	% Alive in light[a] Mean ± SE	DF	P
Control	80	15.56 ± 2.19a	56	<0.0001
Permethrin	80	28.84 ± 2.19c	56	<0.0001
Chlorpyrifos	80	28.48 ± 4.60bcd	56	<0.0001
Propoxur	80	21.24 ± 2.19ab	56	<0.0001
Imidacloprid	80	24.97 ± 2.19bc	56	<0.0001
Fipronil	80	41.72 ± 5.80d	56	<0.0001

[a] Mean over the entire test. Means followed by the same letters were not significantly different (P <0.05).

Table 20. Repellency of insecticides against the German cockroach strain E determined in Ebeling choice boxes

Treatment	n	% Alive in light[a] Mean ± SE	DF	P
Control	80	15.56 ± 3.34a	54	<0.0001
Permethrin	80	85.92 ± 3.32d	54	<0.0001
Chlorpyrifos	80	34.29 ± 3.71c	54	<0.0001
Propoxur	80	21.54 ± 3.32ab	54	<0.0001
Imidacloprid	80	22.56 ± 3.32b	54	<0.0001
Fipronil	80	27.03 ± 7.59abc	54	0.0008

[a] Mean over the entire test. Means followed by the same letters were not significantly different (P <0.05).

Table 21. Repellency of insecticides against the German cockroach strain G determined in Ebeling choice boxes

Treatment	n	% Alive in light[a]	DF	P
		Mean ± SE		
Control	80	6.68 ± 1.77a	54	0.0004
Permethrin	80	27.16 ± 1.77c	54	<0.0001
Chlorpyrifos	80	16.15 ± 1.77b	54	<0.0001
Propoxur	80	15.69 ± 1.77b	54	<0.0001
Imidacloprid	80	18.41 ± 1.77b	54	<0.0001
Fipronil	80	4.37 ± 4.79a	54	0.3663

[a] Mean over the entire test. Means followed by the same letters were not significantly different ($P < 0.05$).

Table 22. Repellency of insecticides against the German cockroach strain H determined in Ebeling choice boxes

Treatment	n	% Alive in light[a]	DF	P
		Mean ± SE		
Control	80	34.76 ± 2.60c	54	<0.0001
Permethrin	80	27.06 ± 2.60ab	54	<0.0001
Chlorpyrifos	80	29.12 ± 3.27bc	54	<0.0001
Propoxur	80	20.96 ± 2.60a	54	<0.0001
Imidacloprid	80	27.69 ± 2.60ab	54	<0.0001
Fipronil	80	26.40 ± 8.03abc	54	0.0018

[a] Mean over the entire test. Means followed by the same letters were not significantly different (P <0.05).

Table 23. Repellency of insecticides against the German cockroach strain I determined in Ebeling choice boxes

Treatment	n	% Alive in light[a] Mean ± SE	DF	P
Control	120	17.13 ± 1.79a	54	<0.0001
Permethrin	120	53.66 ± 1.78c	54	<0.0001
Chlorpyrifos	120	26.95 ± 1.78b	54	<0.0001
Propoxur	120	27.08 ± 1.78b	54	<0.0001
Imidacloprid	120	20.61 ± 1.78a	54	<0.0001
Fipronil	120	27.41 ± 4.96ab	54	<0.0001

[a] Mean over the entire test. Means followed by the same letters were not significantly different ($P < 0.05$).

Table 24. Relationship between day and performance index of control against German cockroaches repelled in Ebeling choice boxes

Strain	Slope ± SE	Intercept ± SE	r^2	df	*F*	*P*
S	6.41 ± 0.49	-36.87 ± 4.17	0.93	13	171.04	<0.0001
B	4.62 ± 0.83	-26.05 ± 7.03	0.72	13	31.30	0.0001
D	1.50 ± 0.18	-2.71 ± 1.49	0.86	13	73.21	<0.0001
E	4.47 ± 0.81	-23.62 ± 6.88	0.72	13	30.55	0.0001
G	2.10 ± 0.22	-5.03 ± 1.91	0.88	13	87.58	<0.0001
H	3.48 ± 0.30	-25.30 ± 2.54	0.92	13	135.42	<0.0001
I	4.00 ± 0.51	-16.46 ± 4.33	0.84	13	62.02	<0.0001

Table 25. Relationship between day and performance index of permethrin against German cockroaches repelled in Ebeling choice boxes

Strain	Slope ±SE	Intercept ±SE	r^2	df	*F*	*P*
S	1.94 ±0.45	66.53 ±3.83	0.61	13	18.53	0.0010
B	2.15 ±0.46	-39.68 ±3.90	0.65	13	22.07	0.0005
D	0.94 ±0.48	2.53 ±4.03	0.24	13	3.88	0.0725
E	5.36 ±0.30	-60.72 ±2.59	0.96	13	311.80	<0.0001
G	1.85 ±0.20	-9.70 ±1.67	0.88	13	88.92	<0.0001
H	2.26 ±0.49	28.84 ±4.14	0.64	13	21.72	0.0006
I	3.04 ±0.27	-22.82 ±2.32	0.91	13	124.57	<0.0001

Table 26. Relationship between day and performance index of chlorpyrifos against German cockroaches repelled in Ebeling choice

Strain	b ±SE	a ±SE	r^2	df	*F*	*P*
S	0.11 ±0.02	101.59 ±0.46	0.83	13	59.19	<0.0001
B	2.29 ±0.86	100.71 ±9.76	0.78	13	41.77	<0.0001
D	0.52 ±0.04	103.87 ±0.87	0.96	13	322.86	<0.0001
E	2.28 ±0.52	109.37 ±6.41	0.89	13	99.68	<0.0001
G	1.59 ±0.26	94.81 ±3.28	0.92	13	142.02	<0.0001
H	0.68 ±0.10	103.30 ±1.90	0.90	13	112.14	<0.0001
I	2.02 ±0.56	101.08 ±6.79	0.84	13	64.10	<0.0001

