PALATABILITY AND EFFICACY OF EMAMECTIN BENZOATE GEL BAITS ON FOUR PEST COCKROACH SPECIES

By

BARBARA ELLEN BAYER

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To my family and friends. Thank you for all of your support.

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PALATABILITY AND EFFICACY OF EMAMECTIN BENZOATE GEL BAITS ON FOUR PEST COCKROACH SPECIES

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Barbara Ellen Bayer

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Four species of pest cockroaches, the German, *Blattella germanica,* the brownbanded, *Supella longipalpa*, Oriental, *Blatta orientalis* and American, *Periplaneta americana,* were fed gel baits containing emamectin benzoate to determine relative palatability and efficacy. Emamectin benzoate gel baits were formulated as either emamectin A or emamectin B at concentrations of 0.05%, 0.1%, or 0.2%. For all cockroaches tested, there was no significant difference in palatability or efficacy between emamectin A 0.1% or emamectin B at 0.05%, 0.1% or 0.2%, with the exception of percent mortality of American cockroaches; emamectin B 0.05%, which had the lowest percent mortality, was significantly different from emamectin A 0.1%.

Two German cockroach strains, the Daytona bait averse strain and the Orlando normal susceptible strain, of German cockroach were fed a set of experimental gel baits, emamectin A and B at 500 ppm (0.05%) and 1000 ppm (0.1%) emamectin benzoate. There was no significant difference in palatability of baits between strains; both strains consumed similar percentages of gel bait and dog food. Percent mortality from consumption of emamectin gel baits was between 80% and 90% at 6 d.

A second set of experimental emamectin gel baits formulated at 0.05%, 0.1%, or 0.2% emamectin benzoate were fed to the Daytona German cockroach stain, brownbanded

cockroaches, Oriental cockroaches and American cockroaches in order to determine palatability and efficacy. The emamectin gel baits were palatable to all four species. Only brownbanded cockroaches consumed similar percentages of gel bait and dog food. The other three species preferred the emamectin gel baits over dog food, with 78% to 99.7% of total consumption being emamectin gel baits. All four species were highly susceptible to the emamectin gel baits with 85% to 99% mortality at 14 d. Percent mortality from emamectin gel baits was similar to Maxforce FC Select for the Daytona German cockroach strain and brownbanded cockroach. For the Oriental cockroach, percent mortality from emamectin gel baits, emamectin A 0.1% and emamectin B 0.1% and 0.2%, was similar to Maxforce FC Select at 14 d. For American cockroaches, there were no statistical differences between Maxforce FC Select and the emamectin gel baits, emamectin A 0.1% or emamectin B 0.2%, with 98.6% and 96.4%, respectively, at 14 d.

All formulations and dosages of emamectin gel baits were palatable to all cockroach species tested. Additionally, when fed emamectin gel baits, high susceptibility, 85% to 99.5% mortality, was observed for all pest cockroaches tested at 14 d. Therefore, cockroach gel baits containing emamectin benzoate show excellent potential for controlling pest cockroaches.

CHAPTER 1
INTRODUCTION

Cockroaches are known to carry certain fungi, viruses, and bacteria, which cause specific diseases in humans. Additionally, cockroaches produce allergens that can elicit asthma attacks. Worldwide, approximately 300 million people suffer from asthma. More than 250,000 deaths per year are attributed to asthma and this number is expected to increase by ~20% over the next decade (WHO 2006). The urbanization of people around the world, from rural communities into large crowded cities, has been blamed for some of the increased occurrences of asthma. This may be due to increased exposure to pest cockroach allergens. Because of the risk they pose to human heath, cockroaches are considered serious urban pest.

Most cockroaches are feral, rarely encountering humans; however, other cockroaches live in close association with humans and these cockroaches are considered the worst pests. Those most commonly encountered are the German, *Blattella germanica* L., brownbanded, *Supella longipalpa* (Fabricius), Oriental cockroach, *Blatta orientalis* L. and American *Periplaneta americana* L. cockroaches (Whitney et al. 1967, Cornwell 1968, Reierson et al. 1979, Darr et al. 1998, Capinera 2004).

Cockroach control is commonly accomplished with the use of insecticides. With repeated application of an insecticide, insecticide resistance becomes an issue. Consequently, new insecticides must be tested to combat the resistance. Insecticides are available in a variety of formulations, such as sprays, dusts, and more recently, gel baits. Each formulation has positive and negative attributes. With sprays, there is increased insecticide exposure to humans as well as environmental degradation of the insecticide due to heat or moisture. Dusts can have a long residual, but can become airborne and will clump if exposed to too much moisture. Gel baits, which are increasingly relied upon for cockroach control, can be used in sensitive areas where other formulations cannot be used, and limit the amount of insecticide in the environment. However, some cockroach strains have developed an aversion to some of the ingredients in gel baits; therefore, their matrices must be altered to overcome the aversion.

In my study, I tested two sets of experimental cockroach gel bait matrices with a novel insecticide, emamectin benzoate, were tested against the most commonly encountered pest cockroach species. In the first study, an experimental gel bait matrix, with emamectin benzoate, was found to be palatable to both a bait averse strain of German cockroaches as well as a normal strain. Additionally, the percent mortality caused by consumption of emamectin benzoate, on these two strains of German cockroaches was tested. In the subsequent studies, a second set of experimental gel bait matrices, also containing emamectin benzoate, were used to determine palatability and susceptibility of a bait averse strain of German cockroach, as well as the brownbanded cockroach, the Oriental cockroach, and the American cockroach.

CHAPTER 2
LITERATURE REVIEW

Four hundred million yeas ago, 150 million years before dinosaurs appeared, the first arthropods (springtails, spiders, and scorpions) were roaming the earth. Cockroaches entered the fossil record 300-360 million years ago and have remained nearly unchanged in appearance (Blatchley 1920, Appel 1995 Rust et al. 1995, Copeland 2003, Kendall 2005). Of the more than 4,000 described species of cockroaches that currently inhabit the earth (Bell 1984, Mabbett 2004), only 69 live in North America (Atkinson et al. 1991) and of these, just a few are considered pests.

All cockroaches are hemimetabolous. They undergo three life stages: egg, nymph, and adult. Cockroach "eggs are enclosed in an ootheca" which can be carried by the gravid female for a few hours and then dropped or glued to a surface or the ootheca can be carried until nymphal hatch (Mullins and Cochran 1987). Nymphs are similar in appearance to adults but are wingless. Nymphal development is punctuated by molts, the final molt ending in adulthood. Incubation time of oothecae and nymphal development are both temperature and nutrient dependent, with warmer temperatures increasing the rate of development.

Pest Status

Pest cockroaches are those that live and breed in and around human structures (Cornwell 1976). Their "natural habitat" is outdoors and it is the fault of humans that pest cockroaches exist. "We encourage them to come indoors", by providing them with an environment similar to their "natural habitat". Once inside, these cockroaches are considered pests for both aesthetic and health reasons.

Aesthetics

Cockroaches are messy. Their fecal matter stains and discolors surfaces in addition to creating unpleasant odors that humans can detect. They defecate for obvious biological reasons, as well as to communicate. Pheromones in feces assist cockroaches in locating aggregations and can send signals to attract mates. The sex pheromones, periplanone A and B, of the American cockroach, *Periplaneta americana* L, were isolated from fecal material (Persoons et al. 1982). However, sex pheromones are also found in the cuticle like those of the German cockroach female, *Blattella germanica* L. (Schal et al. 1990). These cuticular compounds require physical contact before a sexual response occurs (Charlton et al. 1993). In addition to carrying pheromones, cockroach feces and their cuticle can also be the source of health problems for humans.

Health

Humans can develop physical and psychological problems due to the presence of cockroaches. Inner city children with asthma, who were given skin tests, had a greater reaction to cockroach allergens than to dust mite or cat allergens (Rosenstreich et al. 1997). More than 80% of the children's bedrooms had some detectible level of cockroach allergen and 50% had levels high enough to induce an asthma attack. According to Brenner et al. (1990), cockroach allergens are second, only to house dust mites, in causing reactions in asthmatics. This is especially important considering that "300 million people suffer from asthma and 255,000 people died from asthma in 2005" (WHO 2006).

Cockroaches are able to "carry, maintain, and excrete" viable fungi, protozoa, eggs of helminthes, viruses, and bacteria. This includes several strains of streptococcus and salmonella (Roth and Willis 1957, 1960). While it is difficult attribute human illness directly to cockroaches, correlations have been made that implicate them. Brenner et al. (1987) reviewed several instances, which link cockroaches to diseases including an outbreak of typhus on a ship, gastroenteritis in a hospital, and dysentery in Northern Ireland. Another case, which implicates cockroaches in the spread of disease, Tarshis (1962) describes decreases in the spread of hepatitis in a housing project. The decrease, in this particular housing project, coincided with insecticide treatments and documented decreases in cockroach infestations. At the same time, hepatitis cases were increasing in the immediate area suggesting that cockroaches were helping transmit hepatitis.

Cockroaches can also cause psychological problems; people are embarrassed to have them. Cornwell (1976) stated that “fear or shame” associated with cockroach infestations prevented some people from admitting infestation even existed. The “fear or shame” can cause stress in proportion to the size of the cockroach and/or infestation (Brenner 1995). The stress comes from the implication that if cockroaches are present it is due to an “unsanitary environment”, which may or may not be the case.

Pest Cockroaches

Of the thousands of described species of cockroaches, there are only a few that are considered pest cockroaches. Cockroaches with a pest status are those in close association with humans. Four primary pest species are German cockroach, brownbanded cockroach, *Supella longipalpa* (Fabricius), Oriental cockroach, *Blatta orientalis* L., and American cockroach (Whitney et al. 1967, Cornwell 1968, Reierson et al. 1979, Darr et al. 1998, Capinera 2004). Pest cockroaches are divided into two groups, domestic and peridomestic cockroaches, based upon where they live and breed. Domestic cockroaches are found mostly indoors while peridomestic cockroaches can be found outdoors as well as indoors.

Domestic Cockroaches

Domestic cockroaches almost exclusively live and breed indoors (Darr et al. 1998). They are "completely depend on the human habitat for survival" and there may be co-evolution between humans and the domestic cockroaches (Barcay 2004). While both, brownbanded cockroaches and German cockroaches, are found throughout the United States, the German cockroach poses the larger pest problem because it is encountered more often.

Domestic cockroaches can enter homes by a variety of methods. In apartment homes or condominiums, there can be movement from one adjacent unit to another via shared plumbing (Owens and Bennett 1982) and through wall and ceiling voids. Additionally, oothecae, as well as live individuals, can be transported from one location to another in paper bags, cardboard boxes, and furniture (Cornwell 1968, Barcay 2004). "Many homes and business establishments become infested with German cockroaches when they are introduced inside infested cartons, foodstuffs, and other materials" (Barcay 2004). Once cockroaches are introduced, the availability of food, water, and harborage encourage infestation, especially where sanitation practices are poor. Food can come from dirty dishes left in the sink or from meals eaten at various locations within a structure such as a bedroom, living room, at a desk, etc. Water is obtained from leaking pipes, condensation on plumbing, pet water bowls, or drip pans in refrigerators. Harborage, in general, can be any clutter such as boxes, papers, furniture, appliances, and cracks and crevices. Gravid German cockroaches prefer cracks 4.77 mm in width and nymphs prefer cracks as narrow as 1.59 mm (Koehler et al. 1994).

German Cockroach

Distribution

Originating in Asia (Atkinson et al. 1990, Appel 1995), the German cockroach has a worldwide distribution and is considered to be "one of the worst insect pests" (Blatchley 1920) in heated structures (Cornwell 1968, Ross and Mullins 1995). Cornwell (1968) attributed its distribution to human commerce and war, suggesting that the German cockroach stowed away with humans as they traversed the globe.

Habitat and ecology

German cockroaches prefer a "warm, moist environment" (Cornwell 1968) where they have "daily access to water" (Barcay 2004). This includes houses, apartments, restaurants, supermarkets, food processing plants, motor vehicles, as well as naval and cruise ships (Cornwell 1968, Barcay 2004, Metzger1995). Kitchen and bathroom areas are often preferred harborage sites and large aggregations have been located around refrigerators, stoves, and trashcans (Appel 1995). In heavy infestations, German cockroaches are found throughout structures and are rarely found living outdoors (Cornwell 1968, Appel 1995).

Physical characteristic

The one of the more distinguishing characteristic of German cockroaches are the two dark brown "longitudinal" parallel bands on the yellowish-brown pronotum (Blatchley 1920, Guthrie and Tindall 1968, Barcay 2004). On the late instar German cockroach nymphs, the dark brown bands extend to the mesonota and metanota; the abdomen is also dark brown. Early instar nymphs have a single yellowish-brown spot may be present on the mesonota and metanota with the rest of the body a dark brown color.

Adult German cockroaches possess non-functional wings that extend to the tip of the abdomen. The males are 10-13 mm in length and slender, with the abdomen tapering to the tip of the posterior. The females are slightly darker in color and longer, 12-15 mm, as well as broader (Blatchley 1920, Guthrie and Tindall 1968). The female wings cover the abdomen and extend to just beyond the tip. The ootheca, when present, is 8 mm long and light brown.

Other members of the genus *Blattella* have similar characteristic to the German cockroach. *Blattella vaga* Hebard has a brownish-black colored marking on the head, from the vertex to the clypeus, which distinguishes it from the German cockroach. The Asian cockroach, *Blattella asahinai* Mizukubo, is nearly identical to the German cockroach. However, the Asian cockroach is a strong flier and peridomestic or "feral" (Barcay 2004). Specific morphological differences between the German cockroach and Asian cockroach have been described (Roth 1986, Appel 1995, Richman 2000). Additionally, Carlson and Brenner (1988) described a method for discriminating Asian cockroaches, German cockroaches and *B. vaga* by means of gas chromatography for the quantitative determination of cuticular hydrocarbon components.

Life cycle

Female German cockroaches carry their ootheca, which contains 30 to 40 embryos, until just prior to hatching. Incubation requires ~28 d. Successive oothecae contain fewer developing embryos (Cornwell 1968, Ross and Mullins 1995). Nymphs require an average of 103 d to go through the 6-7 molts before reaching adulthood (Barcay 2004). The adult female will mate ~5 d after emergence and, generally, one mating is sufficient to fertilize all the eggs she will produce (Ross and Mullins 1995). She will create her first ootheca 7 to 10 d after adult emergence. The adult female can live for five to 10 months and produce an average of five oothecae. Males have a shorter life span and can live for three to five months (Ross and Mullins 1995, Barcay 2004).

