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Over-the-Counter Aerosol Insecticide-Induced Sociotomy and Translocation of Cockroach Gel Bait Toxicant in the Pharaoh Ant, *Monomorium pharaonis* (L.) (Hymenoptera: Formicidae)

A Thesis submitted in partial satisfaction
of the requirements for the degree of

Master of Science

in

Entomology

by

Xingwei Feng

June 2025

Thesis Committee:
Dr. Chow-Yang Lee, Chairperson
Dr. Dong-Hwan Choe
Dr. Michael K. Rust

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The Thesis of Xingwei Feng is approved:

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Dedication

To my lovely wife, Yuan.

ABSTRACT OF THE THESIS

Over-the-Counter Aerosol Insecticide-Induced Sociotomy and Translocation of Cockroach Gel Bait Toxicant in the Pharaoh Ant, *Monomorium pharaonis* (L.) (Hymenoptera: Formicidae)

by

Xingwei Feng

Master of Science, Graduate Program in Entomology
University of California, Riverside, June 2025
Dr. Chow-Yang Lee, Chairperson

The Pharaoh ant, *Monomorium pharaonis* (L.), is one of the most distributed invasive tramp ant species. Due to its ability to frequently relocate nest sites after disturbances and often nest in inaccessible areas, managing Pharaoh ant infestation remains challenging. In Chapter 1, a literature review on Pharaoh ants is presented. In Chapter 2, three common OTC insecticidal aerosol products were tested with three application rates on four Pharaoh ant colony sizes. The results revealed essential oil-based insecticides exhibited a higher probability of inducing budding at all colony sizes when applied at a high rate, whereas pyrethroid-based insecticides increased the probability of inducing budding at a low application rate. The number of workers present outside the main nest in the essential oil-based insecticide-treated group decreased with increasing application rate, whereas it increased in pyrethroid-based insecticide-treated groups. The density of workers outside

the main nest appeared to play a role in influencing the final nest site after budding. The number of worker ant cadavers observed on pyrethroid-treated surfaces was significantly greater than on surfaces treated with the essential oil-based insecticide and controls. In Chapter 3, a cross-species mechanism of toxicant transfer was revealed as Pharaoh ants were exposed to bait-killed German cockroaches. German cockroaches were first treated with one of the four cockroach gel baits, namely Advion Trio (0.6% indoxacarb, 0.1% pyriproxyfen, 0.1% novaluron), Maxforce FC Magnum (0.05% fipronil), Alpine Rotation 1 (0.5% dinotefuran), and Vendetta Nitro (0.5% clothianidin, 0.5% pyriproxyfen), before the Pharaoh ants were allowed to forage on the cadavers. Ants fed on cadavers that were killed by Maxforce FC Magnum showed significantly higher queen and brood mortality. Advion Trio initially led to rapid elimination of queens, workers, and brood; however, Pharaoh ant colonies later recovered as the workers abandoned treated cockroach cadavers and switched to a regular diet. In the Vendetta Nitro-treated group, pyriproxyfen was delivered to the queens and significantly reduced the size of mature oocytes. However, no structural damage was observed on any oocyte, as all exhibited a visible nucleus, multiple nurse cells, and a well-developed follicular epithelium.

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Introduction

The Pharaoh ant, *Monomorium pharaonis* (L.), is one of the most widely distributed invasive tramp ant species (Wetterer 2010), exhibiting characteristics such as highly polygynous, unicolonial, short queen lifespan, sterile workers, and colony reproduction via budding (Passera 1994). This species nests exclusively indoors and is known for infesting residential and commercial structures, though outdoor activities were observed in the warmer climate (Oi et al. 1994, Man and Lee 2012). Due to its close association with human activity and ability to mechanically transmit pathogens, the Pharaoh ant has become an important insect pest especially in healthcare facilities (Beatson 1972, Lee 2002, Man and Lee 2012).

Given the potential health risks associated with the Pharaoh ant as a mechanical vector, effective control strategies are essential to manage infestations. However, its budding behavior poses a significant challenge, as most control methods cause physical or chemical disturbances, and often trigger colony fragmentation and migration, spreading the infestation (Buczkowski et al. 2005, Buczkowski and Bennett 2009, Tay and Lee 2015). Therefore, successful treatment must minimize disturbances while ensuring the elimination of the entire colony.

Many homeowners are unaware of the budding behavior of Pharaoh ants, and they often attempt to manage the infestation themselves instead of looking for pest management professionals (PMP) because many of them cannot afford the service (Wang and Bennett 2010). In household settings, over-the-counter (OTC) insecticidal aerosols

are most commonly used by homeowners due to the affordability, ease of acquisition and simplicity of use (Bennett et al. 1983). However, most OTC aerosol products contain pyrethroids, which are known to induce Pharaoh ant colony budding (Buczkowski et al. 2005, Tay and Lee 2015), worsening the infestation.

Current control strategies for Pharaoh ant infestations emphasize the use of toxic baits (Oi et al. 1994), as this method enables the thorough distribution of toxicants throughout the colony via trophallaxis while minimizing disturbances that could trigger budding. Among the commonly used active ingredients in baits, juvenile hormone analogues (JHAs) such as fenoxycarb and pyriproxyfen reduce queen fertility and interrupt larval development (Williams and Vail 1993, Tay and Lee 2014), which is ideal for Pharoah ant infestation management, as workers can distribute the toxicant without worker mortality. However, since JHA mainly targets the brood, it may take a few generations to eliminate the colony. Therefore, the slow-acting bait toxicant provided gradual but long-term control. In contrast, the fast-acting bait toxicant provided rapid, localized control for a shorter duration and Pharaoh ant colonies may recover from the treatment (Oi et al. 2000).

Given the widespread presence of Pharaoh ants, effective control strategies remain challenging. The process of budding complicates Pharaoh ant infestation management and limits control strategies. Understanding the mechanism underlying this behavior is crucial for an effective control outcome. In addition, while various control methods have been employed to manage Pharaoh ant infestations, baiting demonstrates a promising

performance due to its ability to target the entire colony including the hidden colony fragments.

Meanwhile, baiting is also one of the most employed control method against the German cockroach, *Blattella germanica* (L.), an indoor pest well known for its resilience and transmit pathogens, that poses a considerable challenge in pest management (Menasria et al. 2014, Fardisi et al. 2019). In addition, the potential of horizontal transfer of bait toxicants may further enhance the bait performance as mortality could occur on cockroaches that do not directly feed on the bait through contacting and feeding on the contaminated cockroach cadavers (Hamilton et al. 2023).

The global distributions of both Pharaoh ants and German cockroaches have a great overlap, with infestation hotspots reported in Eastern USA and Europe (Wetterer 2010, Lee and Wang 2021). Co-infestations of both species within a structure have been reported in sensitive environments where insecticide spray and dust application are limited, such as hospitals and insectaries (Burden and Smittle 1975, Gliniewicz et al. 2003, Gliniewicz et al. 2006). In such settings, bait formulations offer effective control while other methods cannot be employed (Burden and Smittle 1975). When cockroach gel baits are used to manage German cockroach infestations, Pharaoh ants may scavenge bait-killed cockroaches, facilitating cross-species horizontal transfer of bait toxicants and potentially affecting Pharaoh ant colony growth.

To advance the control practices and understanding of Pharaoh ant, this thesis was structured around two primary objectives:

- Chapter 2: Investigating the Pharaoh ant colony movement pattern after encountering over-the-counter insecticide aerosol treated surfaces. We examined one essential oil- and two pyrethroid-based aerosols by testing four Pharaoh ant colony sizes with three application rates and monitoring their effects on the colony over time.
- Chapter 3: Evaluating the impact of cockroach gel bait toxicants on Pharaoh ant colony growth after ants fed on intoxicated German cockroaches. We treated German cockroaches with four commonly used commercial gel baits, then allowed Pharaoh ants to feed on the resulting cockroach cadavers to observe whether colony growth was suppressed at the end of the study.

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Chapter 1. Literature Review

Pest ants

Ants represent one of the most successful evolutionary lineages, having emerged approximately 120 million years ago and diverging into around 20,000 species that can colonize most terrestrial environments (Ward 2006). With the rise of urbanization, many ant species have been unintentionally introduced into the urban environment, becoming tramp ants and raising significant concerns for public health and well-being (Rust and Su 2012). In addition, the control of pest ants is very costly. In recent years, pest ants have consistently ranked as the top pest in the structural pest control industry, generating an estimated $29.58 billion annually for pest management professionals in the United States (Angulo et al. 2022).

Many pest ant species can cause serious damage. *Camponotus* spp. are severe pests of wooden structures, causing replacement of 100-250 utility poles at a cost of 4-11 million dollars in New York and Pennsylvania along (Shields et al. 2000). In addition of being nuisance in residential structures, the Argentine ant, *Linepithema humile* (Mayr), can also cause economic damage on agricultural production by protecting honeydew-excreting hemipterans from natural enemy (Daane et al. 2007, Angulo et al. 2024). The red imported fire ant, *Solenopsis invicta* Buren. can sting and may cause severe sequelae, including hypersensitivity reactions, secondary infections, rare neurologic sequelae, and even impact mental health (Kemp et al. 2000, Wang et al. 2018). As one of the most distributed pest ant species, the Pharaoh ant, *Monomorium pharaonis* (L.), can infest most indoor environments and serve as mechanical vector for pathogenic organisms

(Alharbi et al. 2019). Other examples of pest ant species commonly found in the United States are *Paratrechina longicornis* (Latrielle)*, Pheidole megacephala* (Fabricius)*, Tetramorium caespitum* (L.)*, Anoplolepis gracilipes* (Smith) (Klotz et al. 2008).

Biology of ants

All ants are eusocial, exhibiting a division of labor, cooperative brood care, and overlapping generations (Klotz et al. 2008). The developmental stages of ants include eggs, larvae, pupae, adults. While all adults undergo metamorphosis, most members belong to the non-reproductive worker caste, and the rest belong to the reproductive caste (Ward 2006).

Ant colonies may be monogynous or polygynous. In monogynous ants such as *Leptothorax muscorum* (Nylander), only a single fertile queen is present in each colony, and workers tend to be more aggressive than those from polygynous colonies, which contain multiple queens (Heinze and Smith 1990, Fournier et al. 2016). This difference arises from genetic diversity: monogynous colonies have lower genetic diversity, whereas polygynous colonies exhibit higher genetic diversity, resulting in greater relatedness among individuals (Kellner et al. 2007, Fournier et al. 2016).

Worker ants can be either polymorphic or monomorphic. In polymorphic species like *S. invicta*, worker body size ranges from 1.6 mm to 5 mm, whereas monomorphic workers, such as those of *M. pharaonis*, are uniformly 2 mm long (Klotz et al. 2008). Workers perform essential colony functions, including brood care, nest organization, foraging, and defense. The task specialization is associated with genetic lineage, though

lineage and body size influence task allocation independently (Schwander et al. 2005). Thus, multiple factors shape task specialization.

The diet of ants varies, but most species prefer sugar-rich foods. Upon returning to the nest, workers feed directly on food, carry or store it in their crops, and share it with other colony members through trophallaxis. Both developmental stages and queens rely heavily on trophallaxis for nutrients, as they cannot and do not feed directly (Wilson 1971).

Sociotomy of ants

Sociotomy, or budding, in ants is the process by which a reproductively competent group—typically including workers, reproductives, and broods—separates from the parent colony and migrates to a new location to establish a new colony (Vail and Williams 1994). This behavior has been frequently documented in many tramp ant species which is crucial for colony survival, particularly under stressful conditions (Vails and Williams 1994).