Table 27. Relationship between day and performance index of propoxur against German cockroaches repelled in Ebeling choice boxes

Strain	Slope ± SE[a]	Intercept ± SE	b ± SE[b]	a ± SE	r^2	df	*F*	*P*
S	-	-	0.70 ± 0.05	92.39 ± 0.94	0.97	13	361.67	<0.0001
B	-	-	2.56 ± 0.63	63.12 ± 4.23	0.88	13	92.01	<0.0001
D	-	-	0.71 ± 0.39	31.24 ± 2.29	0.45	13	9.28	0.0101
E	-	-	6.81 ± 4.78	91.16 ± 28.54	0.72	13	30.70	0.0001
G	-	-	1.54 ± 0.25	58.96 ± 1.98	0.91	13	124.18	<0.0001
H	-	-	4.29 ± 0.89	79.84 ± 5.96	0.93	13	164.58	<0.0001
I	1.80 ± 0.43	67.36 ± 3.64	-	-	0.59	13	17.61	0.0012

[a] Linear regression model.

[b] Rectangular regression model.

Table 28. Relationship between day and performance index of imidacloprid against German cockroaches repelled in Ebeling choice boxes

Strain	Slope ± SE[a]	Intercept ± SE	b ± SE[b]	a ± SE	r^2	df	*F*	*P*
S	-	-	6.85 ± 2.29	101.65 ± 15.23	0.90	13	107.45	<0.0001
B	4.64 ± 0.46	-15.60 ± 3.95	-	-	0.89	13	100.02	<0.0001
D	-	-	2.62 ± 1.12	46.02 ± 5.46	0.74	13	34.17	<0.0001
E	-	-	36.00 ± 56.01	216.03 ± 261.95	0.78	13	42.58	<0.0001
G	-	-	18.52 ± 26.63	40.79 ± 38.51	0.62	13	19.73	0.0008
H	3.61 ± 0.32	-14.58 ± 2.75	-	-	0.91	13	124.89	<0.0001
I	-	-	6.22 ± 2.32	90.71 ± 14.48	0.87	13	83.54	<0.0001

[a] Linear regression model.

[b] Rectangular regression model.

Table 29. Relationship between day and performance index of fipronil against German cockroaches repelled in Ebeling choice boxes

Strain	b ± SE	a ± SE	r^2	df	*F*	*P*
S	0.61 ± 0.19	108.56 ± 4.01	0.68	13	24.99	0.0003
B	0.59 ± 0.18	108.28 ± 3.82	0.68	13	25.46	0.0003
D	1.29 ± 0.62	116.58 ± 10.45	0.61	13	18.54	0.0010
E	1.91 ± 1.30	123.79 ± 19.74	0.55	13	13.32	0.0033
G	1.24 ± 0.52	115.95 ± 8.87	0.66	13	22.91	0.0004
H	0.85 ± 0.31	111.56 ± 5.93	0.66	13	23.12	0.0004
I	1.76 ± 1.00	121.99 ± 15.54	0.59	13	17.16	0.0014

Fig. 1. Ebeling choice box

Fig. 2. Performance index of control to the German cockroach determined in Ebeling choice boxes