Brownbanded Cockroach

Distribution

Brownbanded cockroaches are believed to have originated in Africa (Atkinson et al. 1990). First discovered in Florida in 1903, it has since been found throughout the continental United States as well as tropical and subtropical regions of the world (Blatchley 1920, Cornwell 1968, Atkinson et al. 1990, Barcay 2004). Brownbanded cockroaches' distribution is attributed to its tendency to hide in and attach egg cases to furniture with subsequent movement of humans (Cornwell 1968, Pest Management 1989, Rust et al. 1995, Barcay 2004).

Habitat and ecology

Brownbanded cockroaches inhabit "homes, apartments, hotel, and hospital rooms" more than "stores, restaurants, and kitchens" (Pest Management 1989). They are only found outdoors in Africa (Cornwell 1968). Brownbanded cockroach water requirement is much lower than that of German cockroaches, and preferring warm, ≥27ºC, and dry areas. These factors allow them to infest diverse locations within a building such as bedrooms, shelves, spaces behind pictures, ceiling voids, light fixtures, electronics, and inside all types of furniture (Cornwell 1968, Pinto 1988, Barcay 2004, Capinera 2004).

Physical characteristic

The most distinguishing marks on the brownbanded cockroach are their lateral cream-colored strips, which are visible in both nymphs and adults. These strips transverse, or nearly transverse, dark brown regions on the apical portion of the wings on adults. On the nymphs, the bands run "across the posterior margin of the mesonotum" and "the first abdominal segment" (Cornwell 1968). Nymphs have amber colored abdomens. The pronotum, for both the adults and nymphs are dark brown with clear margins and cream-colored legs. There can be a great amount of color variation within the species (Blatchley 1920, Cornwell 1968); however, males are generally a lighter in color than the females. Males are also more slender than females and about 13-14.5 mm long. Their wings extend to cover the tip of the abdomen. The females are slightly smaller than the males in length, 10-12 mm, but have much broader abdomens. The wings on the female do not cover the abdomen, but leave the sides and tip exposed. The ootheca is ~5 mm in length and has a reddish-brown to yellowish tint (Cornwell 1968, Barcay 2004).

Life cycle

Females will carry the ootheca, which contains up to 18 embryos, for 24 to 36 hours, then will attach it an object in a protected location. Incubation requires an average of 70 d. Nymphs go through six to eight molts, which require an average of 160 d. Females mate three days after adult emergence and produce their first egg case about seven days later. She can produce up to 14 oothecae and live for three months. The males will mate about five days after adult emergence and live for four months (Cornwell 1968, Pest Management 1989, Barcay, 2004).

Peridomestic Cockroaches

Peridomestic cockroaches are found living and breeding outdoors around structures as well as, indoors (Darr et al. 1998). Outdoors, peridomestic cockroaches are found near structures, in sewers, under leaf detritus, stones, bark, in tree holes, palm trees, woodpiles, pine mulch, and vegetation (Suiter et al. 1992). They can move indoors in a variety of ways such as via cracks and crevices in the foundation of a structure. Additionally gaps around doors, windows, and plumbing are easily accessible to these cockroaches. It is also possible to transport them inside with laundry or food packaging. Once inside, conditions that help establish and maintain an infestation are access to food, water and harborage. Food may consist of foodstuffs thrown away in a trashcan to the glue used in books. Water can come from condensation on pipes, leaky pipes, or drip pans in refrigerators.

American Cockroach

Distribution

The American cockroach originated in Africa (Cornwell 1968, Roth 1982, Atkinson et al. 1990). Its spread from Africa was greatly aided by commerce, as this cockroach is the most common one found on ships (Blatchley 1920, Cornwell 1968, Barcay 2004). It "has been introduced to most countries of the world" and is widely distributed in tropical and subtropical regions (Blatchley 1920, Cornwell 1968, Bell 1984). According to Bell (1984), the American cockroach lives outdoors in tropical and subtropical regions and indoors in temperate regions.

Habitat and ecology

The American cockroach is most commonly associated with sewer systems but can be found under leaf debris, woodpiles, mulch, dumps, latrines, palm trees, sheds, and alleys (Cornwell 1968, Roth 1982, Barcay 2004). They can also occur in greenhouses where they will feast on young plants (Blatchley 1920). The American cockroach will move indoors. Once inside, it is associated with basements and areas where food is prepared or stored (Cornwell 1968, Barcay 2004). This can include restaurants, grocery stores, bakeries, as well as factories, hospitals, hotels and zoos (Cornwell 1968, Barcay 2004). According to Barcay (2004), they are active at temperatures of ≥21º C and temperatures below -6º C will kill them. However, it has been reported that active American cockroaches have been found in trash heaps that were covered with snow (Barcay 2004).

Physical characteristic

The most distinguishing feature of American cockroaches is their pronotum, which has a brown bilobed spot surrounded by pale yellow (Blatchley 1920). Their wings are a reddish-brown and in the male, the wings extend beyond the tip of the abdomen. On the female, the wings just reach the tip of the abdomen (Blatchley 1920, Cornwell 1968, Guthrie and Tindall 1968, Barcay 2004). The females, which average 34.7 mm in length, are slightly longer and broader than the males, which average 33.6 mm in length (Barcay 2004). According to Cornwell (1968), nymphs in the first to fifth instars are a uniform brown color. From the sixth instar on, pale patches appear on the pronotum.

Periplaneta spp. are similar in appearance, however; they can be distinguished from each other. The American cockroach is slightly larger, has thin cerci, and brownish-yellow legs while *P. australasiae* (Fabricius), *P. fuliginosa* (Serville), and *P. brunnea* Burmeister are all slightly smaller, have thicker cerci, and have dark brown legs. Additionally, *P. australasiae* has bright yellow markings surrounding a “sharply defined bilobed black spot” on the pronotum as well as bright yellow “basal margins” on the wings (Cornwell 1968). *Periplaneta fuliginosa* are uniformly dark brown on pronotum and wings. Finally, *P. brunnea*, which is the most similar in appearance to the American cockroach, can be distinguished by the dark brown coloration of the wings. Furthermore, *P. brunnea* has a pale yellow pronotum, like that of the American cockroach, however, the brown spots on the pronotum are less defined and touch the margins.

Life cycle

Female *P. americana* will carry the ootheca, which contains up to 16 embryos, for a few hours to several days (Cornwell 1968, Barcay 2004). The female will attempt to conceal oothecae, possibly by digging a hole in a substrate (soil, wood, or cardboard) and then covering the hole with debris which is held in place by her salvia (Roth 1982). Incubation requires an average of 44 d. Nymphal development takes ~600 d during which nymphs go through 10-13 molts (Cornwell 1968, Barcay 2004). The female will produce her first ootheca ~13 d after adult emergence and can live for three months up to two years. Adult male *P. americana* can live for three months up to about a year.

Oriental Cockroach

Distribution

Described by Blatchley (1920) as "the most noisome and disagreeable insects", the Oriental cockroach is believed to have originated in North Africa and spread by means of commerce (Cornwell 1968). According to Atkinson et al. (1990), the Oriental cockroach is found in 20 of the 48 contiguous states, but not in Florida or most of the rest of the Southeastern United States. They can be found in two South American countries, Chile and Argentina (Cornwell 1968), and in more temperate regions of the world.

Habitat and ecology

Oriental cockroaches are located in and around structures, prefer cooler temperatures, and are most active at 20 - 29° C (Cornwell 1968). Outdoors, they gravitate to moist areas in the shade such as under leaf debris, stones, and tree bark, as well as in lumber and trash piles (Cornwell 1968, Pest Management 1995). Oriental cockroaches are relatively cold tolerant and can survive at 2°C for more than 42 d (le Patourel 1993). The Oriental cockroach can move indoors by way of laundry, food packaging, and gaps around doors, windows, or pipes, and cracks in the foundation (Thoms and Robinson 1986, Pest Management 1995, Barcay 2004). Indoors, they prefer moist, damp, cool areas such as crawl spaces and basements (Blatchley 1920, Pest Management 1995, Barcay 2004). They are also found in warmer areas such as wall and porch voids (Thoms and Robinson 1986, Pest Management 1995) as well as around "radiators, ovens, and hot-water pipes" (Cornwell 1968). The Oriental cockroach can use garbage chutes, electrical conduits, and plumbing to move from lower areas of a structure to upper levels (Pest Management 1995).

Physical characteristic

Adult and nymph Oriental cockroaches are a very dark brown to black, giving rise to the common name of "black beetle" in England. Oriental cockroach males are 17-29 mm in length and have wings that extend and cover "¾ of their abdomen" (Guthrie and Tindall 1968, Pest Management 1995, Barcay 2004).The females average 32 mm in length and their wings are reduced to pads. The adult female can be distinguished from nymphs by the venations on her wing pads. The nymphs have "no distinguishing marks" (Pest Management 1995). The ootheca is ~10 mm in length and a dark reddish-brown (Cornwell 1968, Barcay 2004).

Life cycle

A field collected strain of Oriental cockroach maintained within the lab at 27° C and 45% relative humidity was observed (Short and Edwards 1991). According to this study, Oriental cockroach females carry their ootheca, which contains ~14 embryos, for 24 to 36 hours. She then either drops or glues the ootheca somewhere warm and near food (Pest Management 1995, Barcay 2004). Incubation requires ~45 d. Male nymphal development requires about six and a half months during which time they go through seven to nine molts. Female nymphal development requires an additional month and eight to 10 molts. The female will produce her first ootheca 12 d after adult emergence and she can live for up to three months. The males can live slightly longer, up to four months.

Most other sources indicate that the number of embryos per ootheca is 16 (Barcay 2004, Blatchley 1920, Pest Management 1995). However, Short and Edwards (1991) stated that while the Oriental cockroaches' biology had been well documented, there was "a surprising amount of contradiction in the literature about several aspects of the developmental and reproductive biology of this species". They ran their experiments at 27° C, which Cornwell (1968) reported was in the "preferred temperature" range of Oriental cockroach activity. Cornwell (1968) however, reported biology averages taken at temperatures of 30-36° C, well out of the "preferred temperature" range for Oriental cockroach activity, which seems to support Short and Edward's assertion.

Control Methods

> "For no other insects have so many quack remedies been urged and are so many newspaper remedies published … In fact, rather than put faith in half of those which have been published, it were better to rely on the recipe current among the Mexicans: …Catch three (cockroaches) and put them in a bottle and so carry them to where two roads cross. Here hold the bottle upside down, and as they fall out repeat aloud three *credos.* Then all the cockroaches in the house for which these three came will go away" (Blatchley 1920).

Cockroach pests need to be controlled because of the risks they pose to human health. Most insecticides used to control cockroaches disrupt insect nervous systems; others can interfere with the cuticle and even interrupt the process of molting into adulthood. Consideration of insecticidal mode of action is important when trying to control cockroaches.

Synthetic Insecticides

After World War II, synthetic insecticides were developed extensively to control insect pest, such as cockroaches. There are varieties of modes of action for the numerous synthetic insecticides.

Sodium channel

Sodium channels are voltage gated and when stimulated to open they allow the influx of sodium ions, which can cause excitatory reactions in the nervous system. Pyrethroids are synthetic versions of naturally occurring pyrethrin insecticides. Pyrethroids are some the most common insecticides and there are a large number of them including, allethrin, cyfluthrin, lambda-cyhalothrin, delthmethrin, and permethrin. They function as sodium channel agonists, binding to the channel and causing neuron-excitation, which results in rigid paralysis. There are two generations of pyrethroids. Type I pyrethroids do not contain an α-cyano group, they have a fast knockdown, and but little residual activity. Type II pyrethroids have an α-cyano group, a slower knockdown, and longer residual activity (Barcay et al. 2004, Yu 2007).

Oxidiazine, which includes the active ingredient indoxacarb, is a relatively new insecticide that functions at the sodium channel as an antagonist. It binds to the sodium channel, holding it closed, which results in flaccid paralysis. Indoxacarb is called a pro-insecticide because it is relatively inert until ingested. Once inside the insect, it is metabolized to its toxic from *N*-decarbomethoxyllated metabolite (DCJW) (Yu2007).

Dichloro-Diphenyl-Trichloroethane (DDT), an organochlorine, functions at the sodium channel as an agonist. It binds to the channel and causes neuron-excitation resulting in rigid paralysis. It was first synthesized in 1874; however, its insecticidal activity was not discovered until about 1940 (Cornwell 1976, Yu 2007). Both a dust and a spray formulation were used extensively during and after WWII, for control of everything from body lice to mosquitoes (Cornwell 1976). However, not all organochlorines act on the sodium channels.

Chloride channels

Chloride channels are ligand gated and located in the central nervous system (CNS) as well as the peripheral nervous system (PNS). When stimulated, they open to allow chloride ions to enter and cause neuron-inhibition. The chloride channels open when glutamate in the PNS or γ-aminobutyric acid (GABA) in the CNS binds to the postsynaptic neuron. Cyclodeines, such as aldrin and chlordane, act on the GABA gated chloride channels as antagonists. They bind to the channel and prevent chloride ions from entering, resulting in neuron-excitation and rigid paralysis. Phenylpyrazole, which includes the active ingredient fipronil, is also a chloride channel antagonist. It binds to the postsynaptic neuron preventing the influx of chloride ions. This causes an over stimulation of the nervous system and rigid paralysis. However, Avermectins, such as abamectin and emamectin benzoate, act as chloride channel agonists. They bind to the channel and prevent it from closing. This allows chloride ions to flow into the cell and results in neuron-inhibition and flaccid paralysis (Yu 2007). Avermectins are microbial lactones; however, not all insecticides in this class work on chloride channels.

Acetylcholine receptors

Acetylcholine (ACh), an excitatory neuro transmitter in the central nervous system, is released from the presynaptic neuron; it crosses the synaptic gap, and then binds to the postsynaptic neuron at the acetylcholine receptor (AChR). ACh is removed from the postsynaptic neuron by acetylcholinesterase (AChE). Spinosyn, another microbial lactone, function as an agonist at the nicotinic AChR. It binds to the receptor which results in neuro excitation and rigid paralysis. Nicotinoids, such as the active ingredients nicotine and imidacloprid, also function as an agonist at the nicotinic AChR.

Acetylcholinesterase inhibitors

Organophosphates (OP), such as dichlorvos and chlorpyrifos, and carbamates, such as aldicarb and bendiocarb, function by mimicking ACh and binding to AChE. This makes AChE unavailable to ACh and causes neuro-excitation and rigid paralysis (Barcay 2004, Yu 2007).

Insect growth regulators

Insect growth regulators, such as methoprene and noviflumuron, do not act on the nervous system but instead interfere with naturally occurring hormones within the insect. They can mimic juvenile hormone, ecdysone, or affect chitin synthesis, which can result in sterilization, malformations, and unsuccessful molting for the insect (Barcay 2004, Yu 2007). Chitin synthesis can also affect a nymph's ability to molt successfully. In adults, it can cause females to abort oothecae and shorten the life span of males (Barcay 2004).