In little fire ants, *Wasmannia auropunctata* (Roger), the main mode of colony formation relies on budding as all reproductives are genetically identical in nearby colonies (Fournier et al. 2005, Chifflet et al. 2018). The longhorn crazy ants, *P. longicornis*, abandon the original nest site when encounter physical disturbances (Kamarudin et al. 2011). In the white-footed ants, *Technomyrmex brunneus* Forel, because numerous intercastes, worker-like reproductives, exclusively mate within the

nest and enable rapid colony growth, colonies bud frequently to expand (Sunamura et al. 2025).

Monomorium pharaonis (L.)

Identification

Pharaoh ant workers are monomorphic, and they are approximately 2 mm long. Each antenna consists of 12 segments with a 3-segmented club (Klotz et al. 2008). The last 2-3 abdominal segments are black, and the rest of the body is orange (Klotz et al. 2008). Workers are often misidentified with thief ant, *Solenopsis molesta* Say., due to similar color and size. However, thief ants can be distinguished by their antennae, which have 10 segments with a 2-segmented club (Klotz et al. 2008). Queens are approximately 4 mm long, and the body color is primarily presented as rust-orange. Males are black and about 3 mm in length.

Distribution of *Monomorium pharaonis*

Linnaeus first described Pharaoh ants as *Formica pharaonis* in Egypt in 1758 (Bolton 1987). The origin of Pharaoh ants remains a topic of debate. Some researchers suggested that they originated in Africa (Bernard 1953, Fowler et al. 1993), while others argued that they originated in tropical Asia (Bolton 1987, Wetterer 2010). However, determining the origin of Pharaoh ants is challenging due to frequent misidentifications and limited sampling and information dissemination in the early years (Kohn and Vlcek 1985, Wetterer 2010).

Pharaoh ants are among the most widespread and successful tramp ant species worldwide. The primary infestation hotspots are Europe and the United States (Klotz et al. 2008, Wetterer 2010). Pharaoh ants have been recorded throughout the United States, except Alaska and Nevada (Wetterer 2010).

Biology of *Monomorium pharaonis*

Pharaoh ants exhibit key characteristics of tramp ants, including unicoloniality, high polygyny, lack of intraspecific aggression, small monomorphic workers, short lifespans, worker sterility, and reproduction via sociotomy (Passera 1994). These traits enable Pharaoh ants to rapidly establish a reproductively competent colony shortly after introduction to a new environment (Holway et al. 2002). In temperate regions, Pharaoh ants nest and forage exclusively indoors, although they may exhibit outdoor activity in warmer climates (Oi et al. 1994, Klotz et al. 2008, Wetterer 2010, Man and Lee 2012). Indoors, Pharaoh ants prefer inaccessible nesting sites, such as beneath wall panels, within attics, inside wall voids, and behind electrical outlets (Harris 1991, Vail 1996, Klotz et al. 2008). Additionally, they often establish nests close to water sources (Harris 1991).

Pharaoh ants feed on various food sources, including proteins, lipids, and carbohydrates (Haack et al. 1995, Chong et al. 2002, Eow and Lee 2007, Sarkar and Karmakar 2023). Among these, proteins and carbohydrates are the most frequently consumed nutrients. Carbohydrates provide energy for workers, while proteins are essential for raising sexual broods (Abbott 1978, Haack et al. 1995, Chong et al. 2002).

Lipids also serve as an energy source for workers and third instars, but their conversion into readily usable energy requires more effort (Chong et al. 2002). Edwards and Abraham (1990) observed that Pharaoh ants exhibited a significant preference for one food type after being fed another type for several weeks. Buczkowski et al. (2005) also reported a reduction of foraging activity in the Pharaoh ant colony after constantly being fed the same diet. This "satiation" and "alternation" response likely ensures the colony receives a varied and balanced nutrient intake.

Pharaoh ants exhibit central place foraging behavior, where foragers search for food, collect it, and return to the main nest to share it (Jackson et al. 2006). Vail and Williams (1994) reported that Pharaoh ants can forage up to 45 meters. Locating food sources and recruiting other workers from a distance requires pheromone trails along the way (Sudd 1960). In Pharaoh ants, at least 600 workers are needed to form a functional and attractive pheromone trail (Sumpter and Beekman 2003). However, 21% of workers cannot detect pheromone trails, while 17% can always locate them. These 17% of workers play a key role in reinforcing the pheromone trails as they utilize them (Jackson et al. 2006, Evison et al. 2012). Pharaoh ants' pheromone trails also provide polarity information (Jackson et al. 2006), a trait similarly observed in *Trigona spinipes* (F.), where polarized short odor-trails are used for recruitment communication (Nieh et al. 2004, Czaczkes et al. 2015). Additionally, Pharaoh ants produce aversive pheromones to deter other workers from following unrewarding foraging paths (Robinson et al. 2005). Beyond foraging, pheromone trails also play a key role in colony migration, potentially guiding the colony to new nesting sites (Evison et al. 2012).

Importance of *Monomorium pharaonis*

Pharaoh ants are among the most widespread and successful tramp ant species worldwide (Wetterer 2010). Due to their close association with human activity, they may raise serious health concerns about public health and well-being (Beatson 1972, Edwards and Baker 1981, Hölldobler and Wilson 1990, Lise et al. 2006, Alharbi et al. 2019). Pharaoh ants serve as mechanical vectors of pathogens in residential or healthcare facilities (Beatson 1972, Alharbi et al. 2019, Do Nascimento et al. 2020). Alharbi et al (2019) isolated 16 different species of Gram-negative bacteria from 2 residential districts, with the highest percentage of occurrence being *Shigella sonnei* (Levine)*, Acinetobacter lwoff* Brisou and Prevot*, Serratia marcescens* (Bizio)*,* and *Salmonella choleraesius* (Smith)*.* In hospital environments, pathogenic bacteria such as *Salmonella, Pseudomonas, Staphylococcus, Streptococcus,* and *Clostridium* were frequently found on the Pharaoh ants (Beatson 1972, Do Nascimento et al. 2020)*.* Pharaoh ant infestations occur frequently in hospitals due to constant temperature and humidity, intense flow of people, and sufficient food sources (Lutinski et al. 2015). In addition to serving as mechanical vectors for pathogenic bacteria, Pharaoh ant is an important source of inhalant allergens in bronchial asthma (Kim et al. 2005).

Caste composition and regulation

A reproductively competent Pharaoh ant colony often consists of fertile queens, workers, and mixed stage broods. The caste composition within each colony was reported by

Buczkowski and Bennett (2009) of a ratio of 2.48 eggs: 1.03 larvae: 1.94 pupae: 12.86 workers: 1 queen. However, this ratio is not fixed as Pharaoh ants may regulate caste composition to ensure rapid colonial growth (Buczkowski and Bennett 2009, Schmidt et al. 2011). The caste regulation is mainly affected by the presence of fertile queens (Edwards 1987, 1991, Tay et al. 2014, Boonen and Billen 2017). When fertile queens are present in the colony, they produce queen pheromones that signal workers to cannibalize sexual broods, preventing the development of new sexual adults (Edwards 1985, 1987, 1991, Oliveria et al. 2020). On the other hand, workers begin to rear sexual brood when the fecundity of queens drops, or fertile queens are absent (Edwards 1987, 1991, Eow et al. 2004). Edwards (1987) reported that JHA-treated queens lost the ability to inhibit sexual brood rearing, indicating that the inhibitory action of queens is mediated by the number of eggs they lay. Later, Eow et al. (2004) proposed that this inhibitory action was only present in the earlier colony development as the number of queens exponentially increased as the observation period extended, causing the author to suggest both worker population and brood quantity affect the number of fertile queens in the colony. However, Tay et al. (2014) revealed that brood quantity has a limited impact on the final queen population, and worker population has no effect on the queen population. It is the initial number of queens in an incipient colony that significantly affects the queen population growth (Tay et al. 2014).

The fecundity of fertile queens of Pharaoh ants depends on the presence of larvae (Børgesen and Jensen 1995, Børgesen 2000, Klotz et al. 2008). Børgesen and Jensen (1995) found that the number of eggs laid is positively correlated with the number of

larvae in the colony, and queens maintain low levels of egg production in the absence of larvae. Queens without larvae feed on larval secretions collected and stored in the crops of workers to delay the decline in fecundity. Later, Børgesen (2000) revealed lack of larval secretion inhibits the uptake of vitellogenin by the ovarioles, thereby disrupting oocyte development.

Sociotomy in *Monomorium pharaonis*

As a tramp ant species, Pharaoh ant colonies reproduce through budding (Edwards and Baker 1981, Passera 1994, Alharbi et al. 2019). Budding in Pharaoh ants is often triggered by disturbances such as interspecific aggression, insufficient resources, and physical or chemical disturbances (Buczkowski et al. 2005, Buczkowski and Bennett 2009, Tay and Lee 2015). To develop a reproductively competent colony fragment through budding, only 50 workers and at least 1 fertile queen are required while the presence of brood is not necessary (Tay et al. 2014). This behavior plays a crucial role in colony success, particularly when survival is threatened, making Pharaoh ant infestation management challenging.

Control of *Monomorium pharaonis*

To control Pharaoh ants, it is desirable to eliminate the entire or the majority of the colony within a building structure (Oi et al. 2000). However, infestation of Pharaoh ants often involves multiple colony fragments, and the colonies are often hidden and nest in

inaccessible areas (Harris 1991, Vail 1996, Klotz et al. 2008), which makes control strategies limited.

Current control methods for Pharaoh ants emphasize baiting with active ingredients that have delayed toxicity, as this approach allows toxicants to be thoroughly distributed throughout the colony via trophallaxis (Oi et al. 2000). Common active ingredients used in Pharaoh ant baits include juvenile hormone analogs (JHAs), fipronil, neonicotinoid, etc. (Lee 2000, Stanley 2004, Rupes et al. 2008). In addition, indoxacarb bait is also commonly employed in pest ant infestations due to its delayed toxicity (Stanley 2004).

JHAs are compounds that mimic the function of juvenile hormones and act on the insect endocrine system to prevent insect maturation (Sullivan and Goh 2008). Due to their delayed toxicity, JHAs are frequently employed in Pharaoh ant control. The most commonly applied JHAs against Pharaoh ants—methoprene, fenoxycarb, and pyriproxyfen—have been shown to reduce egg production in fertile queens (Edwards 1975, Williams and Vail 1993, Lee et al. 2003, Tay and Lee 2014). In addition, methoprene and pyriproxyfen have been reported to cause abnormalities in ovary morphology and disrupt oocyte development (Edwards 1975, Tay and Lee 2014).

Fipronil is a neurotoxin that binds to GABA receptor and block the chloride channel, which prevents hyperpolarization to reach inhibitory postsynaptic potential and cause central nervous system hyperexcitability, ultimately leads to death. Fipronil-based baits have demonstrated effective control outcomes against pest ants, including Pharaoh ants (Lee 2000).

Neonicotinoids compete with acetylcholine, a neurotransmitter that initiates neuro signaling, and act as a nAChR competitive modulator. Common neonicotinoids used in baits include imidacloprid, clothianidin, and thiamethoxam. Rupes et al. (2008) reported a successful control outcome against Pharaoh ants using imidacloprid baits from 0.001 to 0.1%.

Indoxacarb is a relatively new insecticide active ingredient produced by DuPont and registered under EPA in the US in 2000 (Stanley 2004). This compound can be readily metabolized into DCJW by esterase. This metabolite acts as a sodium channel inhibitor and disrupts insect neuron signaling. Because only the metabolite form of indoxacarb is toxic, the toxicant can be thoroughly distributed within the colony via trophallaxis.