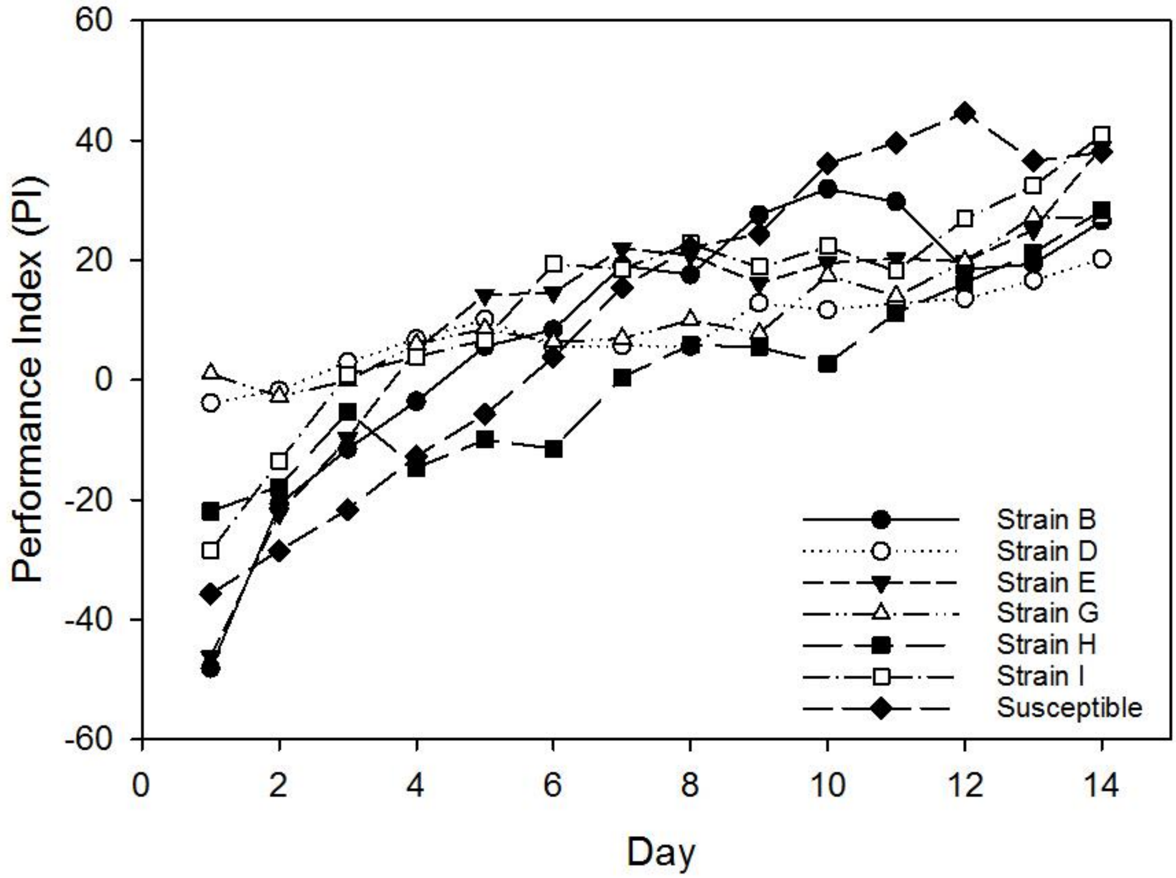


Fig. 3. Performance index of permethrin to the German cockroach determined in Ebeling choice boxes

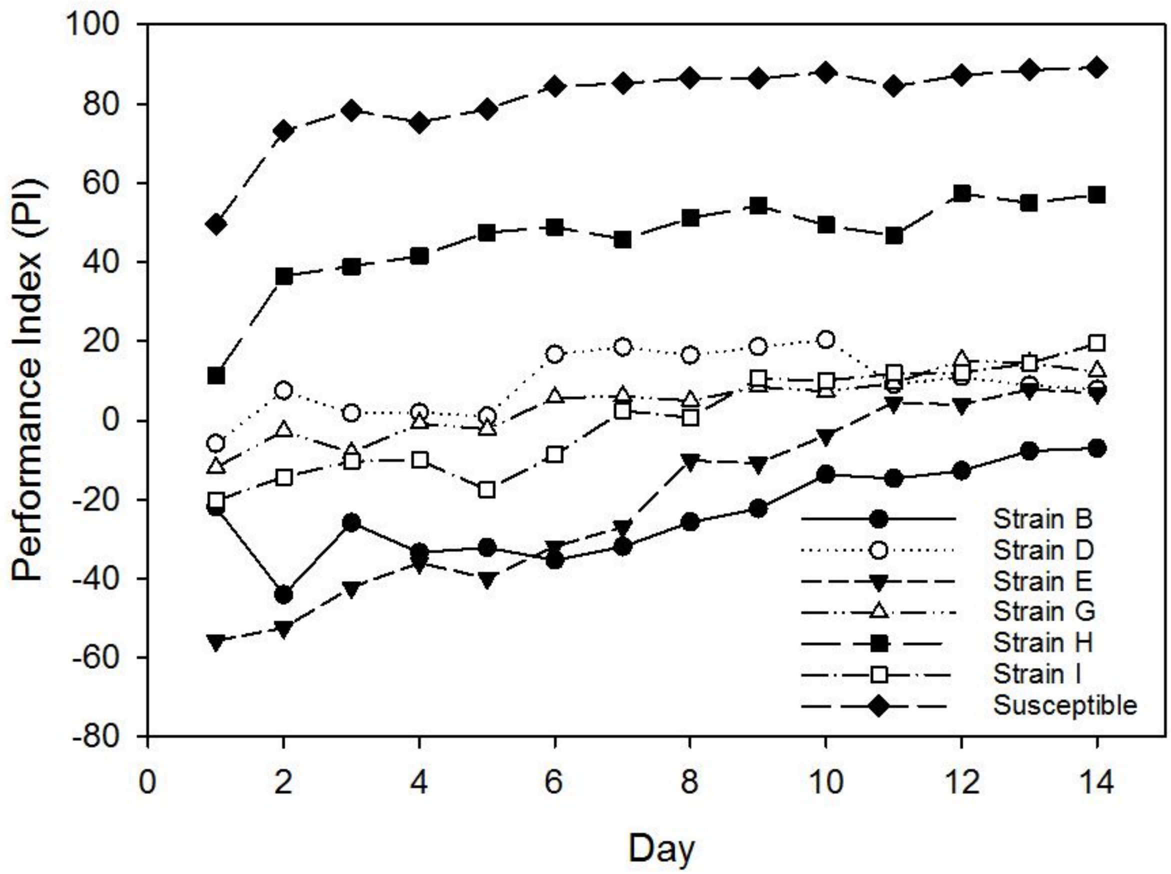


Fig. 4. Performance index of chlorpyrifos to the German cockroach determined in Ebeling choice boxes