Mitochondrial toxins

Other insecticides can interfere with an insect's ability to produce adenosine triphosphate (ATP), which is the main source of cellular energy (Campbell et al. 2003). Some insecticides can act at one of several points within the tricarboxylic acid (TCA) cycle. Other insecticides, such as hydramethylnon, can act at points on the electron transport chain, by inhibiting the flow of electrons down the chain, by inhibiting or uncoupling oxidative phosphorylation along the electron transport chain (Yu 2007).

Natural Insecticides

Natural insecticides are the oldest insecticides used for insect control. They have been used for well over a century (Reierson 1995). Prior to World War II, they were used extensively for cockroach control (Cornwell 1976). These were inorganic materials, such as boric acid, diatomaceous earth, arsenic, phosphorus and sodium fluoride, as well as organic materials such as pyrethrins made from crushed dried Chrysanthemum flowers (Cornwell 1976, Ebeling 1995, Barcay 2004). These materials were mixed with food to make baits or used as dusts. Inorganics can have long residuals and low resistance, but can also be slow acting (Bennett et al. 1988).

Both, boric acid and diatomaceous earth act as cuticle disrupters. They are abrasive and absorptive to the insect cuticle. This has the affect of causing the insect to desiccate due to water loss. Phosphorous is an acetylcholinesterase inhibitor while, arsenic and sodium fluoride effect energy production.

Use of natural insecticide dusts greatly decreased with the development of synthetic insecticide “sprays, fogs and aerosols” which were less labor intense and had a faster speed of action (Ebeling 1995). With the onset of resistance to synthetic insecticides, dusts regained some popularity.

Formulations

Insecticides are dispersed by a variety formulations and one insecticide (active ingredient) can be used in more than one type of formulations. Each type of formulation has both positive and negative qualities. Therefore, consideration should be taken when deciding which formulation to use in a given environment.

Wettable powders

Wettable powders (WP) consist of an active ingredient adhered to a diluent with a wetting agent which are then suspended in water (Yu 2007). It is sprayed onto surfaces or injected into harborages (Koehler et al. 1995, Barcay 2004). Wettable powders generally have good residual, especially on porous surfaces. However, they tend leave a visible residue on dark colored materials.

Emulsifiable concentrates (EC)

Emulsifiable concentrates (EC) consist of the active ingredient, a solvent, and a surfactant mixed with water. Like WPs, ECs can be sprayed onto surfaces or into harborages. Unlike WPs, they do not stain surfaces. In addition, they do not adhere well to non-porous surfaces like "stainless steel and ceramic tile" and are absorbed by porous surfaces. They can become unstable when applied on materials with high a pH (Barcay 2004), performing best on finished wood, vinyl tile and porcelain (Koehler et al. 1995).

Microencapuslation

Microencapsulates consist of an active ingredients surrounded, or encapsulated, by plastic. The active ingredient is released over time. These insecticides generally have low odor and a long residual activity. While mortality can be observed within a few hours, it can require a couple of days for initial mortality. Since release is slow and over time, cockroaches may be exposed to sub-lethal doses, thereby decreasing the time required for insecticide resistance to develop (Koehler et al. 1995, Barcay 2004).

Dusts

One of the oldest formulations for delivering insecticide is dust. A dust generally consists of the active ingredient and a diluent. Dusts are most often used in cracks and crevices (Yu 2007). They have a low odor and a long residual. They can also be used around electrical outlets and equipment (Koehler et al. 1995, Barcay 2004). Dust also have slow knockdown, they can drift, and are "considered the most difficult of the cockroach control formulations to apply correctly" (Koehler et al. 1995).

Baits

One of the more important formulations for cockroach control is baits. Baits have been used for more than a century and Blatchley (1920) described a bait made from phosphorous paste, which contained "sweetened flour paste" and 1 - 2% phosphorus, which was to be spread on "paper or cardboard and placed in the runways of the roaches". However, it was not until the early 1980s that cockroach baits (gel baits) were seriously considered for cockroach control. Gel baits consist of an active ingredient, a feeding stimulant, and a carrier (Yu 2007). Gel baits can be used in sensitive areas where other formulations are prohibited while reducing the amount of insecticide placed in the environment. Proper gel bait placement is important, since gel baits must often compete with other food sources. Additionally, use of other insecticides, especially repellants, on or around gel baits can affect control.

Resistance

Resistance is "the development and heritable ability of insects to tolerate doses of toxicants that would prove lethal to the majority of individuals in a normal population of the same species" (Braness 2004) and which results in control failures. The first documented case of insecticide resistance was from Melander (1914). In 1908, small populations of scale insects were still alive after sulfur-lime application. In 1910, about a 10% survival rate in the scale population was observed, this was followed by a 50% survival rate in 1912. This classic example shows how high selective pressure and natural genetic variation within a population can lead to resistance. Resistance can develop with any level of selection pressure. Selection pressure can include a variety of mechanism that can increase resistance within a population (Hoy 1999):

- The repeated use of one insecticide, class of insecticides or insecticides with the same mode of action for a prolonged period.
- The treatment of a large geographical area with one insecticide
- Not leaving refugia of susceptible insects.

- Low migration.
- Treating all life stages with same insecticide.
- Multivoltine with overlapping generations
- Use of insecticides with long residuals.

Not surprisingly, German cockroaches are subjected to most, if not all, of these selection pressures. Resistance "mitigation" may be possible; however, with increased selection pressure, resistance is generally considered inevitable (Hoy 1999). There are three categories of resistance: biochemical, physiological, and behavioral (Georghiou 1972). For our purpose, only biochemical and behavioral resistance will be discussed. Biochemical resistance includes the detoxification of insecticides, enzyme activation, and decreased "sensitivity of target enzymes" Georghiou (1972). While, behavioral resistance is the ability to decrease the duration of contract with toxicant and an alteration of host or habitat preferences.

Biochemical Resistance

The selective pressure placed on insects can result in biochemical resistance. Cytochrome P450 is one mechanism that increases insecticide detoxification. Some insecticide resistant strains of German cockroaches have cytochrome P450 levels that were 2.5 and 4.5 fold higher than the cytochrome P450 level of a normal strain of German cockroaches (Valles and Yu 1996, Scharf et al. 1998). In addition to insecticide detoxification, biochemical resistance can occur with target site insensitivity. Insensitivity of the sodium channel was observed in an insecticide resistant stain of German cockroaches. Some of the pyrethroid resistance observed, as well as DDT cross-resistance, was attributed to target site insensitivity (Umeda et al. 1988).

Behavioral Resistance

Behavioral resistance in insects is an action that allows a population to avoid contact with toxic compounds (Lockwood et al. 1984, Sparks et al. 1989) and is a result of "hypersensitivity" or "hyperirritability" (Yu 2007). One example of behavioral resistance has been noted in the horn fly, *Haematobia irritans* (L.) (Byford et al. 1987, Sparks et al. 1989). Resistance strains of the horn fly moved from face, shoulders and back to the bellies of cattle with pyrethroids-impregnated ear tags. In another example, some strains of German cockroach refused to consume gel baits with certain sugars.

Glucose aversion

In 1993, Silverman and Bieman described a strain of German cockroaches that refused to consume gel baits. They found that there was not repellency to the insecticide but rather to a sugar contained in the bait matrix. Several strains had developed a behavioral resistance (aversion) to the consumption of glucose (Silverman and Ross 1994), a sugar that was previously considered a phagostimulant for a number of insects (Bernays 1985). It was determined that one gene was controlling the aversion and it was "an autosomal incompletely dominant trait", not linked to sex and the cockroaches that carried just one allele for the gene expressed the aversion (Silverman and Bieman 1993, Ross and Silverman 1995). Silverman (1995) found that glucose avoidance had beneficial effects. When fed food containing no glucose, all three strains (a homozygous normal strain, a homozygous bait averse strain and a heterozygous mix of the bait averse and normal strains) were observed to have increases in the number of embryos per ootheca. Additionally, nymphs consumed more food, gained more weight, took less time to mature, and survived to adulthood in greater numbers compared to those fed an 18% glucose diet. It was not clear, whether the different populations of German cockroaches developed the aversion independently of each other or if the aversion developed in a few strains that were transported to other regions. However, the aversion issue was solved by replacing glucose in the gel baits with another sugar, such as fructose.

Multiple sugar aversion

By 1999, new cases of German cockroach gel bait aversion were appearing in isolated locations within Florida, Texas, and New York (Morrison et al. 2004). The common thread was poor sanitation along with a heavy reliance on gel baits for control. By 2006, more than 50% of the pest control operators' accounts were having aversion issues (Koehler 2006). Considering the previous case of bait aversion, an attempt was made to alter the sugar in the bait matrix to resolve the problem, but this was to no avail (Barile 2003). Wang et al. (2004) found the sugar aversion problem varied from one strain of German cockroaches to another. They tested three strains of German cockroaches (one normal lab reared strain and two field collected strains) on six sugars (D-fructose, D-glucose, D-maltose, D-sucrose, D-lactose, D-galactose) to determine if the sugars were feeding stimulants or deterrents. They discovered that four of the six sugars (D-fructose, D-glucose, D-maltose, D-sucrose) were feeding stimulants to the normal lab reared strain. Of the two field collected strains, all of the sugars were feeding deterrents in the strain which had been was collected from apartments where the only method of control, for the five years prior to collection, was gel baits. The other field collected strain, which had been subjected to "pyrethroid sprays, gel baits, bait stations and boric acid dusts by residents, contractors and researchers" for the five years prior to collection, two of the sugars (D-maltose, D-sucrose) were stimulants, 1 (D-fructose) was neither stimulant nor deterrent and the other three were deterrents. This suggests that, much like physiological resistance to insecticides, the cockroaches with the greatest selection pressure exhibit a greater aversion to gel baits.

The newest gel bait aversion, unlike the glucose aversion, appears to be partially sex linked, with female averse individuals passing on strong resistance genes than their male counterparts, as well as being incompletely dominant (Wang et al. 2006).

Avermectins

While avermectins were touched on earlier, here the mode of action is explored more in depth. Additionally, the development of avermectins, especially emamectin benzoate is discussed.

Mode of Action

All avermectins have the same mode of action (Campbell et al. 1983, Dybas et al. 1989). Originally, avermectins were thought to work on the central nervous system; affecting chloride channels by stimulating the release of the neurotransmitter GABA from the presynaptic neuron and enhancing its binding to the postsynaptic membrane. This enhanced binding does not increase the duration that GABA is bound to the site, but rather increases the number of sites available to GABA (Pong et al. 1980, Pong and Wang 1982, Turner and Schaeffer 1989). GABA is a neural inhibitor and its binding causes an influx of chloride ions into the neuron. This influx acts to dampen neural excitation.

The primary mode of action for avermectins is as an agonist in the peripheral nervous system (Jansson and Dybas 1998, Buckingham 2005). At neuromuscular junctions, avermectin binds to the postsynaptic membrane, which again allows for the influx of chloride ions. These dampening effects in the central and peripheral nervous systems result in neuron-inhibition and flaccid paralysis (Campbell et al. 1983, Zufall et al. 1988, Turner and Schaeffer 1989, Yu 2007).

Development

Avermectins are a class of “16 membered macrocyclic lactone ring” insecticides that affect chloride channels. Their initial development came about when scientists at Merck Sharp & Dohme Research Laboratories were looking for microbial fermentation products with anthelmintic activity. An actinomycete (*Streptomyces avermitilis*), which was originally isolated at the Kitasato Institute in Japan from a soil sample, produced two series of compounds, A and B (Campbell et al. 1983). Ultimately, "four homologous pairs" (eight compounds) which could be subdivided into major (A_{1a}, A_{2a}, B_{1a}, B_{2a}) and minor (A_{1b}, A_{2b}, B_{1b}, B_{2b}) groups were developed (Burg et al. 1979, Campbell et al. 1983, Fisher and Mrozik 1989). Avermectins, generally, consist of a mixture of a major and a minor group (≥80% to ≤20% respectively), due to the prohibitive cost of isolating individual groups and because the two groups have similar biological activities (Dybas et al. 1989, Fisher and Mrozik 1989, Jansson and Dybas 1998). From these compounds, ivermectin was pure and "marketed as an antiparasitic" in 1981. In 1985, abamectin became available to control agricultural pests (Campbell 1989). By the mid 1990's abamectin was available for cockroach control (Appel and Benson 1995). Further experimentation in 1984 resulted in the discovery of emamectin benzoate.

Emamectin Benzoate

Emamectin benzoate was first registered in 1999 for use in field crops to control lepidopteran pests (Leibee et al. 1995, Jansson et al. 1996, Jansson et al. 1997, Ishaaya et al. 2002). As a soluble granule, it is sprayed on crops where, initially, it has some contact activity (Chukwudebe et al. 1997). However, emamectin benzoate's translaminar, non-systemic activity allows it to form "a reservoir within treated leaves". It is in this state that it is most effective and emamectin is reported to be "several hundred fold" better at controlling lepidopteran crop pests than abamectin (Fisher 1993). Insect pests consume not only plant material but also the emamectin benzoate, which results in paralysis and death in 3-4 d (Syngenta 2007). Emamectin benzoate has use in aquaculture to control sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) which are parasites of salmon and trout in fish farms (Stone et al. 1999). In this application, the fish are fed the emamectin benzoate in a feed mixture such as Slice™. The sea lice acquire emamectin benzoate when parasitizing the fish. Once in the sea lice it "binds to ion channels of nerve cells and disrupts transmission of nerve impulses" (Schering-Plough 2007).

Research testing emamectin benzoate in pine trees has also been conducted. A liquid formulation was developed for injection into pine trees to control the pine wood nematode, *Bursaphelenchus xylophilus*, and found to have a residual effect for at least 3 years (Takai et al. 2000, 2001, Takai et al. 2003, 2004). Additional experiments testing emamectin benzoate for control of the southern pine engraver beetle and wood borers in Loblolly pines has also been performed (Grosman and Upton 2006). In these experiments, pine trees were injected with emamectin benzoate. Trees were observed for up to 5 months for the presence of egg galleries and attacks by beetles. They found that emamectin benzoate controlled both egg galleries and attacks, but caused vertical lesions at each injection point.

Whether it is biochemical or behavioral, resistance is the reason new insecticides and improved formulations, especially gel baits, need to be developed. Gel baits are heavily relied upon in the pest control industry for their low exposure of humans to insecticides, the ability to target cockroaches where they live and their ability to be used in sensitive areas. Therefore, in this study, I tested at an experimental gel bait matrix with the avermectin insecticide, emamectin benzoate, which has not been used for cockroach control.