Most residual insecticide sprays are not suitable for Pharaoh ant infestation management because such products would only kill a small portion of foragers, and the colony would split and migrate if the Pharaoh ants experience any chemical disturbance, thereby spreading the infestation (Buczkowski et al. 2005, Tay and Lee 2015). However, nonrepellent residual spray with acute toxicity was proven to be an effective control method against Pharaoh ants (Buczkowski et al. 2005). Buczkowski et al. (2005) revealed fipronil residual insecticide spray was able to rapidly eliminate Pharaoh ant colonies without triggering budding.

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Chapter 2: Effects of Over-the-Counter Insecticidal Aerosol Products on Sociotomy of the Pharaoh Ant, *Monomorium pharaonis* (Hymenoptera: Formicidae)

Abstract

The Pharaoh ant, *Monomorium pharaonis* (L.), is a widely distributed invasive tramp ant species. Due to their ability to frequently relocate nest sites when disturbed, Pharaoh ants are particularly challenging to manage. This study examined the colony movement patterns of different colony sizes of the Pharaoh ant in response to chemical disturbances caused by varying over-the-counter aerosol insecticide application rates. Three aerosol products, three application rates, and four colony sizes were tested. Essential oil-based insecticides exhibited a higher probability of inducing budding at all colony sizes when applied at a high rate. In contrast, pyrethroid-based insecticides increased the probability of inducing budding at a low application rate. The number of workers present outside the main nest in the essential oil-based insecticide-treated group decreased with increasing application rate, whereas the number of workers present outside the main nest increased in pyrethroid-based insecticide-treated groups. Furthermore, the density of workers outside the main nest appeared to play a role in influencing the final nest site after budding. The number of worker ant cadavers observed on pyrethroid-treated surfaces was significantly greater than on surfaces treated with the essential oil-based insecticide and controls. The effects of aerosol product type, colony size, and insecticide application rate on Pharaoh ant colony movement are discussed.

Introduction

The Pharaoh ant, *Monomorium pharaonis* (L.), is an important indoor pest ant species known to infest residential and commercial structures. It exhibits tramp ant characteristics such as true polygyny, small and sterile workers, short queen lifespan, and colony reproduction through budding (Edwards and Baker 1981, Passera 1994, Alharbi et al. 2019). Due to its synanthropic nature, the Pharaoh ant can be easily dispersed to new environments to establish a colony, and when disturbed, it splits or relocates the nest location (Hölldobler and Wilson 1990, Lutinski et al. 2015).

Sociotomy, or budding, in ants refers to the process by which a reproductively competent group—typically comprising workers, reproductives, and broods—separates from the parent colony and migrates to a new location to establish a new colony (Vail and Williams 1994). This behavior has been frequently documented in many tramp ant species, such as *Solenopsis invicta* Buren, *Linepithema humile* (Mayr), *Paratrechina longicornis* (Latreille), and *M. pharaonis* (L.) (Vargo and Porter 1989, Passera 1994, Heinze and Tsuji 1995, Suarez et al. 2001, Buczkowski and Bennett 2009a, Kamarudin et al. 2011). Sociotomy is vital for colony survival, especially under stressful conditions (Vail and Williams 1994).

Budding in Pharaoh ants is often triggered by factors such as interspecific competition, aggression, insufficient resources, and physical or chemical disturbances (Buczkowski et al. 2005, Buczkowski and Bennett 2009a, Tay and Lee 2015). They frequently nest in inaccessible areas, such as beneath wall panels, in attics, inside wall voids, and behind electrical outlets (Harris 1991, Fowler et al. 1993, Vail 1996, Klotz et

al. 2008), making Pharaoh ants particularly challenging to control. Control strategies, such as barrier treatments with high-repellency insecticides, can create unwanted disturbances that trigger budding, thereby spreading the infestation (Buczkowski et al. 2005).

Homeowners and tenants often try to manage pest control on their own by using over-the-counter (OTC) insecticide aerosols instead of hiring professional pest control services (Lee 2002, Carlton et al. 2004, Wang and Bennett 2010). While professional services achieve better pest control outcomes, many consumers are deterred by the cost (Wang and Bennett 2010, DeVries et al. 2019). OTC aerosols are popular due to their affordability, availability, and straightforward application (Bennett et al. 1983, DeVries et al. 2019). However, most OTC aerosols available contain pyrethroids, which can induce budding in Pharaoh ants, potentially spreading infestations (Buczkowski et al. 2005, Tay and Lee 2015).

Understanding how abiotic and biotic factors influence budding behavior is essential for designing effective pest management strategies for the Pharaoh ant. Active ingredients, such as cinnamaldehyde, cypermethrin, and bifenthrin triggered colony budding (Buczkowski et al. 2005). Subsequently, Buczkowski and Bennett (2009a) examined the effects of various physical disturbances on Pharaoh ant colony budding and the population dynamics of colony fragments. Tay and Lee (2015) investigated the causes of colony budding, evaluating factors such as physical and chemical disturbances, interspecific competition, and resource depletion. Evison et al. (2012) reported that Pharaoh ants use pheromone trails produced by foragers to select their potential budding

sites. However, these studies were conducted using standard subcolonies of Pharaoh ants of similar colony sizes. In the field, Pharaoh ant colonies can vary in size, ranging from as few as five workers and fifty pieces of brood to a colony of ten thousand workers (Klotz et al. 2008).

In this study, we examined how colony size and aerosol application rates impact the budding of the Pharaoh ant. We employed three widely used commercial insecticide aerosols and monitored their effects on the colony over time.

Materials and Methods

Insects

The study was conducted using *M. pharaonis* obtained from the USDA-ARS, Center for Medical, Agricultural, & Veterinary Entomology in Gainesville, Florida, in 2020. The ants were reared under laboratory conditions of 27 ± 1°C, 70 ± 2% RH, and a 12:12 L:D cycle in arenas coated with fluon at the University of California, Riverside, over a span of four years. Water and a 10% sucrose solution were provided *ad libitum*, and Tetra Goldfish Flakes (Tetra GmbH, Melle, Germany), along with raw tuna obtained from Costco, served as protein sources and were provisioned weekly.

Experimental design

The experimental arena comprised six fluon-coated polyethylene containers (8.7 cm in diameter, 7.8 cm in height), including one central container A, which was connected to four other containers C–F by one-meter lengths of Tygon tubing that served as potential

budding sites. Container B linked to container A through a 5 cm length of Tygon tubing and contained treated filter paper (Fig. 1). All Tygon tubes were sealed with cotton until the end of the acclimatization period to prevent the ants from beginning to bud immediately after being introduced to the setup, which could happen due to physical disturbances.

All containers, except for container B, were provisioned with 0.03 g of canned tuna (Kirkland Signature, Issaquah, WA) and boiled egg yolk on a weekly basis. Water and a 10% sucrose solution were provided *ad libitum*. Additionally, each container included a small piece of corrugated paper (5 × 5 cm) for shelter. All containers, except for container B, were designed identically. All Tygon tubes were thoroughly cleaned using laboratory decontaminant DECON 90 (Decon Laboratories, Hove, England) before the experiment to eliminate any potential chemical residues from the manufacturing process that could influence the results.

In the experimental setup, a subcolony of Pharaoh ants with the following composition was used: (A) 100 workers, 10 mg of mixed brood stage, and 1 queen; (B) 200 workers, 25 mg of mixed brood stage, and 2 queens; (C) 400 workers, 50 mg of mixed brood stage, and 3 queens; (D) 800 workers, 100 mg of mixed brood stage, and 4 queens. The insects were placed in central container A to acclimate for 7 days.

Three common household aerosol products were examined: Zevo Crawling Insect Killer - Ants, Roaches, Spider (geraniol 1.3%, cinnamon oil 0.2%, The Procter & Gamble Company, Cincinnati, OH), Raid Ant Killer 26 (imiprothrin 0.06%, cypermethrin 0.1%, S.C. Johnson & Son, Racine, WI), and Black Flag Dry Ant and Roach Killer (imiprothrin

0.1%, gamma-cyhalothrin 0.025%, Spectrum Brands, Madison, WI). Each aerosol product was applied to a piece of filter paper (5 x 7.5 cm) for 1, 2, or 3 seconds. The insecticide dispense rates for the tested aerosol products were assessed by subtracting the product's weight after 1 second of application from its weight before application. This process was repeated 10 times for each aerosol product. The dispense rates were relatively consistent across all tested products (Zevo: 2.84 ± 0.08 g/s; Raid: 2.84 ± 0.05 g/s; Black Flag: 2.97 ± 0.02 g/s). For the control, water was applied to the filter papers. The treated filter paper was left in the fume hood to dry for 2 hours before being placed in container B at the end of the acclimatization period. The average number of workers present outside the main nest over six weeks was assessed by counting the total number of workers in all unoccupied containers (no nest present) once a week and calculating the weekly mean. Colony movement was evaluated based on which potential budding site (container C-F) the reproductively competent colony fragments occupied. A reproductively competent colony fragment, produced via budding, is defined as a colony fragment consisting of approximately 50 or more workers, at least one queen, and/or the presence of brood (Peacock et al. 1955, Vail and Williams 1994, Buczkowski et al. 2005, Tay et al. 2014). To determine whether the chemical cue reached the main nest and triggered budding, we assessed the repellency and lethality of treated surfaces based on the weekly mean percentage of Pharaoh ant workers in container B over a six-week period and the number of worker cadavers observed in container B at the end of the study, respectively. We chose to record the number of worker cadavers in container B because fewer worker cadavers would indicate lower lethality of the treated surface, and

most workers could return to the main nest and deliver chemical disturbances, or that they were repelled and never contacted the treated surface. Conversely, more worker cadavers would suggest the opposite. The weekly mean percentage of workers present in container B was assessed by recording the number of live ants in container B once a week over the six-week period. A higher worker presence in container B indicates low repellency and possibly lower toxicity, while a lower presence indicates strong repellency. Worker cadavers remained in container B until the total count was recorded at the end of the study. Studies have shown that the number of live workers present in specific locations might relate to where colonization occurs (Fowler et al. 1993, Evison et al. 2012). To determine whether the number of workers present at each of the four potential budding sites would influence the final budding location, the highest number of workers recorded at each site over the six-week period was compared to the final budding location. The weekly mean percentage of live workers present outside the main nest and in container B were collected together in the morning on days 1, 3, 5, and 7 and then weekly for five weeks. Three replicates were conducted for each treatment.

Statistical analysis

To examine the effect of colony sizes, aerosol products, and insecticide application rates on the response of each replicate colony, we performed a discrete-time survival analysis to evaluate the probability of budding occurring during the six-week period (Suresh et al. 2022). We first modeled the hazard probability with a logit link to estimate how different factors affect the timing of the event occurring in each treated and untreated group. The

hazard probability at week t is expressed as: $h(t)=1-(1/(1+\exp(-\eta)))$, where η is the linear predictor from the model (Suresh et al. 2022). After estimating the hazard probability at each time point, we converted hazard probability to survival probability S(t) to determine the cumulative probability of budding not occurring using $S(t) = S(t-1) * (1-h(t))$ (Suresh et al. 2022). Finally, to calculate the cumulative probability of budding occurring, we converted S(t) to C(t) using $C(t)=1-S(t)$.