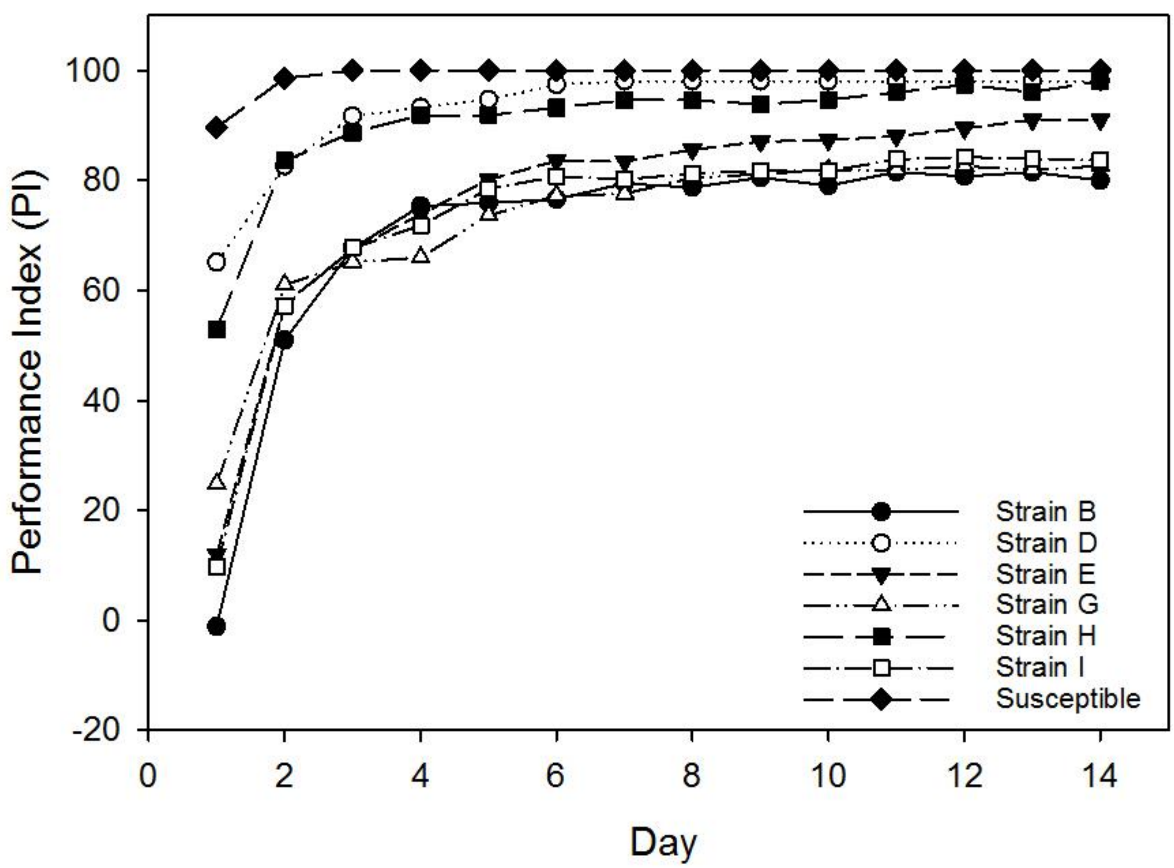


Fig. 5. Performance index of propoxur to the German cockroach determined in Ebeling choice boxes

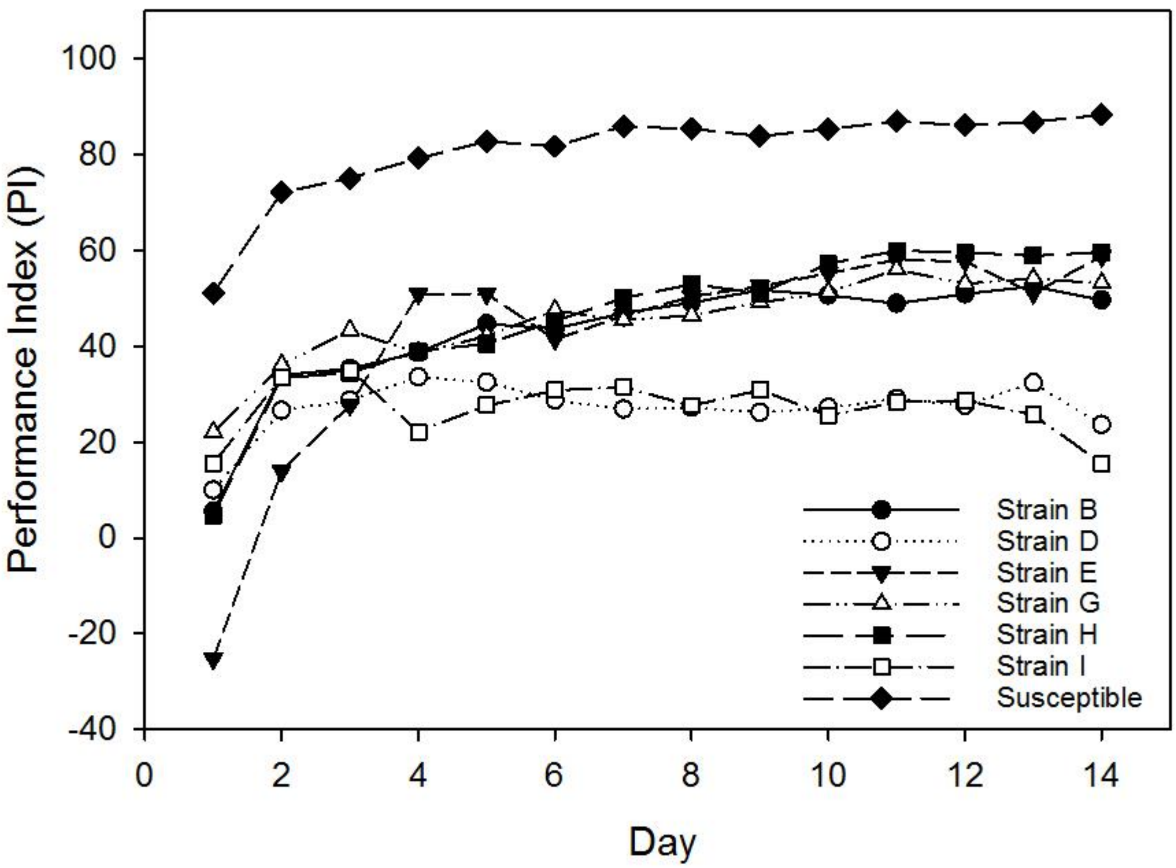


Fig. 6. Performance index of imidacloprid to the German cockroach determined in Ebeling choice boxes