CHAPTER 3
EMAMECTIN BENZOATE ON ORLANDO AND DAYTONA STRAINS OF GERMAN COCKROACH

Introduction

Insects are highly adaptable creatures, able to survive everything from arid deserts to frozen mountaintops. Consequently, some of them are able to survive the various insecticides used to control them. The German cockroach, *Blattella germanica* L., is no exception. According to Whalon et al. (2007), the German cockroach is resistant to 42 active ingredients. Some factors that aid in development of insecticide resistance are high reproductive rate, low migration, and high selection pressure, which is repeated exposure to the same insecticide or class of insecticide. Not surprisingly, the German cockroach benefits from all of those factors. It takes as little as two years (6-8 generations) for German cockroaches to develop resistance under high selection pressure (Cochran 1995). Using laboratory selection strategy, Scharf et al. (1998) identified a high-level resistance evolution in three generations. There are three major categories of resistance (Georghiou 1972): physiological, biochemical and behavioral.

Physiological and biochemical resistance, to an insecticide, occur when an insect is able to survive a level of exposure that is normally lethal and which results in control failures. Because of genetic variability, all populations have some level of resistance; it is believed that the levels are low. However, those levels can increase quickly when selection pressure is high. Mechanisms of resistance include decreased penetration through the cuticle, target-site insensitivity, and increased enzyme production, in which detoxification enzymes detoxify the insecticide thus making it less toxic and easier to excrete from the body (Cochran 1995). One example of this sort of resistance in German cockroaches is pyrethroid resistance in which strains can exhibit increases in enzyme activity and/or target site insensitivity (Umeda et al. 1988, Scharf et al. 1998).

Behavioral resistance occurs when an insect is able to avoid an insecticide due to an increased ability to detect the insecticide or a change in preference. As with physiological and behavioral resistance, behavioral resistance can also result in substantial control failures. This type of resistance occurred in some strains of the German cockroach in the early 1990's. German cockroaches developed an aversion, or change in preference, to consuming gel baits with glucose (Silverman and Bieman 1993). Gel bait manufactures were able to overcome this resistance by substituting glucose with other sugars. However, less than a decade, later bait aversion reappeared in a few strains of German cockroaches that were controlled, almost exclusively, with gel baits (Morrison et al. 2004, Kramer and Miller 2004). These new strains of bait averse German cockroaches had developed an aversion to a numerous sugars and possibly other inert bait matrix ingredients (Wang et al. 2004).

Due to the resistance of German cockroaches, it is important to develop new products to combat them. Emamectin benzoate has been in use against lepidopteran crop pests, and sea lice on salmon. Emamectin benzoate is a novel insecticide for cockroach control. It is in the same class of insecticides, avermectins, as abamectin; however, there is some evidence that emamectin benzoate may have inherently better insecticidal properties than abamectin. Emamectin is "several hundred fold" better at controlling lepidopteran pests than abamectin (Fisher 1993). However, further research will be required to determine if emamectin benzoate is more toxic to cockroaches than abamectin.

My objective for this study was to determine if cockroach gel baits containing emamectin benzoate would be palatable to both a susceptible and bait averse strain of German cockroaches. I also wanted to determine if both, the susceptible and bait averse strains were susceptible to gel baits containing emamectin benzoate.

Materials and Methods

Insecticides

Four experimental formulated emamectin benzoate gel baits, a blank gel bait base and a standard gel bait were tested. The experimental emamectin benzoate gel baits were formulated as either emamectin A or emamectin B at either 500 or 1000 ppm (Syngenta Crop Protection, Greensboro, NC). The blank gel bait base contained no active ingredient. The current industry standard gel bait utilized in this study was Maxforce FC Select with 0.01% (100 ppm) of the active ingredient fipronil (Bayer Environmental Science, Montvale, NJ).

Insects

Orlando (non-bait averse) and Daytona (bait averse) strains of German cockroaches were reared at the University of Florida, urban entomology laboratory (Gainesville, FL) in glass utility jars (25.5 high x 22.0 diameter cm) with the inner top 5 cm greased with a petroleum jelly/mineral oil mixture (2:3) to prevent cockroach escape. Each jar contained cardboard for harborage and was provided water and dry food *ad libitum*. The Orlando strain was fed rodent food (Labdiet 5001, PMI Nutrition Int., Brentwood, MO) and the Daytona strain was fed dog food (Purina One® puppy: growth and development, Nestlé PetCare Company, St. Louis, MO.) due to aversion to rodent food. The cockroaches were maintained at 23.6 ± 2.5°C at 51 ± 16% RH at a photoperiod of 12:12 (L:D). For testing, cockroach nymphs were separated from colonies by anaesthetization with carbon dioxide for less than 5 min, and then sifted using #8 (2.36 mm) and #10 (2.00 mm) testing sieves. Second and third instar nymphs, passing through the #8 sieve and retained in the #10 sieve, were placed in jars with rodent diet or dog food, water, and harborage. All nymphs were allowed to recover for 48 h to recover from anaesthetization prior to utilization in tests. Cockroaches not used in this study were returned to colonies.

Assay Setup

The test arenas were lidded transparent plastic boxes (27 x 19.5 x 9.5 height cm) with the inner top 5 cm greased with a petroleum jelly/mineral oil mixture (2:3) to prevent cockroach nymph escape. The arena contained harborage [a blank white index card (7.6 x 12.7 cm) folded lengthwise and stapled] and a water vial with a cotton stopper. Cockroach nymphs (50) were aspirated from the holding jar, collected in a 50 ml tube, and placed into a test arena. After placement into arenas, cockroach nymphs were starved for 24 h, at which time dead cockroach nymphs and exuviae were removed.

Assay Method

Cockroach nymphs were provided a choice between pre-weighed dog food (~0.28 g) or gel bait (~0.21 g) which were placed on pieces of paper (3.8 x 3.8 cm, Fisherbrand weighing paper). In control assays, dog food replaced gel baits and, therefore, received dog food only. Similar amounts of dog food or gel bait were placed into 30 ml cups, which were then covered with an organdy fabric square and held in place with a rubber band to prevent cockroach access. These moisture controls were used to adjust for loss/gain in consumption calculations. The four portions, two dog foods and two gel baits, were placed in test arenas simultaneously. After 24 h, the four portions were reweighed. The dog food and gel bait were placed back into the arena for the remainder of the study. Cockroach nymph mortality was recorded 6 d after the introduction of food. Mortality was defined as cockroach nymph inability to self-right.

Data Analysis

Consumption was calculated as follows:

$$\text{Consumption} = B_B - \{B_B \times [(MC_B - MC_A) / MC_B]\} - B_A$$

where B_B is the pre-weight of dog food/gel bait before introduction into the arena, MC_B is the pre-weight of moisture control food/gel bait, MC_A is the post-weight of moisture control food/gel bait 24 h after introduction into the arena, and B_A it the post-weight of exposed food/gel bait (Ncherne 2006). Each portion was weighed individually before introduction into the testing arena and 24 h later. Percent gel bait consumption was calculated as follows:

$$\text{Percent consumption} = [GB / (GB + DF)] \times 100$$

where GB is gel bait consumed (mg) and DF is dog food consumed (mg) (Ross 1998). Consumption and mortality data were arcsine square root transformed before being analyzed by analysis of variance (ANOVA) with means separated by the Student Newman Keuls (SNK) test or the Student's *t*-test ($\alpha = 0.05$; SAS Institute 2003).

Results

Consumption

Soon after introduction of the gel baits and/or dog food to the test arenas, cockroaches were observed consuming gel baits and dog food. Between strains, there was no significant difference in percent consumption for dog food or any of the gel baits (bait base, Maxforce FC Select, emamectin A, and emamectin B). Between strains and within each strain, there was no significant difference in percent consumption of emamectin A and emamectin B gel baits.

For the Orlando strain, there was no significant difference in percent consumption of any of the gel bait or dog food ($F = 1.56$, df = 6, $P = 0.1739$) (Table 3-1). For the Daytona strain, the dog food had the lowest percent consumption and was significantly difference from Maxforce FC Select and bait base ($F = 3.74$, df = 6, $P = 0.0030$). However, there no significant difference in percent consumption between any of the gel baits (emamectin A, emamectin B, Maxforce FC Select, or bait base).

Mortality

After the initial introduction of gel baits and dog food, mortality was observed in as little as 24 h. Between strains, Orlando strain had significantly higher percent mortality than Daytona strain for dog food and bait base treatments (Table 3-2). There was no significant difference in percent mortality for emamectin A, emamectin B or Maxforce FC Select between strains. Additionally, between strains and within each strain, there was no significant difference in percent mortality of emamectin A and emamectin B gel baits.

Within strains, for both Orlando and Daytona, there was no significant difference in percent mortality for dog food and bait base (F = 234.96, df = 6, P <0.0001 and F = 593.31, df = 6, P <0.0001, respectively). Maxforce FC Select was significantly different from emamectin A, emamectin B, bait base, and dog food, which had lower percent mortalities. The bait base and dog food had the lowest percent mortality and were significantly different from Maxforce FC Select, emamectin A and emamectin B. There was no significant difference in percent mortality between emamectin A and emamectin B.

For both the Orlando and Daytona strains (Fig. 3-1 and 3-2 respectively), emamectin A and B 1000 ppm had slightly faster speed of action than the emamectin A and B 500 ppm, at 3 d. However, for emamectin A and B at 500 and 1000 ppm, there were no significant differences at 6 d.

Discussion

German cockroaches are highly adaptable creatures, and for this reason, new products to control them need to be developed and tested. Previous studies have confirmed the existence of bait averse German cockroaches (Wang et al. 2004, 2006, Ncherne 2006). In those studies bait averse and normal strains were tested. Wang et al. (2006) tested second generation blank gel baits (Avert with abamectin and Maxforce FC with fipronil) and Ncherne (2006) tested first, second generation (Maxforce FC and Avert) and third generation cockroach gel baits (Maxforce FC Select). Second generation cockroach gel baits were developed in response to glucose aversion and third generation cockroach gel baits were developed in response to the more recent bait aversion. All of these studies found that bait averse strains ate significantly less first and second generation gel baits than the normal susceptible strains. In my study, the Daytona strain consumed a larger percentage of gel bait than the Orlando strain. This was similar to Ncherne's (2006) study with third generation gel baits, in which the Daytona strain consumed greater amounts than the Orlando strain.

Using abamectin gel baits, Cochran (1994) tested 13 strains of fifth and sixth instar German cockroaches and observed a wide range of susceptibility, 31.1% to 97.8% mortality. Also using abamectin gel baits, Negus and Ross (1997) compared six strains of sixth instar German cockroaches and found the susceptibility of two of the six strains to be significantly different from each other. In my study, percent mortality across strains was similar for each of the formulated emamectin benzoate gel baits and Maxforce FC Select at 6 d. The length of the test in Negus and Ross's experiment, which only ran for 3 d, may not have been of an adequate duration. Ross (1993) observed that large nymphs did not reach 80% mortality until after 5 d, when fed abamectin.

Fipronil kills a greater percentage of German cockroaches compared to abamectin (Durier and Rivault 2000, Wang et al. 2004, 2006, Ncherne 2006). The toxic effect of fipronil occurs faster than the effect of abamectin (Gahlhoff et al. 1999, Durier and Rivault 2000, Stejskal et al. 2004). I found similar results in this study. Maxforce FC Select had the greatest percent mortality for both strains at 6 d. However, 100% mortality was observed at 13 d for mid-instar nymphs when given a choice between dog food and abamectin gel bait (Ross 1993). This suggests that while emamectin benzoate could have a slower speed of action compared to fipronil, total mortality may be comparable if given adequate time. Fipronil's faster speed of action may also affect its horizontal kill. In my experiment, fipronil arenas were observed to be relatively free of feces compared to all other arenas. The eating of feces, especially by early instar nymphs, is one type of horizontal kill. "Feces … appeared to play a minor role in the transfer of fipronil" for German cockroaches (Buczkowski and Schal 2001). This is could be due to its fast speed of action, about 4 h (Durier and Rivault 2000), which may not give the German cockroach time to defecate prior to mortality. This possibility, as well as other modes of horizontal transfer and mortality should be further investigated.

As was expected, emamectin A and B at 1000 ppm, for both the Orlando and Daytona strains, had slightly higher morality at 3 d, compared to emamectin A and B at 500 ppm. I would expect a gel bait with a higher concentration of insecticide to work slightly faster than one with a lower concentration. However, there was no significant difference between any of the emamectin gel baits, A and B at 500 and 1000 ppm, at 14 d.

The Orlando strain showed greater susceptibility than the Daytona strain, even though the Daytona strain had higher consumption rates. This could indicate some physiological and /or biochemical resistance to emamectin benzoate in the Daytona strain. However, Orlando also had higher percent morality for the control and bait base, so the higher mortality percentage may be due to natural mortality rather than to resistance.

In conclusion, my study has shown that novel bait base, emamectin A and emamectin B were able to over come putative feeding deterrence in a bait averse strain of German cockroach, and were, essentially, equally palatable to both normal and bait averse strains. This study also showed that when German cockroaches consumed the gel baits with emamectin benzoate, a high level of mortality was obtained at 6 d. However, emamectin benzoate has a slower speed of action than fipronil, which probably effects fipronil's horizontal transmission. A longer study, of at least 13 d, is needed to compare accurately the mortality between fipronil and emamectin gel baits.

Table 3-1. Gel bait preference by *Blattella germanica* nymphs (Orlando and Daytona strains) in a 24 h choice experiment.

	Bait as percent of total consumption (Mean ± sem)[a]				
	Strain		Student's *t*-test (P = 0.05)		
Treatment	Orlando	Daytona	df	*t*-value	*P*-value
Dog Food	50.9 ± 6.42a	51.2 ± 4.09b	18	0.04	0.9694
Bait Base	67.7 ± 3.44a	75.3 ± 2.06a	18	1.90	0.0742
Maxforce FC Select	68.3 ± 4.73a	74.0 ± 3.14a	18	0.99	0.3370
Emamectin A 1000 ppm	59.6 ± 4.03a	62.3 ± 3.37ab	18	0.51	0.6182
Emamectin A 500 ppm	60.5 ± 3.32a	64.6 ± 4.12ab	18	0.78	0.4468
Emamectin B 1000 ppm	61.6 ± 5.90a	63.2 ± 5.55ab	18	0.20	0.8457
Emamectin B 500 ppm	61.9 ± 3.77a	63.4 ± 5.63ab	18	0.23	0.8195

[a]Consumed percentage was obtained by the following formula:
{gel bait consumed (mg) / [gel bait consumed (mg) + dog food consumed (mg)]} x 100.
Means within a column followed by the same letter are not significantly different (P = 0.05; Student Newman Keuls [SAS Institute, 2003]).