To determine the effect of the number of workers at each potential budding site on the location of the final nest site after budding, we calculated the percentage of present workers as the highest number of workers observed during the experiment at each potential budding site, divided by the initial number of workers in the corresponding colony size (100, 200, 400, or 800 workers), and analyzed this using binary logistic regression. The weekly mean percentage of workers present outside the main nest across six weeks (the percentage of workers present outside the nest at each time point was calculated as the total number of workers in all unoccupied containers divided by the initial number of workers in the corresponding colony size; the weekly mean percentage was then determined by averaging these percentages across all time points), the weekly mean percentage of workers present in container B during the experiment (the percentage of workers present in container B at each time point was calculated as the number of live workers in container B divided by the initial number of workers in the corresponding colony size; the weekly mean percentage was then found by averaging these percentages across all time points), and the percentage of cumulative ant cadavers in container B (the number of worker cadavers in container B at the end of the experiment divided by the

initial number of workers in the corresponding colony size) were transformed into arcsine square roots and subjected to one-way ANOVA. Mean values were compared using the Least Significant Difference (LSD) test. Discrete-time survival analysis was conducted using the Survival package (Therneau 2024), and a graph of budding probability was generated using ggplot2 in R (Wickham 2016). Binary logistic regression and one-way ANOVA were performed using IBM SPSS Version 29.0.

Results

Colony movement

Colony size did not significantly affect the discrete-time survival model across 3 different insecticide application rates and aerosols ($P > 0.05$), indicating colony size does not influence Pharaoh ants' response to chemical disturbances. At the 1-second treatment, all aerosols induced a higher probability of budding of Pharaoh ant colony from week 1 to week 6 (Fig. 2A), with Black Flag and Raid showing significantly higher probabilities of budding ($Z = 3.48$, $P < 0.001$; $Z = 2.10$, $P < 0.05$). Deposits of imiprothrin and cypermethrin and imiprothrin and cyhalothrin were more likely to induce and facilitate budding than the geraniol and cinnamon oil treated papers and controls. At the 2-second treatment, all aerosols showed a higher probability of budding over the same period (Fig. 2B). Compared to Zevo's performance in the 1-second treatment, its probability of budding increased and became significantly higher than the controls ($Z = 2.39$, $P < 0.05$). Additionally, the budding probability of colonies treated with Zevo overlapped with that of Black Flag (Fig. 2B), which was also significantly higher than the controls ($Z = 2.42$,

$P < 0.05$). At the 3-second treatment, the budding probabilities of colonies treated with Zevo- and Raid-treated groups was significantly higher than the budding probability of controls ($Z = 2.85$, $P < 0.05$; $Z = 2.26$, $P < 0.05$). In contrast, the budding probability of Black Flag-treated groups significantly decreased ($Z = -3.613$, $P < 0.001$) and became statistically insignificantly different from that of the controls (Fig. 2C), indicating Black Flag had no significant effect on colony movement at the 3-second treatment.

Presence of workers outside the main nest

At the 1-second treatment, the residual deposit of no aerosol caused a significant difference in the percentage of workers present outside the main nest compared to the controls in colony sizes A and C (Table 1). The Zevo-treated group exhibited significantly fewer workers outside the nest in colony size B, while the Raid- and Black Flag-treated groups showed significantly fewer workers in both colony sizes B and D (Table 1). At the 2-second treatment, Zevo resulted in significantly more workers being present outside the nest compared to the controls in colony size A and significantly fewer in colony size D. Raid also showed significantly fewer workers outside the nest in colony size D, whereas Black Flag had no significant effect on worker presence outside the nest in any colony size (Table 1). At the 3-second treatment, no aerosol product caused a significant difference in the percentage of workers present outside the nest compared to the controls in colony sizes A and B (Table 1). However, Raid and Black Flag resulted in significantly fewer workers outside the nest in colony size C (Table 1). Additionally, the Zevo-treated group showed a significant decrease in worker presence compared to the 1-

second treatment in colony size D, ultimately leading to significantly fewer workers being present outside the nest than the controls (Table 1).

In container B, all tested aerosol products significantly reduced the percentage of Pharaoh ant workers on treated surfaces across all colony sizes and insecticide application rates compared to the controls (Table 2).

Number of worker cadavers observed on treated surfaces

Across all tested aerosol products and insecticide application rates in colony size A, the number of worker cadavers in container B was not significantly different from that in the controls (Table 3). In the Raid-treated groups, the number of worker cadavers in container B was significantly higher than in the controls for colony sizes B, C, and D at all tested insecticide application rates (Table 3). The Black Flag-treated groups initially showed no significant difference in the number of worker cadavers across all colony sizes at the 1-second treatment (Table 3). However, at the 2- and 3-second treatments, significantly more worker cadavers were observed in colony sizes C and D, and in colony sizes B, C, and D, respectively. In contrast, the Zevo-treated groups showed no significant differences in the number of worker cadavers across all insecticide application rates and colony sizes (Table 3).

Budding activity

Colony size was not a significant predictor in the logistic regression model for any of the tested aerosol products, indicating that different colony sizes did not influence budding

activity (Table 4). Insecticide application rate was a significant predictor only in Black Flag-treated groups. The number of workers present at each potential budding site significantly influenced the logistic regression model in the controls and Raid-treated groups ($B = 0.328$, $df = 1$, $P < 0.001$; $B = 0.291$, $df = 1$, $P < 0.001$), suggesting that locations with more workers were more likely to become the final budding sites in those groups. Although potential budding sites with a higher number of workers also had an increased likelihood of becoming the final nest location in Black Flag-treated groups, this effect was not statistically significant ($B = 0.132$, $df = 1$, $P = 0.067$).

Discussion

Our results showed that colonies typically took 1 to 3 weeks to respond to the treatment. In contrast, Tay and Lee (2015) demonstrated that directly applying deltamethrin to Pharaoh ant workers and introducing them into the colony led to immediate nest relocation within a day. This difference in response time indicates that the chemical disturbance was not immediately delivered to the colony in this study. It could also be attributed to differences in the experimental setup. Our results showed that all tested aerosol products significantly reduced the exploratory activity of Pharaoh ant workers on treated surfaces (Table 2). However, significantly more ant cadavers were observed in container B in the Raid- and Black Flag-treated groups compared to the Zevo-treated groups and the controls (Table 3), suggesting that Zevo exhibited strong repellency and relatively low contact toxicity, while Raid and Black Flag demonstrated strong contact toxicity. This finding aligns with previous research indicating that pyrethroids act as

contact irritants rather than spatial repellents, like essential oil components do (Yoon et al. 2009, Huang et al. 2020, Gaire et al. 2024). Additionally, it was observed that Pharaoh ant colonies may relocate again after budding to a new site because the queens occasionally moved to other locations. Since the presence of a queen is essential for the development of reproductively competent colonies, the presence of colony fragments fluctuated.

While the changes in the percentage of workers present outside the nest in colony sizes A, B, and C were inconsistent across all insecticide application rates following treatment with different aerosol products, a significantly lower presence of workers was predominantly observed in colony size D. Previous studies have demonstrated that colony sizes do not impact the percentage of Pharaoh ants present outside the nest under control conditions (Lee and Lee 2002, Buczkowski et al. 2005). A similar phenomenon was likewise seen in *Tapinoma sessile* Say, where colony size does not significantly influence foraging activity (Buczkowski and Bennett 2009b). This discrepancy suggests that the tested aerosols primarily affect exploratory behavior in large Pharaoh ant colonies. Since at least 600 Pharaoh ant workers are needed to establish a functional, attractive pheromone trail (Sumpter and Beekman 2003), the presence of workers outside the main nest was less responsive to treatments in colony sizes A, B, and C due to their smaller size.

The presence of more workers significantly influenced the location of final nest sites after budding in Pharaoh ants, as these ants use pheromone trails to relocate to potential nest sites (Evison et al. 2012). Fowler et al. (1993) also observed clustering of

colonization attempts at specific locations, suggesting that colonization may be predictable. In this study, we found that Pharaoh ants tended to bud into potential nesting sites that had previously been visited by more workers under control conditions (Table 4). The same phenomenon was observed after Pharaoh ant colonies were treated with Raid (Table 4). In Black Flag-treated groups, the insecticide application rate was highly correlated with colony movement (Fig. 2C), which poses a key problem in the binary logistic regression model when explanatory variables are highly correlated, creating multicollinearity (Senaviratna and Cooray 2019). As a result, the insecticide application rate became a significant predictor, while the percentage of workers present did not. In contrast, neither predictor significantly influenced the location of the final budding site in Zevo-treated groups, most likely because Zevo induced budding more rapidly than Raid and Black Flag, preventing pheromone trail formation.

The colony movement for each tested aerosol exhibited distinct trends in response to increasing application rates. Previous studies have reported that essential oils show increasing repellency with higher insecticide application rates (Xu 2012), and a similar phenomenon has also been observed with pyrethroids (Wen et al. 2022). In the Zevo-treated group, Pharaoh ant colonies displayed increased movement at higher application rates. In contrast, the Raid- and Black Flag-treated groups, particularly the latter, showed decreased movement across all colony sizes. Other studies have indicated delayed budding behavior following pyrethroid treatment (Buczkowski et al. 2005, Knight and Rust 1990). This phenomenon could be attributed to differences in the properties of the active ingredients. Essential oil components act as true spatial repellents, whereas

pyrethroids function as contact irritants (Yoon et al. 2009, Huang et al. 2020, Gaire et al. 2024). In the Zevo-treated group, the number of worker cadavers on treated surfaces was not significantly different from the controls (Table 3), and significantly fewer live ants were observed in container B (Table 2), indicating that Zevo-treated surfaces had low toxicity but strong repellency. In contrast, the Raid- and Black Flag-treated groups showed significantly more worker cadavers on treated surfaces (Table 3), suggesting that workers were not repelled but contacted the treated surface, became irritated, and were ultimately knocked down and killed, resulting in fewer contaminated workers returning to the nest, thereby delaying nest relocation.

This study investigated the movement patterns of Pharaoh ant colonies of various sizes under different insecticide application rates using OTC aerosols. Aerosol products provide several advantages, including ease of acquisition, simplicity of use, affordability, and immediate lethality, making them the most commonly used formulation in household settings (Bennett et al. 1983, Lee 2002). However, effective control of Pharaoh ants necessitates the elimination of the entire colony, yet these ants often nest in inaccessible locations. Most residual insecticide sprays are not effective for managing Pharaoh ant infestations because they only kill a small portion of foragers. Additionally, if Pharaoh ants sense chemical disturbances, the colony may fragment and migrate, further spreading the infestation (Oi et al. 2000, Buczkowski et al. 2005, Tay and Lee 2015). Current control methods for Pharaoh ants focus on baiting with active ingredients that have delayed toxicity, allowing toxicants to be thoroughly distributed throughout the colony via trophallaxis with minimal disturbances (Oi et al. 2000).

In addition to selecting appropriate control strategies, effective management of Pharaoh ant infestations also relies on inspection and monitoring to detect the presence of the pest and assess the effectiveness of these strategies. Pest ant monitoring is often performed by counting the number of ants found on index cards with food at various locations within an infested structure (Oi et al. 1994, Oi et al. 1996). Given that locations with more worker ants visiting are more likely to become final budding sites, this information can be valuable for optimizing bait placement in Pharaoh ant control, as colonies situated closer to toxic bait are more likely to be impacted (Oi et al. 2000). In contrast, aerosol sprays primarily affect exposed foragers and do not reach hidden nest sites, highlighting the limitations of over-the-counter (OTC) aerosol sprays and the importance of baits in managing Pharaoh ant infestations.