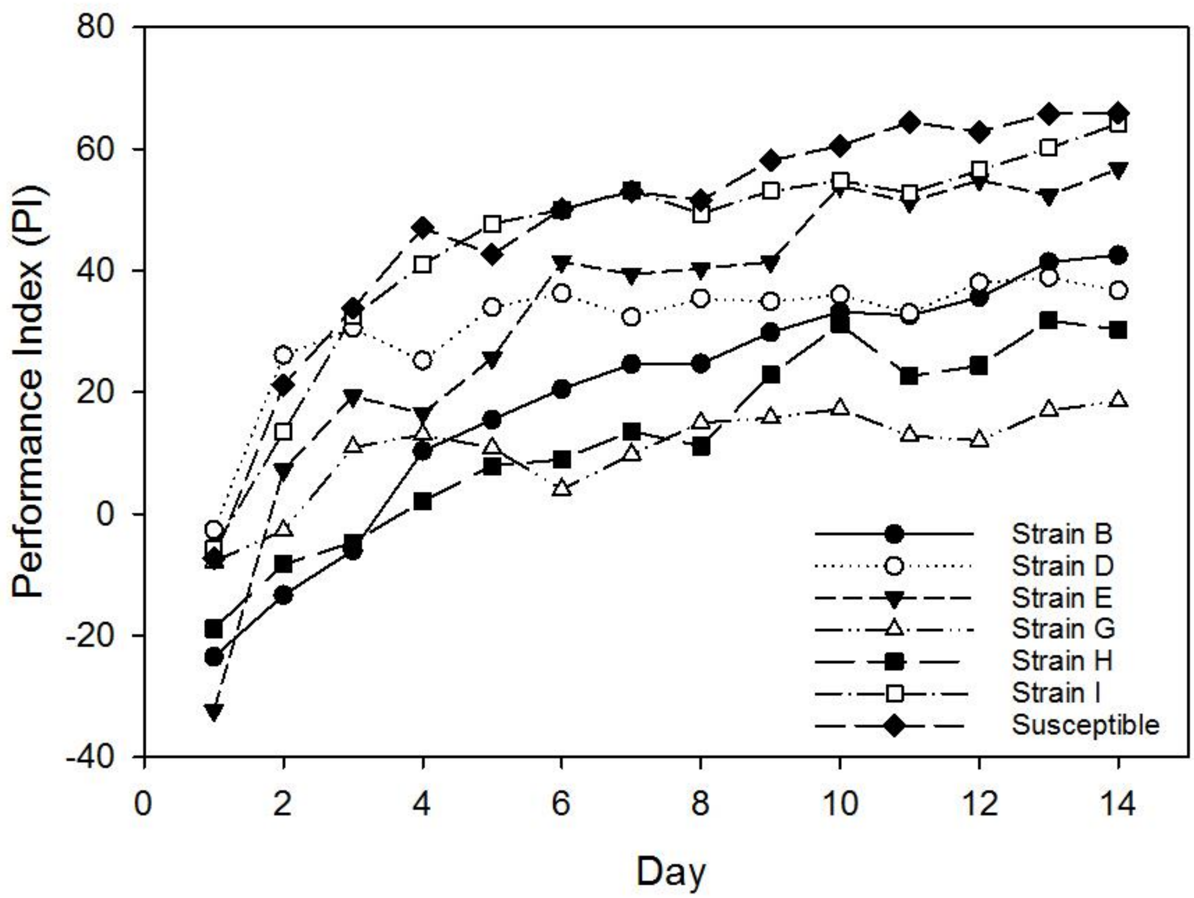
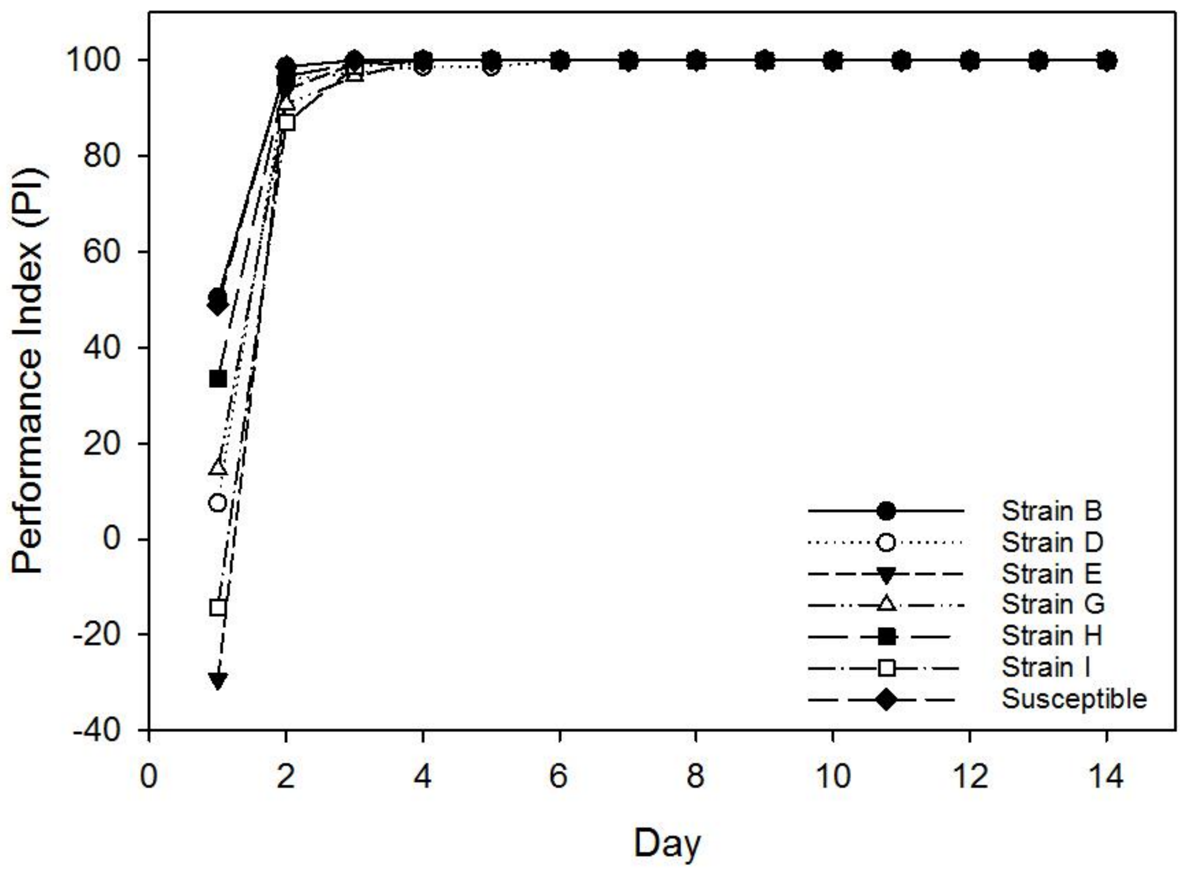