Table 3-2. Mortality at 6 d after bait placement of *Blattella germanica* nymphs (Orlando and Daytona strains).

	Percent Mortality				
	Strain		Student's *t*-test (P = 0.05)		
Treatment	Orlando	Daytona	df	*t*-value	*P*-value
Dog Food	4.7 ± 0.95c	0.4 ± 0.44c	11.4	-4.06	0.0018
Bait Base	7.4 ± 2.16c	1.1 ± 0.48c	8.8	-2.85	0.0116
Maxforce FC Select	99.8 ± 0.22a	99.1 ± 0.49a	11.1	-1.26	0.2342
Emamectin A 1000 ppm	90.3 ± 1.70b	86.8 ± 1.30b	14.9	-1.67	0.1163
Emamectin A 500 ppm	86.4 ± 5.39b	81.6 ± 2.46b	11.2	-0.81	0.4322
Emamectin B 1000 ppm	88.5 ± 2.34b	88.0 ± 2.98b	16	-0.12	0.9048
Emamectin B 500 ppm	87.4 ± 2.87b	86.5 ± 2.14b	14.8	-0.26	0.7990

Means within a column followed by the same letter are not significantly different (P = 0.05; Student Newman Keuls [SAS Institute, 2003]).

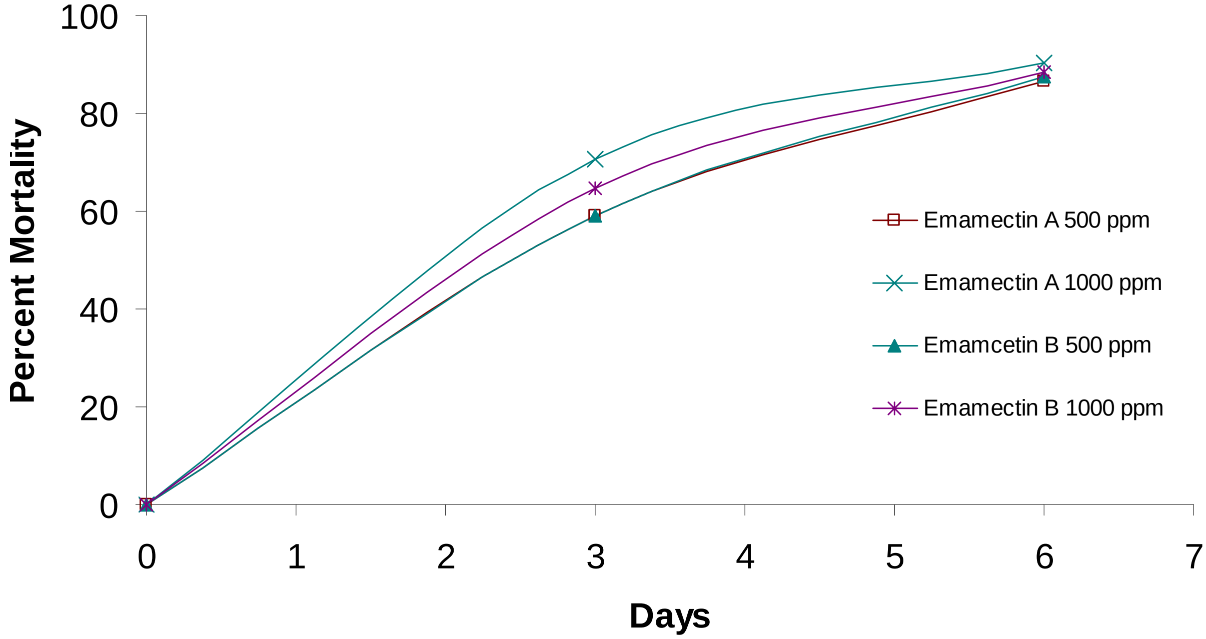

Figure 3-1. Percent mortality at 3 and 6 d after bait placement for the Orlando strain of *Blattella germanica* nymphs.

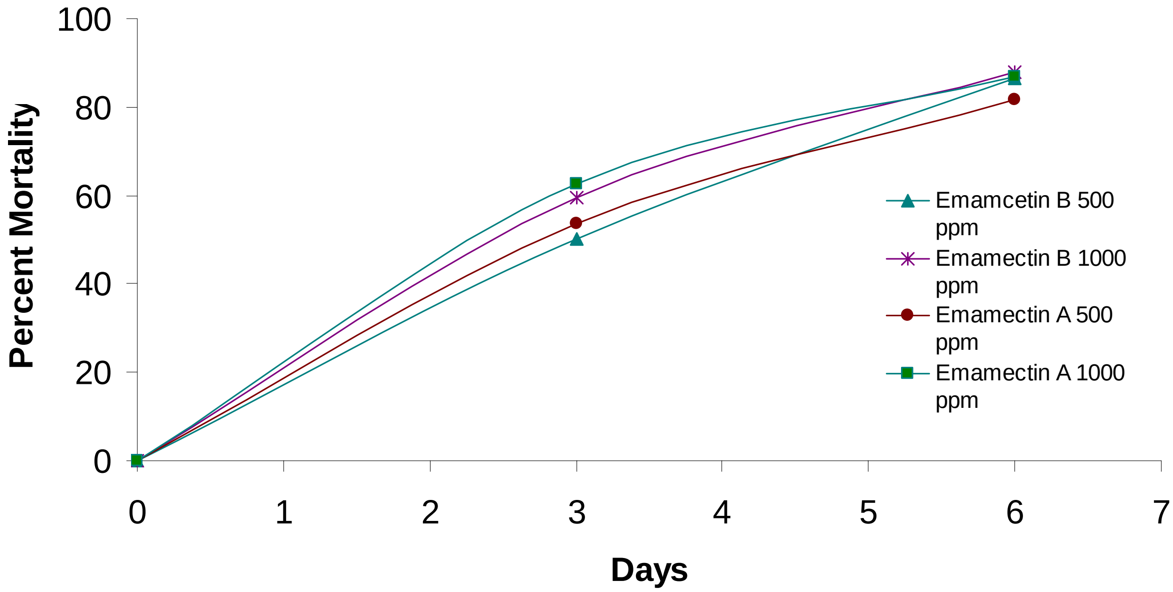

Figure 3-2. Percent Mortality at 3 and 6 d after bait placement for the Daytona strain of *Blattella germanica* nymphs.

CHAPTER 4
EMAMECTIN BENZOATE ON DOMESTIC COCKROACHES

Introduction

Domestic cockroaches, which live and breed almost exclusively indoors, like brownbanded cockroaches, *Supella longipalpa* (Serville) and German cockroaches, *Blattella germanica* L., can be found throughout the United States. These domestic cockroaches have a close association with humans, leading some to believe that there may be co-evolution between domestic cockroaches and humans (Barcay 2004). Because of how closely associated they are with humans, they are considered pests for aesthetic and health reasons. Aside from producing foul smelling odors, they are capable of producing allergens, which can elicit asthma attacks (Rosenstreich et al.1997). They have been implicated as disease vectors, being carriers of a number of bacterial, viral, and fungal pathogens, including salmonella, hepatitis, and e-coli (Roth and Willis 1957, Le Guyader et al. 1989). For these reasons, control of cockroaches is essential.

When controlling cockroaches, harborage location is important. While it is possible to find both the German cockroach and the brownbanded cockroach in the same structure and even the same harborage (Barcay 2004), they tend to inhabit very different areas. The German cockroach is commonly associated with kitchens and bathrooms, where their preference for warm, humid areas with low airflow, and daily access to water can be satisfied. Brownbanded cockroaches also prefer warm areas, but they do not require daily access to water. For this reason, they are able to survive in drier areas of structures. Brownbanded cockroaches are also called the furniture cockroach in some parts of the world, because they have a tendency to reside in furniture of all types. They can also be found under shelves, behind pictures and inside electronics (Pinto 1988).

Once infestations are established, control measures need to be taken. However, resistance can complicate cockroach control. While brownbanded cockroaches are susceptible to a variety of insecticides (Burden 1980, Koehler et al. 1991, Pospischil et al. 1999) and is not known to be resistant to any insecticides (English 2003), development of resistance is always a possibility. German cockroaches are resistant to a large number of insecticides (Whalon et al. 2007) and some strains have even developed an aversion to gel baits (Silverman and Bieman 1993, Silverman and Ross 1994, Wang et al. 2006, Ncherne 2006). Cockroach gel bait aversion is especially troubling as gel bait use has "become a major pest control technique" (Stejska and Aulicky 2006). Gel baits have the benefit of decreasing the amount of insecticide placed in the environment and reducing human and pet exposure to insecticides. It can also be used in sensitive areas, such as hospitals and restaurants.

Because insecticide resistance is generally considered inevitable, it is important to develop and test new insecticides for cockroach control. Emamectin benzoate is currently used to control sea lice on salmon (Stone et al. 1999) as well as to control lepidopteran pests in field crops. Emamectin benzoate is a novel insecticide that is being developed for control of cockroaches. It is in the same class of insecticides, avermectins, as abamectin. However, there is some evidence that emamectin benzoate may have inherently better insecticidal properties than abamectin (Dybas et al. 1989). Emamectin is reported to be "several hundred fold" better at controlling lepidopteran pests than abamectin (Fisher 1993). However, further research will be required to determine if emamectin benzoate is more toxic to cockroaches than abamectin.

The objective of my study was to determine if cockroach gel baits containing emamectin benzoate would be palatable to the brownbanded cockroach as well as to the bait averse Daytona strain of German cockroach nymphs. Additionally, I wanted to determine if gel baits formulated with emamectin benzoate were capable of controlling both species cockroaches.

Materials and Methods

Insecticides

Five experimental formulated emamectin benzoate gel baits, and one standard gel bait were tested. The experimental emamectin benzoate gel baits were formulated as either emamectin A at a concentration of 0.1% or emamectin B at a concentration of 0.05%, 0.1%, or 0.2%. One of the formulated gel baits contained no active ingredient (Syngenta Crop Protection, Greensboro, NC). The current industry standard gel bait utilized in this study was Maxforce FC Select® with 0.01% of the active ingredient fipronil (Bayer Environmental Science, Montvale, NJ).

Insects

Daytona strain (bait averse) German cockroaches (Ncherne 2006) and brownbanded cockroaches were reared at the University of Florida, urban entomology laboratory (Gainesville, FL) in glass utility jars (25.5 high x 22.0 cm diameter) with the inner top 5 cm greased with a petroleum jelly/mineral oil mixture (2:3) to prevent escape. Each jar contained cardboard for harborage, and was provided water and dry food *ad libitum*. Brownbanded cockroaches were fed rodent food (Labdiet 5001, PMI Nutrition Int., Brentwood, MO) and German cockroaches were fed dog food (Purina One® puppy: growth and development, Nestlé PetCare Company, St. Louis, MO.) due to aversion to rodent food. The cockroaches were maintained at 23.6 ± 2.5°C at 51 ± 16% RH at a photoperiod of 12:12 (L:D). For testing, German cockroach nymphs were separated from the colony by anaesthetization for less than 5 min with carbon dioxide and then sifted, using #8 (2.36 mm) and #10 (2.00 mm) testing sieves. Second and third instar German cockroach nymphs, passing through the #8 sieve and retained in the #10 sieve, were placed in jars with rodent food, water, and harborage for 48 h to recover from anaesthetization prior to test. All other cockroaches were returned to the colony. Mixed populations of brownbanded cockroaches (10 adult males, 10 adult females, and 30 nymphs, third to fifth instars) were removed from the colony with feather tip forceps.

Assay Setup

The test arenas were lidded transparent plastic boxes (27 x 19.5 x 9.5 cm height), with the inner top 5 cm greased to prevent cockroach escape. The arena contained harborage [a blank white index card (7.6 x 12.7 cm) folded lengthwise and stapled] and a water vial with a cotton stopper. Fifty German cockroach nymphs or 50 brownbanded cockroaches were placed into test arenas and starved for 24 h. Dead cockroaches and exuviae were removed prior to introduction of dog food and gel baits.

Assay Method

German cockroach nymphs and brownbanded cockroaches were provided a choice between pre-weighed dog food (~0.28 g) or gel bait (~0.50 g) which were placed on pieces of paper (3.8 x 3.8 cm, Fisherbrand weighing paper). In control assays, dog food replaced gel baits and, therefore, received dog food only. Similar amounts of dog food or gel bait were placed into 30 ml cups, which were then covered with an organdy fabric square and held in place with a rubber band to prevent cockroach access. The covered dog food or gel bait was used for moisture controls to adjust for loss/gain in consumption calculations. The four portions, two dog food and two gel bait, were placed in arenas simultaneously. After 24 h, the four portions were reweighed. The dog food and gel bait were placed back into the arena for the remainder of the study.

Cockroach mortality was recorded up to 14 d after the introduction of dog food and gel bait. Mortality was defined as the cockroach nymph's inability to self-right.

Data Analysis

Consumption was calculated as follows:

$$\text{Consumption} = B_B - \{B_B \times [(MC_B - MC_A) / MC_B]\} - B_A$$

where B_B is the pre-weight of dog food/gel bait before introduction into the arena, MC_B is the pre-weight of moisture control food/gel bait, MC_A is the post-weight of moisture control food/gel bait 24 h after introduction into the arena, and B_A it the post-weight of exposed food/gel bait (Ncherne 2006). Each portion was weighed individually before introduction into the testing arena and 24 h later. Percent gel bait consumption was calculated as follows:

$$\text{Percent consumption} = [GB / (GB + DF)] \times 100$$

where GB is gel bait consumed (mg) and DF is dog food consumed (mg) (Ross 1998). Consumption and mortality data were arcsine square root transformed before being analyzed by analysis of variance (ANOVA) with means separated by the Student Newman Keuls (SNK) test or the Student's *t*-test ($\alpha = 0.05$; SAS Institute 2003). Linear regression analysis was performed with consumption as the dependent variable and concentration of emamectin benzoate as the independent variable for brownbanded cockroaches (SAS Institute 2003).

Results

Consumption

Both brownbanded cockroaches and German cockroach nymphs, within about 5 min of introduction, could be seen around both the gel bait and dog food. Brownbanded cockroaches and Daytona cockroach nymphs did not show a preference to either piece of dog food in the control arenas, indicating that there was not a bias for food location (Table 4-1).

For brownbanded cockroaches, there was no significant difference in consumption between any of the gel baits or dog food (*F* = 0.33, df = 6, *P* = 0.9151). However, at the higher concentrations of emamectin benzoate, there was an increase in percent consumption. Regression of consumption versus emamectin benzoate concentration was highly correlated with an R^2 of 0.9875 (Fig. 4-3).