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Figures and Tables

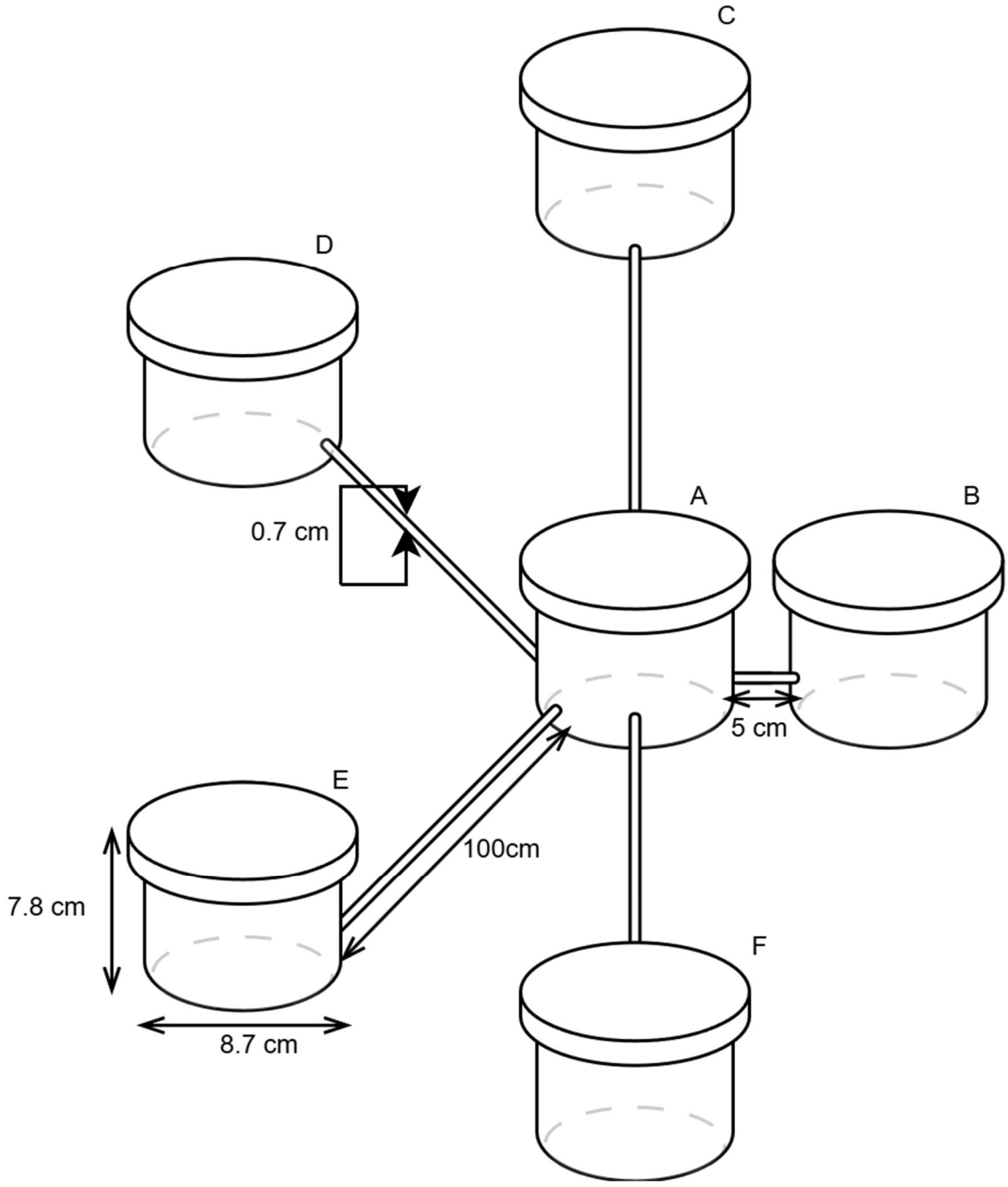


Fig. 1. The experimental setup used in this study. The polyethylene containers are labeled as: (A) Original nest site; (B) Insecticide application site; (C-F) Potential budding sites.

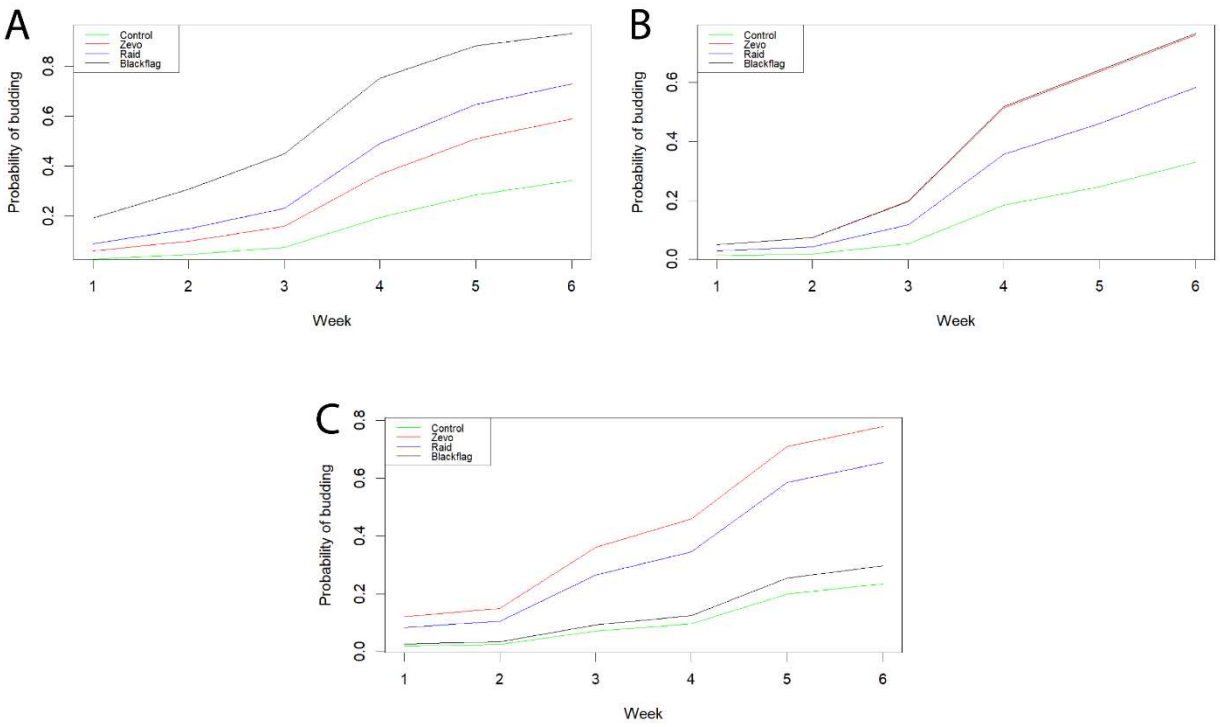


Fig. 2. Probability of ants budding in response to residual deposits of aerosol over 6 weeks. (A) Probability of ants budding in response to residual deposits from a 1-second treatment. (B) Probability of ants budding in response to residual deposits from a 2-second treatment. (C) Probability of ants budding in response to residual deposits from a 3-second treatment.

Table. 1 Weekly mean percentage of workers outside the main nest observed in all nest sites during the 6 weeks at 3 insecticide application rates in response to different aerosol products.

Aerosol	Active ingredients	Application Rate	Mean % workers ± SEM[1,2,3]			
			Colony size[4]			
			A	B	C	D
Control	Water	1s	8.9±1.9 a (a) [a]	6.7±1.0 a (a) [a]	5.1±0.8 a (a) [a]	6.8±1.1 a (a) [a]
		2s	3.1±0.7 ab (b) [a]	3.6±0.8 ab (b) [a]	3.8±0.9 a (a) [a]	6.8±1.2 a (a) [b]
		3s	3.1±0.6 a (b) [a]	4.0±0.9 a (b) [ab]	4.2±0.8 a (a) [ab]	5.3±0.7 a (a) [b]
Zevo	geraniol 1.3% cinnamon oil 0.2%	1s	3.7±0.8 a (ab) [a]	3.6±0.7 b (a) [a]	4.2±0.8 a (a) [a]	4.4±0.8 ab (a) [a]
		2s	7.3±1.5 c (a) [a]	1.9±0.5 b (a) [b]	2.3±0.6 a (b) [b]	2.7±0.5 b (ab) [b]
		3s	3.1±1.1 a (b) [a]	5.3±1.6 a (a) [a]	4.0±0.9 ab (ab) [a]	2.1±0.5 b (b) [a]
Raid	imiprothrin 0.06% cypermethrin 0.1%	1s	5.0±1.0 a (a) [a]	2.5±0.7 b (a) [b]	3.1±0.8 a (a) [ab]	1.6±0.4 c (a) [b]
		2s	1.3±0.4 a (b) [a]	4.7±1.0 a (a) [b]	2.5±0.6 a (a) [ab]	3.0±0.6 b (a) [b]
		3s	2.0±0.5 a (b) [a]	3.2±0.8 a (a) [a]	2.4±0.7 b (a) [a]	4.7±1.4 ab (a) [a]
Black Flag	imiprothrin 0.1% gamma-cyhalothrin 0.025%	1s	4.9±1.0 a (a) [a]	3.3±0.7 b (a) [a]	4.1±0.9 a (a) [a]	2.7±0.5 bc (a) [a]
		2s	4.6±1.0 bc (a) [a]	3.5±0.7 ab (a) [a]	3.7±0.6 a (a) [a]	4.5±1.1 ab (a) [a]
		3s	2.8±0.5 a (a) [a]	3.3±0.8 a (a) [a]	1.9±0.3 b (a) [a]	3.3±0.8 ab (a) [a]

[1]Means followed by the same letter under the same application rate and colony size with different aerosol products indicate no significant difference (P > 0.05; LSD).
[2]Means followed by the same letter in parentheses "( )" under the same aerosol product and colony size with different application rates indicate no significant difference (P > 0.05; LSD).
[3]Means followed by the same letter in parentheses "[ ]" under the same aerosol product and application rate with different colony sizes indicate no significant difference (P > 0.05; LSD).

[4]Colony sizes are labeled as: (A) 1 queen: 100 workers: 0.01 g brood; (B) 2 queens: 200 workers: 0.025 g brood; (C) 3 queens: 400 workers: 0.05 g brood; (D) 4 queens: 800 workers: 0.1 g brood.

Table. 2 Weekly mean percentage of live workers observed in container B during the 6 weeks at 3 insecticide application rates in response to different aerosol products.

Aerosol	Active ingredients	Application Rate	Mean % workers ± SEM[1,2,3]			
			Colony size[4]			
			A	B	C	D
Control	Water	1s	3.1±0.9 a (a) [a]	1.6±0.4 a (a) [a]	1.3±0.3 a (a) [a]	3.0±0.9 a (a) [a]
		2s	1.2±0.4 a (b) [a]	2.1±0.6 a (a) [a]	1.8±0.7 a (a) [a]	1.1±0.4 a (b) [a]
		3s	1.6±0.5 a (ab) [a]	1.8±0.5 a (a) [a]	2.3±0.6 a (a) [a]	2.6±0.6 a (a) [a]
Zevo	geraniol 1.3% cinnamon oil 0.2%	1s	0.1±0.1 b (a) [a]	0.1±0.1 b (a) [a]	0.2±0.2 b (a) [a]	0.2±0.1 b (a) [a]
		2s	0±0 b (a) [a]	0.1±0.1 b (a) [a]	0±0 b (a) [a]	0.1±0.1 b (a) [a]
		3s	0.2±0.1 b (a) [a]	0±0 b (a) [a]	0.1±0.1 b (a) [a]	0.1±0.1 b (a) [a]
Raid	imiprothrin 0.06% cypermethrin 0.1%	1s	0.2±0.1 b (a) [a]	0.2±0.1 b (a) [a]	0.3±0.2 b (ab) [a]	0.4±0.2 b (a) [a]
		2s	0.2±0.1 b (a) [a]	0.2±0.1 b (ab) [ab]	0.4±0.1 c (a) [b]	0.2±0.1 b (ab) [ab]
		3s	0.2±0.1 b (a) [a]	0±0 b (b) [b]	0.1±0.1 b (b) [ab]	0.1±0.1 b (b) [ab]
Black Flag	imiprothrin 0.1% gamma-cyhalothrin 0.025%	1s	0±0 b (a) [a]	0.1±0.0 b (a) [a]	0±0 b (a) [a]	0±0 b (a) [a]
		2s	0.1±0.1 b (a) [a]	0±0 b (a) [a]	0±0 b (a) [a]	0±0 b (a) [a]
		3s	0±0 b (a) [a]	0±0 b (a) [a]	0±0 b (a) [a]	0±0 b (a) [a]

[1]Means followed by the same letter under the same application rate and colony size with different aerosol products indicate no significant difference (P > 0.05; LSD).
[2]Means followed by the same letter in parentheses "( )" under the same aerosol product and colony size with different application rates indicate no significant difference (P > 0.05; LSD).