Fig. 7. Performance index of fipronil to the German cockroach determined in Ebeling choice boxes



References

Abd-Elghafar, S. F., A. G. Appel, and T. P. Mack. **1990**. Toxicity of several insecticide formulations against adult German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 83: 2290–2294.

Appel, A. G. 1990. Laboratory and field performance of consumer bait products for German cockroach (Dictyoptera: Blattellidae) control. J. Econ. Entomol. 83: 135–159.

Appel, A. G. 1992. Performance of gel and paste bait products for German cockroach (Dictyoptera: Blattellidae) control: Laboratory and field studies. J. Econ. Entomol. 85: 1176–1183.

Appel, A. G. 1997. Nonchemical approaches to cockroach control. J. Agric. Entomol. 14: 271–280.

Appel, A. G. 2003. Laboratory and field performance of an indoxacarb bait against German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 96: 863–870.

Appel, A. G. 2004. Contamination affects the performance of insecticidal baits against German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 97: 2035–42.

Appel, A. G., and M. J. Tanley. **2000**. Laboratory and field performance of an imidacloprid gel bait against German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 93: 112–118.

Archbold, E. F., M. K. Rust, and D. A. Reierson. **1987**. Comparative life histories of fungus-infected and uninfected German cockroaches, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). Ann. Entomol. Soc. Am. 80: 571–577.

Barson, G., and N. Renn. **1983**. Hatching from oothecae of the German cockroach (*Blattella germanica*) under culture conditions and after premature removal. Entomol. Exp. Appl. 34: 179–185.

Bloomquist, J. R. 1993. Toxicology, mode of action and target site-mediated resistance to insecticides acting on chloride channels. Comp.Biochem. Physiol. 106: 301–314.

Brenner, R. J. 1995. Medical and economic significance, pp. 77–92. *In* Rust, M.K., Owens, J.M., Reierson, D.A. (eds.), Understanding and Controlling the German Cockroach. Oxford University Press, New York.

Chumley, C. K. 2010. Ohio challenges EPA ban on chemical that kills bedbugs, Heartl. Inst.

Clarke, T. H., and D. G. Cochran. **1959**. Cross-resistance in insecticide-resistant strains of the German cockroach, *Blattella germanica* (L.). Bull. Wid Hith Org. 20: 823–833.

Cochran, D. G. 1995. Insecticide resistance, pp. 171–192. *In* Rust, M.K., Owens, J.M., Reierson, D.A. (eds.), Understanding and Controlling the German Cockroach. Oxford University Press, New York.

Cole, L. M., R. A. Nicholson, and J. E. Casida. **1993**. Action of phenylpyrazole insecticides at the GABA-gated chloride channel. Pestic. Biochem. Physiol. 46: 47–54.

Cox, C. 2001. Imidacloprid. J. Pestic. Reform. 21: 1–30.

Cox, C. 2002. Piperonyl butoxide. J. Pestic. Reform. 22: 12–20.

Delaplane, K. S. 2000. Pesticide usage in the United States: history, benefits, risks, and trends. Univ. Georg. Coll. Agric. Environ. Sci. Bull. 1121/Reprinted Novemb.

Dingha, B., L. Jackai, R. H. Monteverdi, and J. Ibrahim. **2013**. Pest control practices for the German cockroach (Blattodea: Blattellidae): a survey of rural residents in North Carolina. Florida Entomol. 96: 1009–1015.

Dymond, N. L., and I. M. Swift. **2008**. Permethrin toxicity in cats: A retrospective study of 20 cases. Aust. Vet. J. 86: 219–223.

Ebeling, W. 1975. Urban entomology. Div. Agric. Sci. Univ. Calif. (http://www.entomology.ucr.edu/ebeling/ebel14.html).

Ebeling, W., D. A. Reierson, and R. E. Wagner. **1967**. Influence of repellency on the efficacy of blatticides. II. Laboratory experiments with German cockroaches. J. Econ. Entomol. 60: 1375–1390.

Ebeling, W., D. A. Reierson, and R. E. Wagner. **1968**. Influence of repellency on the efficacy of Blatticides. IV. Comparison of four cockroach species. J. Econ. Entomol. 61: 1213–1219.

Ebeling, W., R. E. Wagner, and D. A. Reierson. **1966**. Influence of repellency on the efficacy of blatticides. I. Learned modification of behavior of the German cockroach. J. Econ. Entomol. 59: 1374–1388.