For Daytona cockroach nymphs, dog food had the lowest percent consumption and was significantly difference from the gel baits (emamectin A 0.1%, emamectin B 0.2%, 0.1%, and 0.05%, bait base and Maxforce FC Select) (*F* = 9.85, df = 6, *P* <0.0001). There was no significant difference in consumption between any of the gel baits.

For German cockroach nymphs and brownbanded cockroaches, there was no significant difference in percent consumption between emamectin A 0.1% and the emamectin B 0.05%, 0.1%, and 0.2%. Therefore, the inert ingredients in emamectin A do not act as feeding deterrents or stimulants.

Mortality

For Daytona cockroach nymphs and brownbanded cockroaches, mortality was observed within the first 24 h. For both brownbanded cockroaches and Daytona strain nymphs, there was no significant difference in percent mortality between the bait base and dog food (Table 4-2). The bait base contained no active ingredient; therefore, mortality was expected to be similar to dog food. For Daytona cockroach nymphs and brownbanded cockroaches, there was no significant difference in percent mortality between Maxforce FC Select, emamectin A 0.1%, or emamectin B 0.05%, 0.1%, and 0.2%) (*F* = 226.66, df = 6, *P* <0.0001 and *F* = 149.77, df = 6, *P* <0.0001, respectively).

For brownbanded cockroaches, emamectin B 0.2% had the fastest speed of action among the emamectin gel baits, with 50% mortality at ~2 d and 90% mortality at ~5 d (Fig. 4-1). Emamectin A at 0.1% and emamectin B at 0.1% had similar speeds of action with 50% mortality also at ~2 d and 90% mortality in about 6 to 8 d. Emamectin B 0.05% had the slowest speed of action through 14 d, with 50% mortality in 2 to 3 d and 90% mortality in ~9 d.

For the bait averse German cockroaches, emamectin benzoate gel baits all had similar speeds of action initially with 50% mortality at ~2 d (Fig. 4-2). However, 90% mortality for Emamectin A 0.1% and emamectin B 0.1% and 0.05% was ~7 d and for emamectin B 0.2% 90% mortality was at ~11 d.

Discussion

Daytona cockroach nymphs consumed greater percentage of gel baits than dog food. Most information on bait averse German cockroach strains pertains to bait failures due to sugar aversion (Silverman and Bieman 1993, Wang et al. 2004). However, one study found that the Daytona (bait averse) strain of German cockroaches consumed more dog food than first generation (Maxforce®) or second generation (Avert® and Maxforce® FC) cockroach gel baits (Ncherne 2006). Conversely, the study also found that third generation cockroach gel baits (Maxforce FC Select and Advion®) were consumed more than dog food. Similarly, in my study, I found that the Daytona strain consumed more Maxforce FC Select, bait base, emamectin A and emamectin B gel baits than dog food.

Brownbanded cockroaches consumed about an equal percentage gel baits and dog food. While there is limited information on consumption for brownbanded cockroaches, Cohen et al. (1987) were able to determine that brownbanded cockroaches would self-select protein at 15.5% and glucose at 84.5%. When given a single diet cube at 20:80 (protein: glucose) nymphal growth was stunted and with single diets of 0: 100 or 100: 0 (protein: glucose), there was little consumption. However, when give a choice between two cubes with nutritionally complete diets, with equal amounts of protein and glucose (50: 50), brownbanded cockroaches consumed "about equally from both cubes". In my study, brownbanded cockroaches consumed similar percentages of dog food, which has a crude protein ratio of 28.0% (Purina One 2007), and gel baits: bait base, Maxforce FC Select, emamectin A, and emamectin B. This suggests that all the gel baits and dog food were nutritionally equal for brownbanded cockroaches.

Avermectin at high concentrations is a feeding inhibitor (Cochran 1985). However, in my study, there was a good correlation between consumption and concentrations of emamectin benzoate for brownbanded cockroaches. With an increasing concentration of emamectin benzoate, consumption increased (Fig. 4-3). This is may indicate that emamectin benzoate is a feeding stimulus for this species. However, further research into the feeding preferences is required for the brownbanded cockroach.

Brownbanded cockroaches are highly susceptible to emamectin benzoate. Tests with various insecticides on brownbanded cockroaches show good efficacy (Whitney et al. 1967, Burden 1980, Pospischil et al. 1999). Similarly, in my study, high percent mortalities (≥96%) were observed for all formulated gel baits.

The Daytona (bait averse) strain German cockroach is susceptible to emamectin gel baits, which are in the same class of insecticide as abamectin. Using abamectin gel baits, Cochran (1994) tested 13 strains of 5th to 6th instar German cockroaches and observed a wide range of susceptibility levels from, 97.8 to 31.1%. Also using abamectin gel baits, Negus and Ross (1997) compared six strains of sixth instar German cockroaches and observed susceptibility levels in of two of the six strains to be significantly different from each other. Abamectin gel baits were used on mid-sized nymphs; 100% mortality was observed after 14 d (Ross 1993). Similar to Ross's findings, for the Daytona strain, I observed between 95% and 99% mortality 14 d after introduction of emamectin A, emamectin B or Maxforce FC Select gel baits. The length of the test in Negus and Ross's experiment, which only ran for 3 d, may not have been of an adequate duration. Additionally, in the Cochran (1994) study and the Negus and Ross (1997) study, unidentified bait averse strains could explain the observed mortality in the different strains.

High levels of susceptibility have been observed in brownbanded cockroaches when exposed to insecticides, often within 3 d after treatment (Whitney et al. 1967, Burden 1980, Pospischil et al. 1999). The LT_{50} for abamectin at 0.0550% on brownbanded cockroaches was observed to be 4.54 d (Koehler et al. 1991). Likewise, in my study, by 2 d, emamectin A 0.1% and emamectin B 0.1% and 0.2% had ~50% mortality (Fig. 4-1). Emamectin B 0.05% had 50% mortality at ~3 d. By 14 d, all emamectin gel baits had 96% to 99.5% mortality.

The German cockroach is susceptible to emamectin benzoate, possible at similar or better levels as abamectin. The LT_{50} for German cockroaches at 0.0500 and 0.100% abamectin was observed to be 1.550 and 2.067 d, respectively (Koehler et al. 1991). Likewise, in my study, emamectin A 0.1%, emamectin B 0.05%, 0.1%, and 0.2% mortality was between 40% and 60% at 2 d (Fig. 4-2). At 7 d, emamectin gel bait, A and B at 0.05% and 0.1%, mortalities were greater than 90%. At 14 d, all emamectin gel baits had 95.7% to 99.4% mortality.

In conclusion, my study gel baits with a novel insecticide, emamectin benzoate, palatable to both brownbanded cockroaches and Daytona strain (bait averse) German cockroaches. Additionally, I observed that emamectin benzoate consumed by Daytona strain German cockroaches and brownbanded cockroaches which resulted in high susceptibility. The high susceptibility and palatability make emamectin benzoate gel baits excellent candidates for controlling domestic cockroaches.

Table 4-1. Gel bait preference by *Supella longipalpa*, mixed population, and *Blattella germanica* nymphs (bait averse Daytona strain) in a 24 h choice experiment.

	Bait as percent of total consumption (Mean ± sem)[a]	
Treatment	*Supella longipalpa* (n=4)	*Blattella germanica* (Daytona strain) (n=9)
Dog Food	52.7 ± 4.19a	48.5 ± 5.64b
Blank Bait Base	50.4 ± 11.07a	78.8 ± 2.76a
Maxforce FC Select	61.2 ± 10.47a	79.0 ± 4.87a
Emamectin A 0.1%	62.6 ± 11.07a	81.7 ± 2.32a
Emamectin B 0.05%	56.0 ± 12.72a	84.5 ± 2.99a
Emamectin B 0.1%	58.4 ± 10.96a	85.0 ± 1.89a
Emamectin B 0.2%	66.7 ± 9.63a	78.4 ± 3.24a

[a]Consumed percentage was obtained by the following formula:
{gel bait consumed (mg) / [gel bait consumed (mg) + dog food consumed (mg)]} x 100.
Means within a column followed by the same letter are not significantly different (*P* = 0.05; Student Newman Keuls [SAS Institute, 2003]).

Table 4-2. Percent mortality of *Supella longipalpa*, mixed population, and *Blattella germanica* nymphs (bait averse Daytona strain) 14 d after bait introduction.

	Percent Mortality	
Treatment	*Supella longipalpa* (n=4)	*Blattella germanica* (Daytona strain) (n=9)
Dog Food	7.5 ± 1.26b	3.43 ± 0.72b
Blank Bait Base	9.5 ± 3.59b	3.43 ± 1.04b
Maxforce FC Select	100 ± 0.00a	96.6 ± 2.17a
Emamectin A 0.1%	99.5 ± 0.50a	97.1 ± 1.38a
Emamectin B 0.05%	96.0 ± 2.31a	99.4 ± 0.37a
Emamectin B 0.1%	98.0 ± 1.15a	97.1 ± 1.56a
Emamectin B 0.2%	99.5 ± 0.50a	95.7 ± 1.11a

Means within a column followed by the same letter are not significantly different (P= 0.05; Student Newman Keuls [SAS Institute, 2003]).

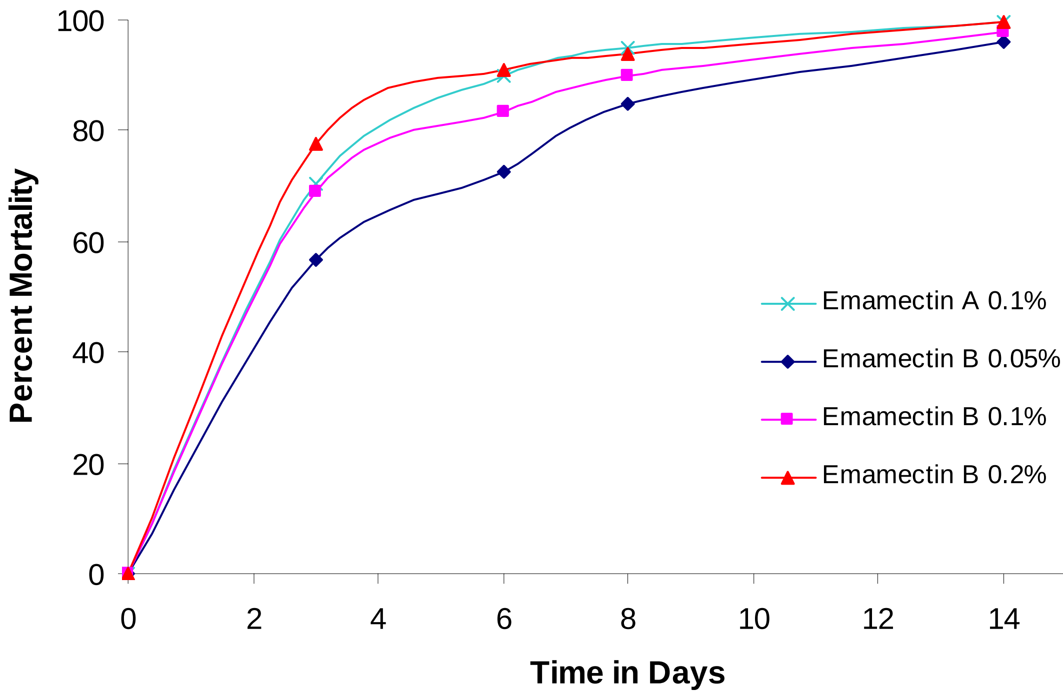

Figure 4-1. Percent mortality for *Supella longipalpa* at 3, 6, 8 and 14 d after introduction of emamectin gel baits.

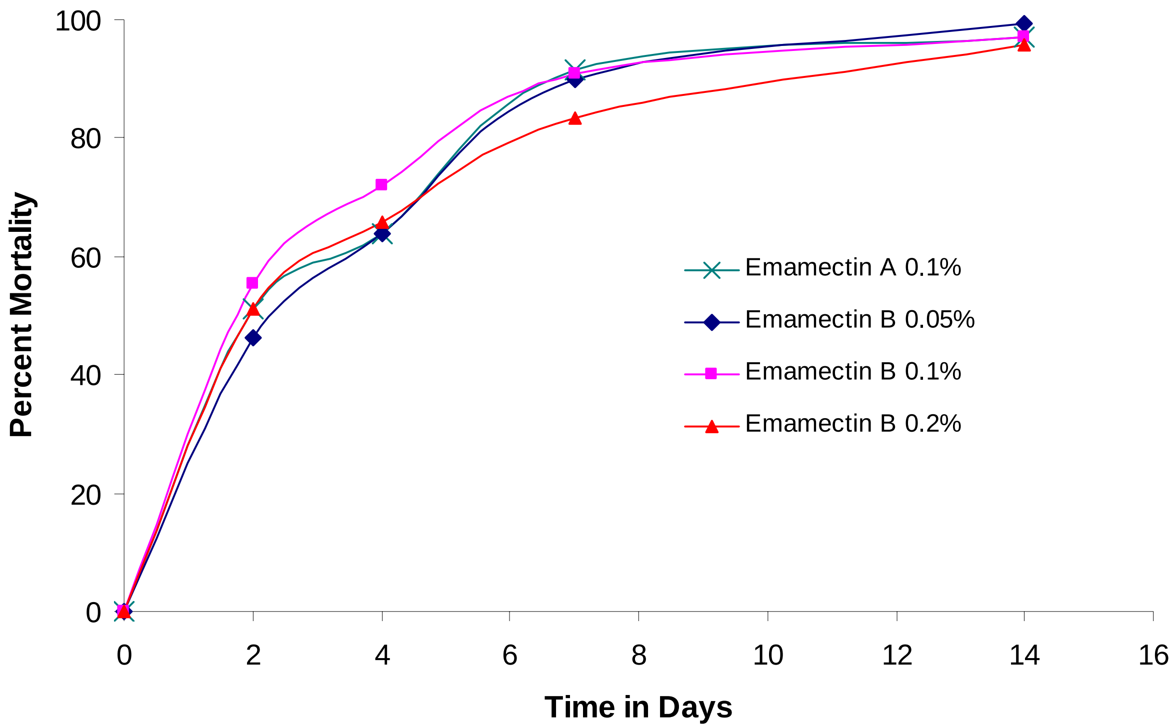

Figure 4-2. Percent mortality for *Blattella germanica* nymphs (bait averse Daytona strain) at 2, 4, 7 and 14 d after introduction of emamectin gel baits.