[3]Means followed by the same letter in parentheses "[ ]" under the same aerosol product and application rate with different colony sizes indicate no significant difference (P > 0.05; LSD)
[4]Colony sizes are labeled as: (A) 1 queen: 100 workers: 0.01 g brood; (B) 2 queens: 200 workers: 0.025 g brood; (C) 3 queens: 400 workers: 0.05 g brood; (D) 4 queens: 800 workers: 0.1 g brood.

Table. 3 Mean percentage of cumulative worker cadavers observed in container B at 3 application rates in response to different aerosol products after 6 weeks.

Aerosol	Active ingredients	Application Rate	Mean % cumulative worker cadavers ± SEM[1,2,3]			
			Colony size[4]			
			A	B	C	D
Control	Water	1s	15.7±4.5 a (a) [a]	19.2±3.3 a (a) [a]	10.8±4.1 a (a) [a]	16.3±4.1 a (a) [a]
		2s	16±4.5 a (a) [a]	11.3±2.2 a (ab) [a]	12.2±3.3 a (a) [a]	11.7±4.4 a (a) [a]
		3s	16.3±2.6 a (a) [a]	7.2±0.3 a (b) [b]	8.5±2 a (a) [b]	7.7±2.7 a (a) [b]
Zevo	geraniol 1.3% cinnamon oil 0.2%	1s	17.3±9.1 a (a) [a]	16.7±7.0 a (a) [a]	20.4±9.8 a (a) [a]	11.2±6.1 a (a) [a]
		2s	14.7±11.3 a (a) [a]	21.2±4.2 ab (a) [a]	15.8±3.4 ab (a) [a]	14.2±6.4 ab (a) [a]
		3s	31±15.5 a (a) [a]	8.5±3.8 a (a) [a]	13.9±5.2 ab (a) [a]	19.7±1.4 ab (a) [a]
Raid	imiprothrin 0.06% cypermethrin 0.1%	1s	26±12.5 a (a) [a]	39±19.5 b (a) [b]	28.3±3.5 a (a) [a]	41.1±1.3 b (a) [ab]
		2s	34±4.2 a (ab) [ab]	25.2±3.7 b (b) [a]	39.8±3.6 c (a) [b]	30.7±5.6 bc (a) [ab]
		3s	47±4.0 a (b) [a]	29.2±3.3 b (b) [a]	29.8±8.5 bc (a) [a]	26.8±7.2 b (a) [a]
Black Flag	imiprothrin 0.1% gamma-cyhalothrin 0.025%	1s	26.7±3.8 a (a) [a]	24.7±8.2 a (a) [a]	29.4±10.9 a (a) [a]	21.7±1.3 a (a) [a]
		2s	20±4.7 a (a) [a]	20.8±4.4 ab (a) [a]	24.8±4.4 b (a) [a]	34.7±5.3 c (a) [a]
		3s	21.3±13.4 a (a) [a]	32±6.4 b (a) [a]	38.5±7.3 c (a) [a]	25.4±7.2 b (a) [a]

[1]Means followed by the same letter under the same application rate and colony size with different aerosol products indicate no significant difference (P > 0.05; LSD).

[2]Means followed by the same letter in parentheses "( )" under the same aerosol product and colony size with different application rates indicate no significant difference (P > 0.05; LSD).
[3]Means followed by the same letter in parentheses "[ ]" under the same aerosol product and application rate with different colony sizes indicate no significant difference (P > 0.05; LSD).
[4]Colony sizes are labeled as: (A) 1 queen: 100 workers: 0.01 g brood; (B) 2 queens: 200 workers: 0.025 g brood; (C) 3 queens: 400 workers: 0.05 g brood; (D) 4 queens: 800 workers: 0.1 g brood.

Table. 4 Binary logistic regression for the influence of colony size, percentage of visiting workers, and application rate on colony movement in response to different aerosol products. B = B value; S.E = standard error; df= degree of freedom; * = $P < 0.05$.

Aerosol	Variable	B	S.E	df	*P*-value
Control	Colony size			3	0.604
	Percentage of visiting workers	0.328	0.089	1	< 0.001*
	Application rate			2	0.481
Zevo	Colony size			3	0.468
	Percentage of visiting workers	0.027	0.063	1	0.675
	Application rate			2	0.37
Raid	Colony size			3	0.19
	Percentage of visiting workers	0.291	0.08	1	< 0.001*
	Application rate			2	0.101
Black Flag	Colony size			3	0.108
	Percentage of visiting workers	0.132	0.072	1	0.067
	Application rate			2	0.044*

Chapter 3: Toxicant Translocation and Colony Impact in the Pharaoh Ant (*Monomorium pharaonis*) (Hymenoptera: Formicidae) After Consumption of Gel Bait-Killed German Cockroach (*Blattella germanica*) (Blattodea: Ectobiidae) Cadavers

Abstract

This study investigated the translocation of cockroach bait toxicants to the Pharaoh ant, *Monomorium pharaonis* (L.), via the feeding on cadavers of German cockroaches, *Blattella germanica* (L.), killed by commercial gel baits. The cockroaches were first treated with one of the four cockroach gel baits, namely Advion Trio (0.6% indoxacarb, 0.1% pyriproxyfen, 0.1% novaluron), Maxforce FC Magnum (0.05% fipronil), Alpine Rotation 1 (0.5% dinotefuran), and Vendetta Nitro (0.5% clothianidin, 0.5% pyriproxyfen), before the Pharaoh ants were allowed to forage to the cadavers. The effects of the toxicant on the Pharaoh ant's colonies were examined. Ants fed on cadavers that were killed by Maxforce FC Magnum showed significantly higher ($P < 0.05$) queen and brood mortality. Advion Trio initially led to rapid elimination of queens, workers, and brood; however, the Pharaoh ant colony later recovered as the workers abandoned treated cockroach cadavers and switched to a regular diet. In the Vendetta Nitro-treated group, pyriproxyfen was delivered to the queens and significantly ($P < 0.05$) reduced the size of mature oocytes. However, no structural damage was observed on any oocyte, as all exhibited a visible nucleus, multiple nurse cells, and a well-developed follicular epithelium. The horizontal transfer efficacy of different cockroach gel bait toxicants is discussed.

Introduction

The Pharaoh ant, *Monomorium pharaonis* (L.), is one of the most important cosmopolitan indoor pest species. Reports have shown that infestations of Pharaoh ants frequently occur in healthcare and food preparation facilities (Edwards and Baker 1981, Yap and Lee 1994, Man and Lee 2012, Tay et al. 2014). To effectively manage Pharaoh ant infestations, it is crucial to select appropriate control strategies, as Pharaoh ant colonies may split and bud when disturbed, worsening the infestation (Buczkowski et al. 2005, Tay and Lee 2015). Studies have shown that non-repellent residual treatments and baiting can control Pharaoh ants while preventing budding (Buczkowski et al. 2005). Current Pharaoh ant control strategies emphasize the use of bait, as residual sprays only kill a small portion of foragers, while bait toxicants can be distributed throughout the colony (Vail 1996). Therefore, baits targeting Pharaoh ants should incorporate their diet preference to maximize the toxicant uptake (Edwards and Abraham 1990).

Besides the Pharaoh ant, the German cockroach, *Blattella germanica* (L.), well known for its resilience and association with public health risks (Page et al. 2006, Tang et al. 2019, Schal and DeVries 2021), also poses a considerable challenge in pest management (Fardisi et al. 2019). German cockroach infestations commonly occur in residential homes, hospitals, restaurants, and supermarkets, where food, water, and shelter are readily available (Alcamo and Frishman 1980, Tang et al. 2019). They are ideal carriers for transmitting diseases and pathogens (Fotedar et al. 1991, Menasria et al. 2014). Given their synanthropic nature and potential to spread diseases in human environments, effective control methods are essential to managing German cockroach

infestations. A survey conducted in North Carolina found that most residents use aerosol sprays, baits, pump sprayers with concentrated sprays, and dust when encountering German cockroach infestations (Dingha et al. 2013).

The global distributions of both Pharaoh ants and German cockroaches have a great overlap, with infestation hotspots reported in Eastern USA and Europe (Wetterer 2010, Lee and Wang 2021). Co-infestations of both species within the same structure have been reported in sensitive environments where insecticide spray and dust application are limited, such as hospitals and insectaries (Burden and Smittle 1975, Gliniewicz et al. 2003, Gliniewicz et al. 2006). In such settings, bait formulations offer effective control while other methods cannot be employed (Burden and Smittle 1975).

Baiting offers several advantages, including simplicity of use and less chemical exposure to the environment (Schal 2011, Appel and Rust 2021). In addition, the potential of horizontal transfer of bait toxicants may further enhance the bait performance (Hamilton et al. 2023). In German cockroaches, mortality can occur to cockroaches that do not directly interact with the bait through necrophagy, coprophagy, emetophagy, and physical distribution of bait within the area (Kopanic and Schal 1999, Buczkowski and Schal 2001, Hamilton et al. 2023). Similar toxicant distribution mechanisms also occur in ants, where workers translocate toxicants mainly through trophallaxis and necrophoresis (Choe and Rust 2008).

Given that Pharaoh ants often feed on live insects and insect cadavers in homes (Berenbaum 1994, Eow and Lee 2007, Koltz et al. 2008, Wetterer 2010), the potential for horizontal transfer of bait toxicants may happen when Pharaoh ants consume cadavers of

German cockroaches that are killed by bait. Such cross-species toxicant exposure has been demonstrated in the horizontal transfer of anticoagulant rodenticides, where nontarget wildlife feeding on contaminated rodent cadavers suffer accidental mortality or bioaccumulate toxicants through the food chain (Mendenhall and Pank 1980, Eason et al. 1999, Hoare and Hare 2006, van den Brink et al. 2018). However, little is known about this cross-species toxicant transfer pathway in insects. Given that Pharaoh ants and German cockroaches can infest the same structure, we conducted this study to investigate how Pharaoh ant colonies were impacted by bait toxicants of several cockroach gel baits after the colonies fed on cadavers of German cockroaches that were killed by the baits, aiming to determine whether the ants would feed on the cockroach cadavers and whether these toxicants could be horizontally transferred through this pathway to a degree sufficient to affect colony growth.