EPA. **2000**. Propoxur (Baygon). US Environ. Prot. Agency. (https://www3.epa.gov/ttn/atw/hlthef/propoxur.html).

EPA. **2009**. Permethrin facts. US Environ. Prot. Agency. (http://nepis.epa.gov).

EPA. **2016**. Chlopyrifos. US Environ. Prot. Agency. (https://www.epa.gov/ingredients-used-pesticide-products/chlorpyrifos#additional information).

Extension Toxicity Network. **1984**. Chlorpyrifos-Chemical fact sheet 9/84. (http://pmep.cce.cornell.edu/profiles/insect-mite/cadusafos-cyromazine/chlorpyrifos/chlorpyr_cfs_0984.html).

Extension Toxicity Network. **1985**. Permethrin (Ambush, Pounce) chemical profile 4/85. (http://pmep.cce.cornell.edu/profiles/insect-mite/mevinphos-propargite/permethrin/insect-prof-permethrin.html).

Extension Toxicity Network. **1993a**. Permethrin. (http://pmep.cce.cornell.edu/profiles/extoxnet/metiram-propoxur/permethrin-ext.html).

Extension Toxicity Network. **1993b**. Chlorpyrifos. (http://pmep.cce.cornell.edu/profiles/extoxnet/carbaryl-dicrotophos/chlorpyrifos-ext.html#45).

Extension Toxicity Network. **1993c**. Propoxur. (http://pmep.cce.cornell.edu/profiles/extoxnet/metiram-propoxur/propoxur-ext.html).

Extension Toxicity Network. **1996a**. Extoxnet PIP-Propoxur. (http://extoxnet.orst.edu/pips/propoxur.htm).

Extension Toxicity Network. **1996b**. Extoxnet PIP - Imidacloprid. (http://extoxnet.orst.edu/pips/imidaclo.htm).

Georghiou, G. P. 1972. The evolution of resistance to pesticides. Annu. Rev. Ecol. Syst. 3: 133–168.

Gish, C. D., and N. J. Chura. **1970**. Toxicity of DDT to Japanese quail as influenced by body weight, breeding condition, and sex. Toxicol. Appl. Pharmacol. 17: 740–751.

Gould, G. E., and H. O. Deay. **1940**. The biology of six species of cockroaches which inhabit buildings. Purdue Univ. Agr. Exp. Stat. Bull.

Grace, J. K., and D. L. Wood. **1987**. Delusory cleptoparasitosis: Delusions of arthropod infestation in the home. Pan-Pac. Entomol. 63: 1–4.

Guillebeau, P., N. Hinkle, and P. Roberts. **2001**. Summary of losses from insect damage and cost of control in Georgia 2001. 1–58.

Hainzl, D., and J. E. Casida. **1996**. Fipronil insecticide: novel photochemical desulfinylation with retention of neurotoxicity. Proc. Natl. Acad. Sci. U. S. A. 93: 12764–12767.

Holbrook, G. L., J. Roebuck, C. B. Moore, M. G. Waldvogel, and C. Schal. **2003**. Origin and extent of resistance to fipronil in the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). J. Econ. Entomol. 96: 1548–1558.

IRAC. **2012**. IRAC mode of action classification scheme. Insectic. Resist. Action Comm. 1–23.

Jolly JR, A. L., J. W. Avault JR, K. L. Koonce, and J. B. Graves. **1978**. Acute toxicity of permethrin to several aquatic animals. Trans. Am. Fish. Soc. 8487: 37–41.

Komiyama, M., and K. Ogata. **1977**. Observations of density effects on the German cockroaches, *Blattella germanica* (L.). Med. Entomol. Zool. 28: 409–415.

Kristensen, M., K. K. Hansen, and K.-M. V. Jensen. **2005**. Cross-resistance between dieldrin and fipronil in German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 98: 1305–1310.

Liu, N., and Y. Xin. **2000**. Insecticide resistance and cross-resistance in the house fly (Diptera: Muscidae). J. Econ. Entomol. 93: 1269–1275.

Matsuda, K., S. D. Buckingham, D. Kleier, J. J. Rauh, M. Grauso, and D. B. Sattelle. **2001**. Neonicotinoids: Insecticides acting on insect nicotinic acetylcholine receptors. Trends Pharmacol. Sci. 22: 573–580.

Metzger, R. 1995. Behavior, pp. 49–76. *In* Rust, M.K., Owens, J.M., Reierson, D.A. (eds.), Understanding and Controlling the German Cockroach. Oxford University Press, New York.

Narahashi, T., X. Zhao, T. Ikeda, V. L. Salgado, and J. Z. Yeh. **2010**. Glutamate-activated chloride channels: Unique fipronil targets present in insects but not in mammals. Pestic. Biochem. Physiol. 97: 149–152.

Nasirian, H., H. Ladonni, M. Shayeghi, H. Vatandoost, M. R. Yaghoobi-Ershadi, Y. Rassi, M. Abolhassani, and M. R. Abaei. **2006**. Comparison of permethrin and fipronil toxicity against German cockroach (Dictyoptera: Blattellidae) strains. Iran. J. Public Health. 35: 63–67.

Ngoh, S. P., L. E. W. Choo, F. Y. Pang, Y. Huang, M. R. Kini, and S. H. Ho. **1998**. Insecticidal and repellent properties of nine volatile constituents of essential oils against the American cockroach, *Periplaneta americana* (L.). Pestic. Sci. 54: 261–268.

NPTN. **1996**. Fipronil. Environ. Prot. (http://www.livingwithbugs.com/PDFiles/fipronil.pdf).