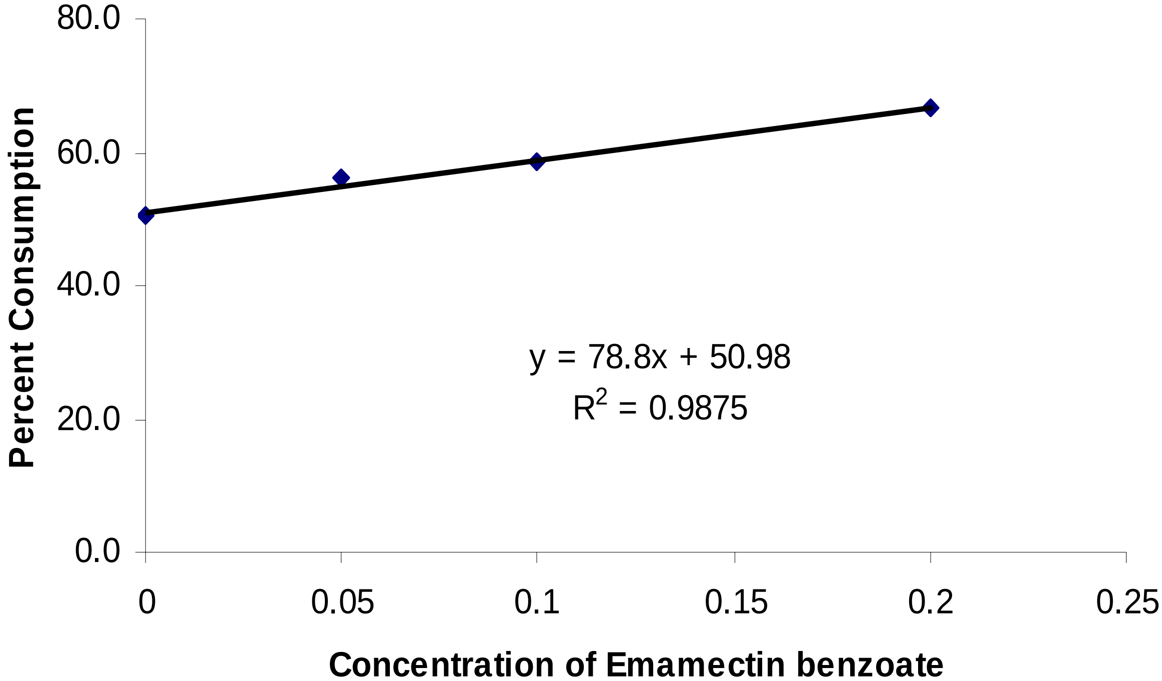

Figure 4-3. Percent consumption for *Supella longipalpa* cockroach, at increasing concentrations of emamectin benzoate.

CHAPTER 5
EMAMECTIN BENZOATE ON PERIDOMESTIC COCKROACHES

Introduction

Of the more than 4,000 described species of cockroaches, only 69 inhabit North America (Bell 1984, Atkinson et al. 1991, Mabbett 2004) and of those, only about 25% are found living "in close association with humans" (Barcay 2004). The majority of these cockroaches are considered peridomestic, living and breeding outdoors near human structures and occasionally entering and infesting structures, or feral, living and breeding outdoors away from human activity and rarely encountering humans. Of the peridomestic cockroaches, both the American and Oriental cockroach are considered pests. Their pest status is due in part to their tendency to move indoors, as well as, their ability to transmit pathogens.

The most common peridomestic cockroach is the American, *Periplaneta americana* L., which is distributed widely throughout the United States, living mostly outdoors in tropical and subtropical regions and moving indoors in more temperate regions (Bell 1984). American cockroaches prefer warm, humid, damp areas, and are closely associated with sewers. Outdoors, they can also be found under leaf debris, woodpiles, in mulch, in dumps, palm trees, and crawl spaces (Cornwell 1968, PCT 1995, Barcay 2004, Jacobs 2007). Indoors, the American cockroach can be found in basements, steam tunnels, sheds, and latrines. They can also be found where food is prepared or stored, such as restaurants, grocery stores, bakeries, as well as factories, hospitals, hotels, and zoos (Cornwell 1968, Roth 1982, Barcay 2004).

Another peridomestic cockroach is the Oriental cockroach, *Blatta orientalis* L., which is found in temperate areas of the United States, preferring cool, damp environments, and temperatures below 29° C (Cornwell 1968, Thoms and Robinson 1986, Barcay 2004). Outdoors, Oriental cockroaches can be found in crawl spaces, the cracks and crevices of walls and porch voids (Thoms and Robinson 1986, Pest Management 1995). Indoors, the Oriental cockroach prefers moist, damp, cool areas, such as basements, (Blatchley 1920, Pest Management 1995, Barcay 2004) but is also found near "radiators, ovens, and hot-water pipes" (Cornwell 1968). The Oriental cockroach may be transported indoors via laundry or food packaging, or they may enter structures through gaps around doors, windows, pipes, or cracks in the foundation (Thoms and Robinson 1986, Pest Management 1995, Barcay 2004). Additionally, the Oriental cockroach can use garbage chutes, electrical conduits, and plumbing to move from lower areas of a structure to upper levels (Pest Management 1995).

Cockroach infestations can cause psychological problems; due to the embarrassment they cause. The American cockroach can cause "serious mental anguish" and "its presence is aggravated by swift movements and flight … across a kitchen counter and flying from ceiling to wall" (Bell 1984). Cornwell (1976) stated that the "fear or shame" associated with cockroach infestations prevented some people from admitting an infestation existed. The "fear or shame" can cause stress in proportion to the size of the cockroach and/or infestation (Brenner 1995). The stress comes from the implication that if cockroaches are present it is due to an "unsanitary environment", which may or may not be the case.

Cockroaches can also cause serious health problems. Cockroaches produce allergens that may elicit asthma attacks. Studies on inner city children with asthma observed that these children were more allergic to cockroach allergens than to either dust mite or cat allergens and about 50% of the bedrooms tested had enough cockroach allergens to elicit asthma attacks (Rosenstreich et al. 1997). Cockroaches are also able to "carry, maintain, and excrete" viable fungi, protozoa, eggs of helminthes, viruses, and bacteria, including several strains of streptococcus and salmonella (Roth and Willis 1957, 1960). Both American and Oriental cockroaches have been associated with bacteria that cause pneumonia, food poisoning, and tuberculosis (Roth and Willis 1957, 1960, Barcay 2004). Because of aesthetic and health issues, cockroach control is essential.

The objective of my study was to determine if cockroach gel baits containing emamectin benzoate would be palatable to both American and Oriental cockroaches. Additionally, I wanted to determine if gel baits formulated with emamectin benzoate were capable of controlling both of these cockroach species.

Materials and Methods

Insecticides

Five experimental formulated emamectin benzoate gel baits, and one standard gel bait were tested. The experimental emamectin benzoate gel baits were formulated as either emamectin A at a concentration of 0.1% or emamectin B at a concentration of 0.05%, 0.1%, or 0.2%. One of the formulated gel baits contained no active ingredient (Syngenta Crop Protection, Greensboro, NC). The current industry standard gel bait utilized in this study was Maxforce FC Select® with 0.01% of the active ingredient fipronil (Bayer Environmental Science, Montvale, NJ).

Insects

Oriental and American cockroaches were reared at the University of Florida, urban entomology laboratory (Gainesville, FL) in glass utility jars (25.5 high x 22.0 cm diameter) with the inner top 5 cm greased with a petroleum jelly/mineral oil mixture (2:3) to prevent escape. Each jar contained cardboard for harborage, and were provided water and dry rodent food (Labdiet 5001, PMI Nutrition Int., Brentwood, MO) *ad libitum*. Cockroaches were maintained at 23.6 ± 2.5°C at 51 ± 16% RH at a photoperiod of 12:12 (L: D). For testing, Oriental cockroach nymphs, second to fourth instars, and American cockroaches, adults and third to fifth instar nymphs, were removed from the colony with feather tip forceps.

Assay Setup

The test arenas were lidded transparent plastic boxes (27 x 19.5 x 9.5 cm height), with the inner top 5 cm greased to prevent cockroach escape. For American cockroaches, 235 mL of corncob grit (Kay-Kob Bedding and Liter, KAYTEE Products, Incorporated, Chilton, WI.) was spread on bottom of arena to absorb excess moisture. Both American and Oriental cockroach arenas contained harborage [a blank white index card (7.6 x 12.7 cm) folded lengthwise and stapled] and a water vial with a cotton stopper. A mixed population of American cockroaches (5 adult males, 5 adult females and 10 nymphs) or 10 Oriental nymphs were placed into arenas and starved for 24 h. Dead cockroaches and exuviae were removed prior to introduction of food and gel baits.

Assay Method

Oriental and American cockroaches were provided a choice between pre-weighed dog food (Purina One® puppy: growth and development, Nestlé PetCare Company, St. Louis, MO.) (~0.28 g) or gel bait (~0.50 or ~1.50 g, respectively) which were placed on pieces of paper (3.8 x 3.8 cm, Fisherbrand weighing paper). In control assays, dog food replaced gel baits and, therefore, received dog food only. Similar amounts of dog food or gel bait were placed onto pieces of paper and into 30 ml cups, which were then covered with an organdy fabric square and held in place with a rubber band to prevent cockroach access. The covered food was used for moisture-loss controls and used to adjust for loss/gain in consumption calculations. The four portions, tow dog foods and tow gel baits, were placed in the arena simultaneously. After 24 h, the four portions were reweighed. The exposed dog food and gel bait were placed back into the arena for the remainder of the study. Cockroach mortality was recorded up to 14 d after the introduction of food. Mortality was defined as cockroach inability to self-right.

Data Analysis

Consumption was calculated as follows:

$$\text{Consumption} = B_B - \{B_B \times [(MC_B - MC_A) / MC_B]\} - B_A$$

where B_B is the pre-weight of dog food/gel bait before introduction into the arena, MC_B is the pre-weight of moisture control food/gel bait, MC_A is the post-weight of moisture control food/gel bait 24 h after introduction into the arena, and B_A it the post-weight of exposed food/gel bait (Ncherne 2006). Each portion was weighed individually before introduction into the arena and at 24 h after introduction. Gel bait consumption was calculated as follows:

$$\text{Percent consumption} = [GB / (GB + DF)] \times 100$$

where GB is gel bait consumed (mg) and DF is dog food consumed (mg) (Ross 1998). Consumption and mortality data were analyzed by analysis of variance with means separated by Student Newman Keuls (SNK) or Student's *t*-test ($\alpha = 0.05$; SAS Institute 2003).

Results

Consumption

Within ~5 min of food portion placement into the arena, both the Oriental and American cockroaches were observed around both the gel baits and dog food. The Oriental cockroaches did not show a preference for either piece of dog food in the control arenas, indicating that there was no location bias (Table 5-1). The American cockroaches seemed to have a slight preference for the dog food side of the arena over the gel bait side.

For Oriental and American cockroaches, dog food had the lowest percent consumption, was significantly difference from all gel baits ($F = 7.30$, df = 6, $P < 0.0001$ and $F = 88.51$, df = 6, *P* <0.0001, respectively). However, there was no significant difference in percent consumption between any of the gel baits.

Mortality

For both Oriental and American cockroaches, dog food and bait base percent mortalities were not significantly different from each other, but with the lowest percent mortality, they were significantly different from all other gel baits (emamectin A 0.1%, emamectin B 0.05%, .01% and 0.2%, and Maxforce FC Select) (Table 5-2). The bait base contained no active ingredient; consequently, percent mortality was expected to be similar to dog food.

For Oriental cockroaches, there was no significant difference in percent mortality for Maxforce FC Select, emamectin A 0.1%, or emamectin B at 0.1% and 0.2% ($F = 81.83$, df = 6, $P < 0.0001$). Likewise, there was no significant difference in percent mortality between emamectin A 0.1% and emamectin B 0.05%, 0.1%, and 0.2%. However, mortality of cockroaches exposed to Maxforce FC Select was significantly different from emamectin B 0.05%.

For American cockroaches, there was no significant difference in percent mortality between Maxforce FC Select, emamectin A 0.1% or emamectin B 0.2% ($F = 149.57$, df = 6, $P < 0.0001$). Additionally, there was no significant difference in percent mortality between the emamectin B gel baits at 0.05%, 0.1% or 0.2%. Furthermore, emamectin A 0.1% and emamectin B 0.1% and 0.2% were not significantly different from each other. However, emamectin B at 0.05% and 0.1%, with the lowest percent mortality at 85% and 92.14%, respectively, was significantly different from Maxforce FC Select. There was also a significant difference in percent mortality between emamectin A 0.1% and emamectin B 0.05% (Table 5-2).

For both the Oriental and American cockroaches, there was no significant difference in percent consumption between emamectin A 0.1% and emamectin B at 0.1%. Additionally, there was no significant difference in percent mortality between emamectin A 0.1% and emamectin B at 0.1%.

For American cockroaches, emamectin B 0.2% had the fastest speed of action among the emamectin gel baits, with ~50% mortality at 4 to 5 d (Fig. 5-1). Emamectin A at 0.1% and emamectin B at 0.1% had similar speeds of action with 50% mortality at ~5 d. Emamectin B 0.05% had the slowest speed of action through the 14 d, with 50% mortality at 7 d.

For Oriental cockroaches, emamectin B 0.2% had the fastest speed of action, among the emamectin gel baits, with about 50% mortality at ~4 d and 90% mortality at ~7 d (Fig. 5-2). Emamectin A 0.1% and emamectin B 0.1% had similar speeds of action with about 50% mortality at 7 d. Emamectin 0.05% had the slowest speed of action with 50% mortality at 10 d. However, at 14 d, there was no significant difference in percent mortality.

Discussion

"American cockroaches climb over the top of their food and often carry it away" (Frishman 1988). In my study, this behavior was also observed. Dog food was also removed from paper, which were labeled prior to initial weighing, and could often be found in the harborage. Dog food found inside harborage was observed to have greater consumption than dog food found outside of harborage. Due to movement of dog food within control arenas, individual pieces were paired with the closest piece of paper. Therefore, location bias could not be assessed.

American and Oriental cockroaches preferred the gel baits (bait base, Maxforce FC Select, emamectin A, and B) to dog food. American cockroaches will feed on a wide assortment of materials including glue, leather, plants, fruit, and starches including nuts, bookbinding, and paper (Bell 1984, Jacobs 2007). They are more attracted to some food materials than to others (Lofgren and Burden 1958, Ahmed 1976). Conversely, little information exists on food preferences for the Oriental cockroach other than "prefers to feed upon starchy foods" (Suiter and Koehler 1991) and "prefer decaying foods" (Barcay 2004). In my study, American and Oriental cockroaches overwhelming preferred the gel baits (>96% and >87% consumption, respectively) to dog food, feeding almost exclusively on the gel baits (Table 5-1), suggesting that American and Oriental cockroaches are attracted to the material in gel baits used in this study more than the material in the dog food.