Materials and Methods

Insects

The study was conducted using *M. pharaonis* obtained from USDA-ARS, Center for Medical, Agricultural, & Veterinary Entomology in Gainesville, Florida, in 2020 and reared in centrifuge tubes (12 cm in length, 1.5 cm in diameter) filled with unbleached paper towel strips under laboratory condition of 27 ± 1°C, 70 ± 2% RH, and 12:12 L:D cycle in arenas (32 cm in length, 19 cm in width, 11 cm in height) coated with fluon at the University of California, Riverside over 4 years. Water and 10% sucrose solution

were provided ad libitum, and Tetra Goldfish Flakes (Tetra GmbH, Melle, Germany) and raw tuna obtained from Costco as protein sources were provisioned once a week.

The German cockroaches used were from the UCR susceptible strain. The UCR strain was acquired from the Orlando normal strain over 40 years ago and reared in 121-liter garbage bins equipped with electrical barrier at 24 ± 2 °C, 40 ± 10 RH, and 12:12 L:D cycle without any insecticide exposure. Dog food (Purina Dog Chow, Nestlé Purina Petcare, St. Louis, MO), water, and corrugated cardboard harborage were provided ad libitum.

Experimental design

The experimental setup consists of three round polyethylene containers (8.7 cm in diameter, 7.8 cm in height), with two containers A and C connected to a central container B, each via a 5 cm Tygon tube on either side (Fig. 1). Ten 3rd and 4th instar German cockroaches from UCR susceptible strain were introduced to container A and starved for 24 hours. After starvation, 0.3 g of bait was provisioned to the German cockroaches, allowing them to feed on the bait for 7 days. In the control groups, German cockroaches were killed in the freezer at -20°C for 24 hours. A Pharaoh ant subcolony consisting of 2 fertile queens, 100 workers, and 50 mg mixed brood stage was introduced into container B 4 days after baiting the cockroaches, provisioned with water, 10% sucrose solution, and a piece of corrugated paper (5 x 5 cm) as harborage, allowing 3 days of acclimatization. Container C served as a clean arena where a regular diet consisting of hard-boiled egg and canned tuna (Kirkland Signature, Issaquah, WA) was provided and replaced every

week. Tygon tubes were blocked by pieces of cotton until the experiment started to prevent Pharaoh ants interacting with German cockroaches and the clean diet during the 3 days acclimatization period. and cotton was removed from the Tygon tube after acclimatization. Total mortality of German cockroaches was achieved in all treatments after 7 days of baiting.

Four common commercial cockroach gel bait products were evaluated: Advion Trio (0.6% indoxacarb, 0.1% pyriproxyfen, and 0.1% novaluron; Syngenta Crop Protection, Greensboro, NC), Maxforce FC Magnum (0.05% fipronil; Bayer Environmental Science, Research Triangle Park, NC), Alpine Rotation 1 (0.5% dinotefuran; BASF Corporation, Research Triangle Park, NC), and Vendetta Nitro (0.5% clothianidin and 0.5% pyriproxyfen; MGK, Minneapolis, MN). The number of workers present in container A and C was recorded once a week for 12 weeks to determine whether the Pharaoh ant workers avoid contaminated cockroach cadavers. Worker mortality was accessed by counting the number of live ants once a week. Brood mortality was evaluated based on the reduction in total brood weight every week. Brood weight was measured using a digital electronic balance with a capacity of 220 g and a precision of 0.0001 g, by placing the brood in a weighing boat. Queen mortality was accessed by counting the number of live queens in each replicate on a weekly basis.

At the end of the study, the live queens were dissected under a stereo microscope (35×) the ovary morphology and the size of oocytes were under the Leica DMS1000 digital microscope using Leica LAS X software (version 5.1.1). To observe the presence of oocyte nucleus, nurse cells, and follicular epithelium, ovaries were immediately fixed

in Bouin's solution after dissection, stained with 4′,6-diamidino-2-phenylindole (DAPI), which marks DNA, and mounted on slides. Observations were made using the ZEISS Apotome 3 fluorescence microscope (400×) using ZEN Microscopy software (version 3.11). The presence of workers in containers A and C and the mortality of workers, brood, and queens were collected weekly for 12 weeks. The experiment was replicated 8 times.

Data analysis

The weekly mean percentage of reduction in worker population ((1-number of live ants/initial number of workers in each subcolony)/total number of observations at each data collection point) and brood weight between different treatments ((1-observed brood weight/initial brood weight in each subcolony)/total number of observations at each data collection point) were transformed ($\log_{10} + 2$) and subjected to one-way ANOVA. Three additional one-way ANOVA were used to compare (1) the means of number of workers present in the container A and C from each treatment (total number of live ants that visited either container A or C/total number of observations on either container A or C), and (2) number and (3) size of mature oocytes. Mean values were compared using Tukey's HSD test. The impact of the baits on the survivorship of Pharaoh ant queens was analyzed with Kaplan-Meier survival analysis, and the survival curves between different treatments were compared using log-rank tests. All analyses were performed using SPSS Version 30.0 at $\alpha = 0.05$.

Results

Presence of workers in container A (contaminated) and C (clean)

In the control groups, the number of workers visiting both container A and C during the experimental period is not significantly different from each other (Fig. 2). In comparison, all treatments exhibited significantly higher activity ($P < 0.05$) in container C than in container A within each treatment, except for the Maxforce FC Magnum-treated group, where the number of ants present in container C was not significantly different from that in container A (Fig. 2). All treatments, except for the Maxforce FC Magnum-treated group, which had significantly fewer ($P < 0.05$) ants, showed no significant difference in the number of workers present in container C compared to that of the controls (Fig. 2). In container A, all treatments showed significantly fewer ants compared to the controls ($P < 0.05$), except for the Vendetta Nitro-treated group, where the number of ants present was not significantly different from that in the controls (Fig. 2).

Worker mortality

At the 1st week post-treatment, the percentage reduction in the worker population was significantly greater in all treated groups than that of the controls until the 8th week (Table 1). By the 8th week, Maxforce FC Magnum, Alpine Rotation 1, and Vendetta Nitro exhibited significantly greater percentage reductions in ant counts, whereas the Advion Trio-treated groups had recovered from the treatment, with their percentage reductions in ant counts no longer significantly different from those of the controls, and this effect further sustained up to the 12th week. At the 12th week, the percentage

reduction in ant counts was not significantly different from that of the controls in the Maxforce FC Magnum- and Alpine Rotation 1-treated groups, whereas a significantly greater percentage reduction was observed in the Vendetta Nitro-treated groups (Table 1).

Brood mortality

All tested baits exhibited significantly greater reduction in brood weight at 1st week post-treatment (Table 2). By the 4th week, Maxforce FC Magnum, Alpine Rotation 1, and Vendetta Nitro caused significantly greater percentage reduction in brood weight, whereas the percentage reduction of brood weight in Advion Trio-treated groups was no longer significantly different from that of the controls until the week 12 (Table 2). At week 12, only Maxforce FC Magnum provided a significant reduction in brood weight (Table 2).

Queen mortality

All treatments caused a significant decrease ($P < 0.05$) in survivorship compared to the controls (Fig. 3). Maxforce FC Magnum performed better than the other baits against Pharaoh ant queens, exhibiting a significantly lower ($P < 0.05$) survival probability with a mean survival time of 6.8 weeks compared to 8.7 -11.9 weeks for the other treatments (Fig. 3).

Ovary morphology

The reproductive system of queens consists of a visible spermatheca, a pair of oviducts, and a pair of ovaries in which the ovarioles with a mature oocyte are positioned toward the oviduct (Fig. 4A). The same morphology was also observed in Advion Trio- and Alpine Rotation 1-treated groups (Fig. 4B and D). In contrast, significantly fewer and smaller oocytes were found in the Vendetta Nitro-treated group (Fig. 4E, Table 3). Additionally, although the reduction was not significant, fewer and smaller oocytes were also observed in the Maxforce FC Magnum-treated group (Fig. 4C, Table 3).

Oocyte development

All treatments did not affect oocyte development (Fig. 5A-E). In the control group, healthy and well-developed oocytes were characterized by a surrounding follicular epithelium, adjacent nurse cells, and a visible nucleus, which were also observed in all treatment groups. Although oocytes from the Vendetta Nitro-treated group were significantly smaller ($P < 0.05$) than those in the controls (Table 3), no structural damages were observed (Fig. 5E).

Discussion

In the process of this study, foragers acquire bait toxicant by physically contacting and feeding on contaminated cockroaches and distributing the toxicants throughout the colony. The primary toxicant horizontal transfer pathway in Pharaoh ant, either through physical contact or trophallaxis, was not determined in this study. Our results showed that

Advion Trio and Maxforce FC Magnum initially demonstrated a significant reduction in worker population, whereas no significant reduction was observed in Alpine Rotation 1- and Vendetta Nitro-treated groups (Table. 1). However, this effect did not sustain throughout the observation period in Advion Trio-treated group. In fact, Advion Trio-treated group had completely recovered from the treatment with a larger average worker population compared to that of the controls by the end of the study, whereas significant reduction in worker population was observed only in Vendetta Nitro-treated group, which initially did not show any significant worker population reduction (Table 1). This phenomenon could be attributed to the difference in the number of ants visiting container A and C. In Advion Trio-treated group, there were significantly fewer ants visiting container A than those of container C and controls (Fig. 2), indicating workers completely abandoned the contaminated arena after feeding on the contaminated cockroach cadavers and went on to feed on the clean diet (Fig. 2). Maxforce FC Magnum and Alpine Rotation 1 also exhibited significantly fewer workers visiting container A compared to the controls (Fig. 2). On the other hand, the number of workers visiting container A in Vendetta Nitro-treated group is not significantly different from that of the controls (Fig. 2), which indicates that workers did not avoid the contaminated arena (Fig. 2).

Hamilton et al. (2023) reported that fipronil and indoxacarb exhibit better horizontal transfer efficiency than dinotefuran and clothianidin. In addition, fipronil and indoxacarb demonstrate stable residual effects (Buczkowski et al. 2005, Jones and Bryant 2012), which align with the initial rapid elimination of worker, queen, and brood

observed in the Advion Trio- and Maxforce FC Magnum-treated groups (Fig. 3, Table 1, Table 2). By the end of the study, only Vendetta Nitro significantly reduced the worker population (Table 1), likely because of pyriproxyfen, which induces brood mortality, delays larval development, and contributes to worker attrition, with the direct worker mortality caused by clothianidin (Stanley 2004).

A decline in the worker population was also observed in the control groups (Table 1), which is likely due to the worker lifespan. In Pharaoh ants, the longevity of workers ranges from 8 weeks to 10 weeks (Peacock et al. 1955, Edwards 1975, 1986). In this study, the initial cohort of 100 workers, along with those that emerged prior to week 4, likely reached the end of their lifespan between weeks 8 and 12, resulting in the observed decline (Table 1). An increase in the worker population may be expected if the experimental period is extended.

In addition to toxicant ingestion, physical contact with bait toxicants further enhances the efficacy of horizontal transfer, as undigested toxicants can be excreted, and bait remnants may be physically distributed by German cockroaches within the arena (Kopanic and Schal 1999, Durier and Rivault 2000). Among the active ingredients in the tested baits, fipronil exhibits strong contact toxicity (Lee 2000, Stanley 2004, Buczkowski et al. 2005). In this study, Maxforce FC Magnum resulted in significantly lower survivorship of queens compared to all other treatments and was the only treatment that caused a significantly higher brood reduction (Fig. 3, Table 2). In addition to being distributed through trophallaxis, fipronil may also be physically transferred on contact as workers groom the queens and brood.