O'Connor, G. T., and D. R. Gold. **1999**. Cockroach allergy and asthma in a 30-year-old man. Environ. Health Perspect. 107: 243–247.

Oswalt, D. A., A. G. Appel, and L. M. Smith. **1997**. Water loss and desiccation tolerance of German cockroaches (Dictyoptera: Blattellidae) exposed to moving air. Comp. Biochem. Physiol. - A Physiol. 117: 477–486.

Phillips, A. K., A. G. Appel, and S. R. Sims. **2010**. Topical toxicity of essential oils to the German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 103: 448–59.

Pridgeon, J. W., A. G. Appel, W. J. Moar, and N. Liu. **2002**. Variability of resistance mechanisms in pyrethroid resistant German cockroaches (Dictyoptera: Blattellidae). Pestic. Biochem. Physiol. 73: 149–156.

Robertson, J. L., H. K. Preisler, and R. M. Russell. **2007**. PoloPlus: Probit and Logit Analysis. User's Guide. LeOra Softw. Co.

Ross, M. H., and D. E. Mullins. **1995**. Biology, pp. 21–48. *In* Rust, M.K., Owens, J.M., Reierson, D.A. (eds.), Understanding and Controlling the German Cockroach. Oxford University Press, New York.

Roth, L. M. 1970. Evolution and taxonomic significance of reproduction in Blattaria. Annu. Rev. Entomol. 15: 75–96.

Roth, L. M., and E. R. Willis. **1957**. The medical and veterinary importance of cockroaches. Smithsonian Institution, Washington, D. C.

Rust, M. K. 1995. Factors affecting control with insecticides, pp. 149–170. *In* Rust, M.K., Owens, J.M., Reierson, D.A. (eds.), Understanding and Controlling the German Cockroach. Oxford University Press, New York.

Rust, M. K., and D. A. Reierson. **1978**. Comparison of the laboratory and field efficacy of insecticides used for German cockroach control. J. Econ. Entomol. 71: 704–708.

Rust, M. K., and D. A. Reierson. **1993**. Relationship between insecticide resistance and performance in choice tests of field-collected German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 86: 1124–1130.

Sakuma, M. 1998. Probit analysis of preference data. Appl. Entomol. Zool. 33: 339–347.

SAS Institute. **2004**. SAS/STAT 9.1 User's Guide. Cary, NC.

Schrut, A. H., and W. G. Waldron. **1963**. Psychiatric and entomological aspects of delusory parasitosis. J. Am. Med. A. 186: 429–430.

Scott, J. G., D. G. Cochran, and B. D. Siegfried. **1990**. Insecticide toxicity, synergism and resistance in the German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 83: 1698–703.

Siegfried, B. D., and J. G. Scott. **1990**. Mechanism responsible for propoxur resistance in the German cockroach, *Blattella germanica* (L.). Pestic. Sci. 33: 133–146.

Siegfried, B. D., J. G. Scott, R. T. Roush, and B. C. Zeichner. **1990**. Biochemistry and genetics of chlorpyrifos resistance in the German cockroach, *Blattella germanica* (L). Pestic. Biochem. Physiol. 121: 110–121.

Systat Software Inc. 2014. SigmaPlot, version 13.0. San Jose, CA.

Tanaka, A. 1976. Stages in the embryonic development of the German cockroach, *Blattella germanica* Linne (Blattaria, Blattellidae). Kontyu. 44: 512–525.

The New York Timess. **2009**. Ohio wants to use industrial poison on bedbugs at home. A23.

Tomizawa, M., and J. E. Casida. **2005**. Neonicotinoid insecticide toxicology: Mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol. 45: 247–268.

Tsuji, H., and T. Mizuno. **1973**. Effects of a low temperature on the survival and development of four species of cockroaches, *Blattella germanica*, *Periplaneta americana*, *P. fuliginosa* and *P. japonica*. Japan Soc. Med. Entomol. Zool. 23: 185–194.

Valles, S. M. 1998. Toxicological and biochemical studies with field populations of the German cockroach , *Blattella germanica*. Pestic. Biochem. Physiol. 200: 190–200.

Valles, S. M., K. Dong, and R. J. Brenner. **2000**. Mechanisms responsible for cypermethrin resistance in a strain of German cockroach, *Blattella germanica*. Pestic. Biochem. Physiol. 66: 195–205.

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.

Wang, L., Y. Zhang, Z. Han, Y. Liu, and J. Fanga. **2010**. Cross-resistance and possiblemechanisms of chlorpyrifos resistance in *Laodelphax striatellus* (Fallén). Pest Manag. Sci. 66: 1096–1100.

Wei, Y., A. G. Appel, W. J. Moar, and N. Liu. **2001**. Pyrethroid resistance and cross-resistance in the German cockroach, *Blattella germanica* (L). Pest Manag. Sci. 57: 1055–1059.

Weill, M., C. Malcolm, F. Chandre, K. Mogensen, A. Berthomieu, M. Marquine, and M. Raymond. **2004**. The unique mutation in ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. Insect Mol. Biol. 13: 1–7.

Wen, Z., and J. G. Scott. **1997**. Cross-resistance to imidacloprid in strains of German cockroach (*Blattella germanica*) and house fly (*Musca domestica*). Pestic. Sci. 49: 367–371.

WHO. **1989**. Permethrin Health and Safety Guide, World Health Organization.

Wickham, J. C. 1995. Conventional insecticides, pp. 109–148. *In* Rust, M.K., Owens, J.M., Reierson, D.A. (eds.), Understanding and Controlling the German Cockroach. Oxford University Press, New York.

Wu, X. 2016. Toxicity and laboratory performance of insecticides to field-collected German cockroaches (Dictyoptera: Blattellidae). Master thesis. Auburn University, Auburn, Alabama.