American and Oriental cockroaches are susceptible to a variety of insecticides (Ahmed 1976, Burden 1980, Koehler et al. 1991, Valles et al. 1999). In my study, I found that both American cockroaches and Oriental cockroaches were susceptible to emamectin gel baits, which resulted in a high percent mortality, ≥85%, at 14 d (Table 5-2).

Emamectin gel baits have a speed of action that requires 5-7 d for 50% mortality in American cockroaches. Using abamectin at about 0.05%, LT_{50}'s for American cockroaches were observed to be 2.1 and 3.4 d for late instar nymphs and adult males, respectively (Koehler et al. 1991 and Smith and Appel 1996). In my study, I observed that emamectin B 0.05% had the slowest speed of action with >50% mortality being achieved by ~7 d (Fig. 5-1). Higher concentrations of emamectin benzoate resulted in 50% mortality at ~5 d. However, by 14 d, consumption of emamectin gel baits resulted in 85% to 98% mortality of American cockroaches. This slower speed of action for the emamectin gel baits compared to abamectin could be due to the different cockroach ages and sexes. In my study, I used mixed populations, while Koehler et al. (1991) utilized adult male American cockroaches and Smith and Appel (1996) made use of "the last 2 instars". In my study, only 25% of American cockroach test populations were late instar nymphs and adult males.

For the Oriental cockroach, speed of action of emamectin gel baits was highly concentration dependent. The abamectin LT_{50} for Oriental cockroaches was observed to be 2.9 d for adult males at a concentration of 0.05% (Koehler et al. 1991). In my study, I observed 50% mortality of Oriental cockroaches at ~10 d when fed emamectin benzoate at 0.05%. The emamectin gel baits at 0.1% required 6 to 8 d for 50% mortality of Oriental cockroaches, and just 4 d when fed 0.2% emamectin benzoate. However, by 14 d, consumption of emamectin gel baits resulted in >85% mortality of Oriental cockroaches. The slower speed of action may be due to age of cockroaches utilized in each study. In my study, mid-instar nymphs were used, while in the Koehler et al. (1991) study, adult males were used. Koehler et al. (1993) observed that susceptibility of German cockroaches varied by the age and sex, thus, similar susceptibility might occur in Oriental and American cockroaches.

Slower speed of action may assist in horizontal transfer of emamectin benzoate. Given that 50% mortality was not observed until after 4 d, for both American cockroaches and Oriental cockroaches, there should be ample time for defecation (one form of horizontal transfer) prior to mortality. Additionally, American cockroaches are repelled by the presence of other dead American cockroaches and just "two cockroach equivalents" were sufficient to cause repellency for up to 4 weeks (Rollo et al. 1995). If a large number of cockroaches return to harborages and die too quickly, this may cause dispersion of untreated populations.

In conclusion, both American cockroaches and Oriental cockroaches consumed large percentages of emamectin gel baits. These were comparable to the percent consumption of Maxforce FC Select. Consumption of emamectin benzoate resulted in ≥85% mortality, at the lowest concentration, for both species. At higher concentrations, 0.1% and 0.2% emamectin benzoate, 92% to 98% of American cockroaches and 88% to 91% of Oriental cockroaches were dead at 14 d. Speed of action to obtain ~50% mortality required 5 to 7 d for American cockroaches and 4 to 10 d for Oriental cockroaches. Such a slow speed of action could aid in horizontal transfer and ultimately resulted in high mortality for both species. Thus, emamectin benzoate appears an ideal candidate material for controlling both American and Oriental cockroaches.

Table 5-1. Gel bait preference of *Blatta orientalis* and *Periplaneta americana* in a 24 h choice experiment.

	Bait as % of total consumption (Mean ± sem)[a]	
Treatment	*Blatta orientalis*	*Periplaneta americana*
Dog Food	49.5 ± 5.61b	41.8 ± 3.78b
Blank Bait Base	91.8 ± 4.77a	98.6 ± 0.89a
Maxforce FC Select	90.1 ± 2.98a	96.7± 2.57a
Emamectin A 0.1%	87.7 ± 6.56a	99.6 ± 0.13a
Emamectin B 0.05%	87.3 ± 5.39a	99.2 ± 0.44a
Emamectin B 0.1%	92.4 ± 3.22a	99.7 ± 0.12a
Emamectin B 0.2%	92.3 ± 3.68a	99.7 ± 0.13a

[a]Consumed percentage was obtained by the following formula:
{gel bait consumed (mg) / [gel bait consumed (mg) + dog food consumed (mg)]} x 100.
Means within a column followed by the same letter are not significantly different (*P* = 0.05; Student Newman Keuls [SAS Institute, 2003]).

Table 5-2. Percent mortality of *Blatta orientalis* and *Periplaneta americana* at 14 d after bait introduction.

Treatment	Percent Mortality[a]	
	Blatta orientalis	*Periplaneta americana*
Dog Food	1.4 ± 1.43c	0.7 ± 0.71d
Blank Bait Base	0.0 ± 0.00c	1.43 ± 0.92d
Maxforce FC Select	100 ± 0.00a	100.0 ± 0.0a
Emamectin A 0.1%	88.6 ± 6.7ab	98.57 ± 0.92ab
Emamectin B 0.05%	85.7 ± 5.28b	85.00 ± 5.67c
Emamectin B 0.1%	90.0 ± 3.09ab	92.14 ± 2.64bc
Emamectin B 0.2%	91.4 ± 4.59ab	96.43 ± 1.43abc

[a]Mortality percentage was obtained by the following formula:
no. of dead cockroaches dead at 14 d / no. of live cockroaches at 0 hr.
Means within a column followed by the same letter are not significantly different (*P* = 0.05; Student Newman Keuls [SAS Institute, 2003]).

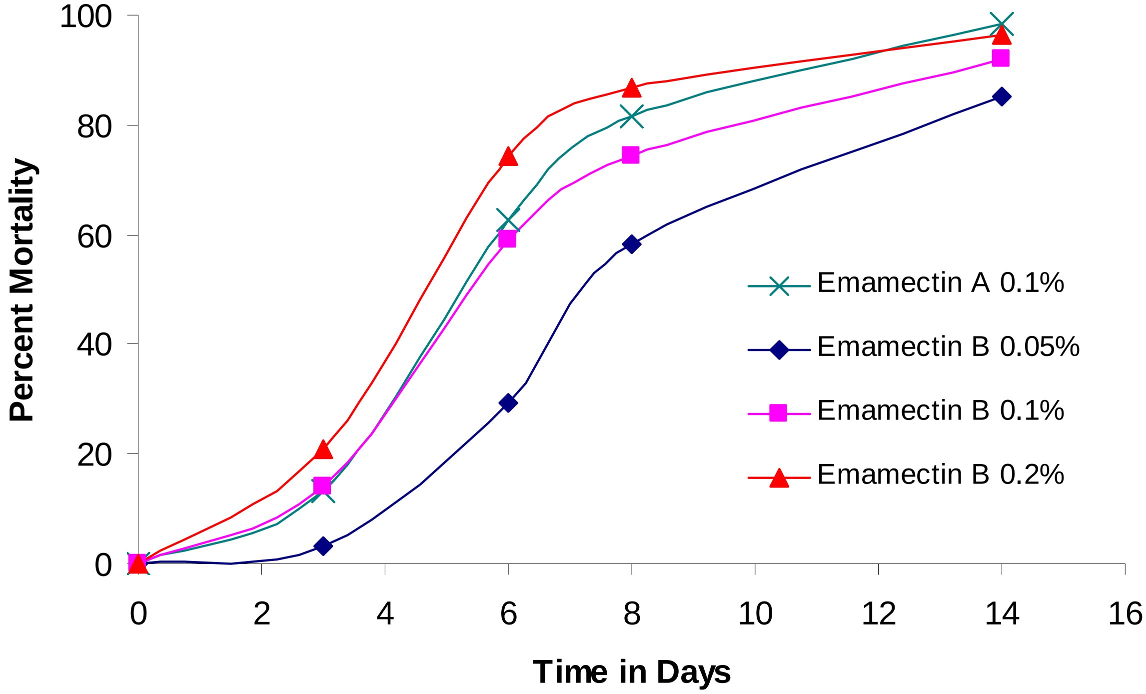

Figure 5-1. Percent mortality for *Periplaneta americana* at 3, 6, 8 and 14 d after introduction of experimental emamectin benzoate baits.

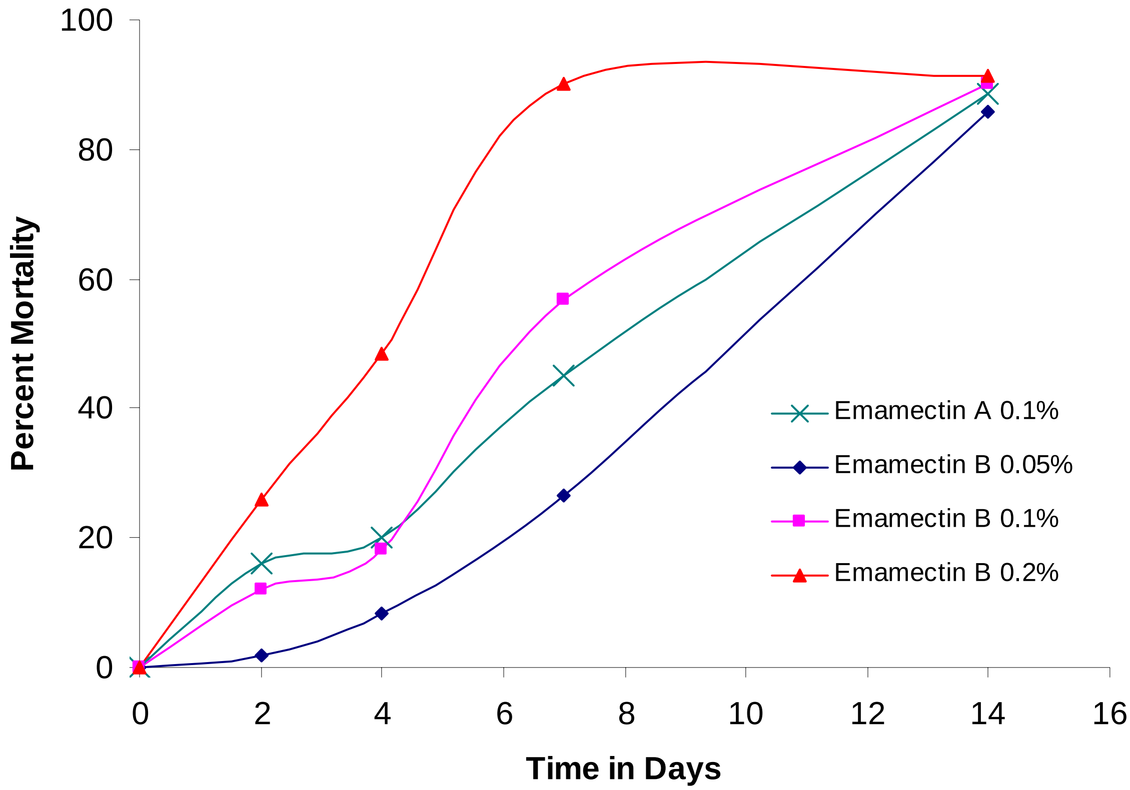

Figure 5-2. Percent mortality for *Blatta orientalis* at 3, 6, 8 and 14 d after introduction of bait.

CHAPTER 6
CONCLUSION

Initially, I compared a bait averse strain and a standard susceptible strain of German cockroach for palatability and efficacy of gel baits containing emamectin benzoate. I observed that the gel baits formulated with emamectin benzoate were palatable to both strains and that there was no statistical difference in percent consumption between the strains. I also observed that both strains were susceptible to emamectin benzoate. Percent mortality was 80% to 90% for the emamectin benzoate gel baits, at 6 d. There was no statistical difference in mortality between the bait averse strain and the normal strain.

In the succeeding experiments, the bait averse strain of German cockroach, brownbanded cockroach, Oriental cockroach and American cockroach palatability and efficacy were observed when the cockroaches were fed gel baits containing emamectin benzoate at several concentrations. For all cockroaches tested, there was no statistical difference between emamectin A 0.1% and emamectin B 0.1% in percent consumption or mortality. The emamectin gel baits were palatable to all species of cockroaches. The bait averse German cockroach, Oriental cockroach and American cockroach all preferred the gel baits to the standard laboratory diet of dog food. Brownbanded cockroaches showed no preference for gel bait or dog food, consuming a similar percent of both materials in choice assays.

I observed good efficacy of the emamectin gel baits. Percent mortality for all cockroach species was between 85% and 99.5% after consuming emamectin benzoate gel baits. Percent mortality for the brownbanded cockroach and the bait averse German cockroach was similar for all formulated gel baits tested: emamectin A, emamectin B, and the standard Maxforce FC Select gel baits. Percent mortality for the Oriental cockroach was similar for emamectin A and emamectin B and only emamectin B 0.05% was statistically different from Maxforce FC Select.

Percent mortality for the American cockroach was similar for the emamectin B gel baits, only emamectin B 0.05% was statistically different from emamectin A 0.1% and only emamectin B 0.05% and 0.1% were statistically different from Maxforce FC Select.

In each of my studies, I observed that the experimental gel bait matrices were palatable to all species and strains of pest cockroaches tested. I also observed high percent mortality for all cockroaches tested when fed emamectin benzoate. Gel baits with emamectin benzoate show excellent commercial potential for controlling both domestic and peridomestic cockroaches.

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BIOGRAPHICAL SKETCH

Barbara Ellen Bayer was born on January 20, 1971, in Fort Myers, Florida. The second of three girls, she grew up in Tampa, Florida, graduating from King High School in 1989. She attended Hillsborough Community College beginning in January 1990, transferring to the University of Florida in May 2003. She earned her B.S. in urban entomology from the University of Florida in 2005.

Barbara has held a variety of jobs, mostly in the accounting field, working at JC Penney from August 1990 to May 1996. She then worked for Housecall Home Health Care for a short period, May 1996 to January 1997. Barbara then went to work with her uncle at Gulfside Supple from January 1997 to August 1999. She then had the honor of working for Famous Tate from August 1999 until August 2005. Barbara was a graduate assistant under Dr. Philip Koehler from August 2005 to August 2007, when she completed here Master of Science (MS) degree. Upon completion of her MS program, Barbara was given the opportunity to continue her education and earn her Ph.D. in entomology at the University of Florida.