Pyriproxyfen and novaluron are not lethal to adult insects and have been shown to have stable residual effects (Ameen et al 2005, Pitts-Singer and Barbour 2017, Joseph 2021), therefore these active ingredients did not directly contribute to the mortality in workers and queens in this study. However, in addition to disrupting larval development, pyriproxyfen can still significantly impact colony dynamics by reducing the fecundity of Pharaoh ant queens (Tay and Lee 2015). Tay and Lee (2015) reported that pyriproxyfen decreases egg production as well as the number and size of oocytes in treated queens. Consistent with these findings, a significant reduction in oocyte size was observed in the Vendetta Nitro-treated group in this study (Table 3). A similar reduction in oocyte size was also reported in Pharaoh ant queens treated with (S)-methoprene (Edwards 1975, Edwards and Clarke 1978). In contrast, Advion Trio did not cause any abnormalities in queen ovaries, likely due to its lower pyriproxyfen content compared to Vendetta Nitro, or because foragers abandoned the arena with contaminated cockroaches, preventing sufficient pyriproxyfen uptake to disrupt oocyte development (Fig. 2). Although the Maxforce FC Magnum-treated group did not show a significant difference in oocyte size compared to the controls, fewer and smaller oocytes were observed (Fig. 4C). This change in ovarian morphology may be attributed to the significant reduction in brood. Børgesen and Jensen (1995) revealed that the fecundity of fertile queens depends on the presence of larvae, as the absence of larval secretions inhibits vitellogenin uptake by the ovarioles, thereby disrupting oocyte development (Børgesen 2000).

In this study, oocytes were stained with DAPI to observe oocyte development. Although a significant reduction in oocyte size was observed in the Vendetta Nitro-

treated group, our results showed that queens from colonies that were exposed to Vendetta Nitro-treated cockroaches had oocytes with visible nuclei and nurse cells present throughout the ovarioles (Fig. 5E). In contrast, Tay and Lee (2015) demonstrated that directly feeding Pharaoh ant colonies with pyriproxyfen mixed with peanut oil led to severe oocyte developmental abnormalities within eight weeks, including the absence of oocyte nuclei, a lack of nurse cells, and underdevelopment of the follicular epithelium. This discrepancy suggests that the amount of pyriproxyfen delivered to the queens in this study was insufficient to induce developmental deformities. This is most likely because Vendetta Nitro also include clothianidin. Given the faster-acting toxicity of clothianidin compared to pyriproxyfen, queen mortality likely occurred before a sufficient amount of pyriproxyfen could induce oocyte developmental abnormalities (Vail and Williams 1995, Lim and Lee 2005, Tay and Lee 2015, Hamilton et al. 2023, Ma et al. 2024).

This study evaluated the horizontal transfer efficacy of commonly used cockroach gel baits in Pharaoh ants by examining the impact of feeding on contaminated cockroaches in the presence of a clean diet. Although incorporating slow-acting IGRs with other faster-acting active ingredients in cockroach gel baits can induce rapid mortality of susceptible cockroaches while eliminating immature stages that interact with other treated cockroaches and do not directly feed on the bait, the results demonstrated that the presence of other active ingredients in the tested baits caused Pharaoh ant queen intoxication before they could acquire sufficient pyriproxyfen to disrupt oocyte development. In comparison, fipronil demonstrated rapid elimination of queens and brood, which would significantly impact colony growth, as all workers are sterile

(Edwards 1986, Passera 1994, Boonen and Billen 2017, Rajakumar et al. 2024). Previous studies have also identified fipronil as a good candidate for managing Pharaoh ant infestations, both as residual spray and in bait formulations (Lee 2000, Buczkowski et al. 2005). In cases where both Pharaoh ant and German cockroach infestations are present, besides reducing German cockroaches, fipronil-based cockroach gel baits could inadvertently control Pharaoh ant infestation.

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Figures and Tables

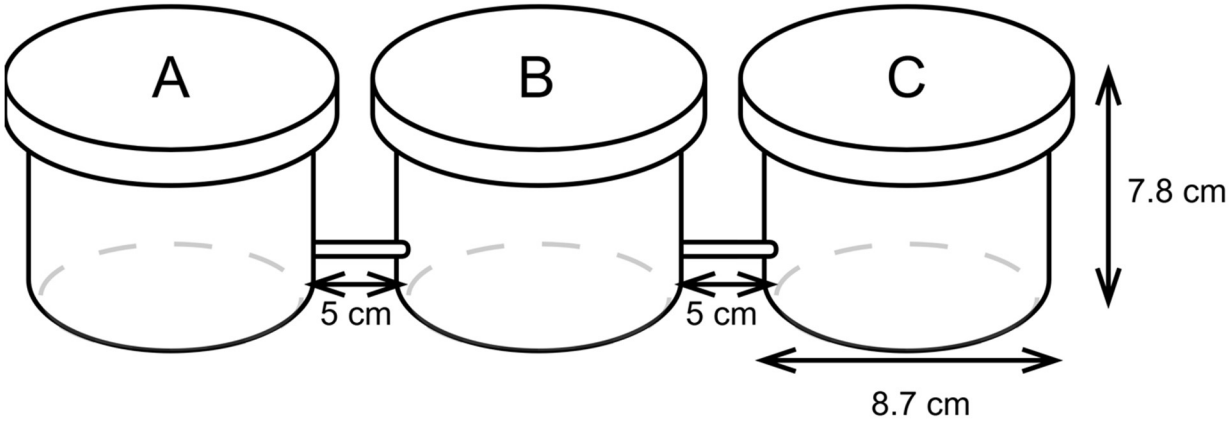


Fig. 1 The experimental setup used in this study. The polyethylene containers are labeled as: (A) Treated cockroach site; (B) Pharaoh ant nest site; (C) Clean site with regular diet.

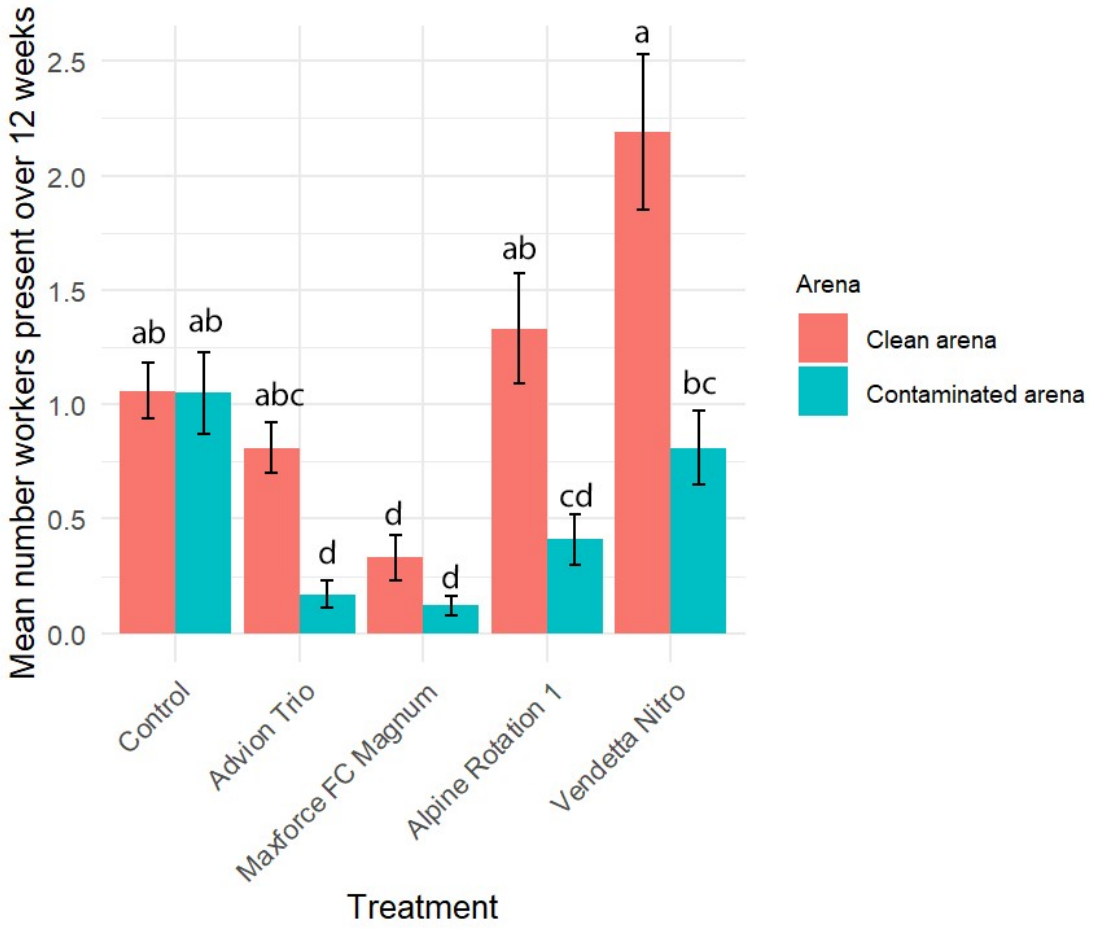


Fig. 2 Impacts of various treatments on the number of workers present in clean and contaminated arena. Means with the same letter indicate no significant difference ($P > 0.05$; Tukey's HSD test).

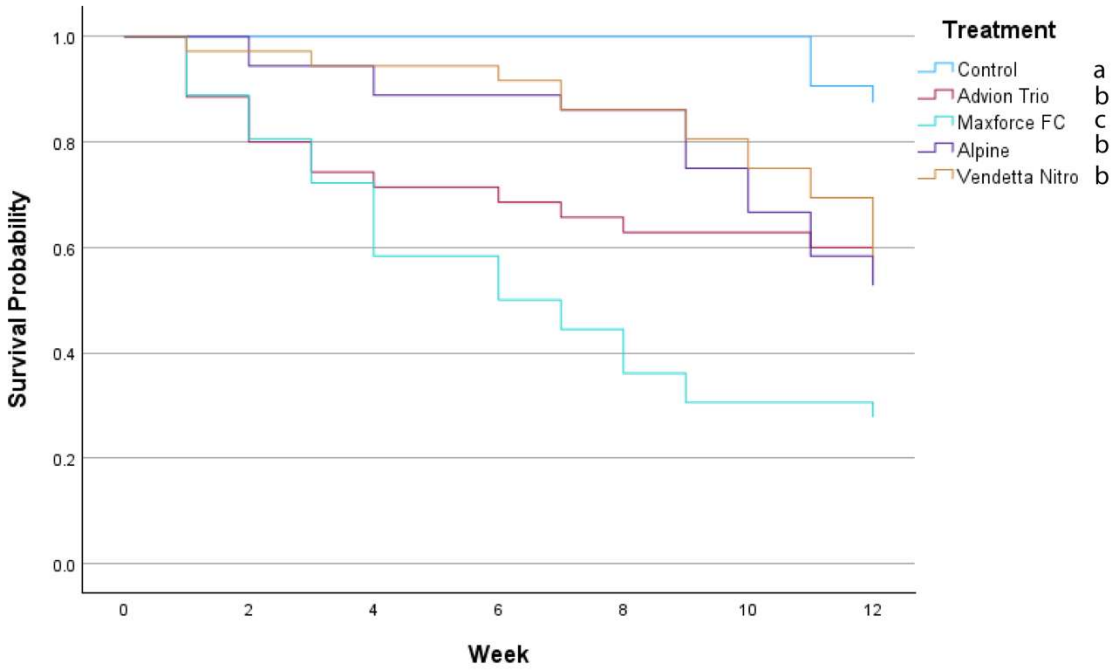


Fig. 3 Survivorship of Pharaoh ant queens in subcolonies exposed to contaminated German cockroach cadavers. Treatment legends followed by the same letter indicate no significant difference ($P > 0.05$; Log-rank test).

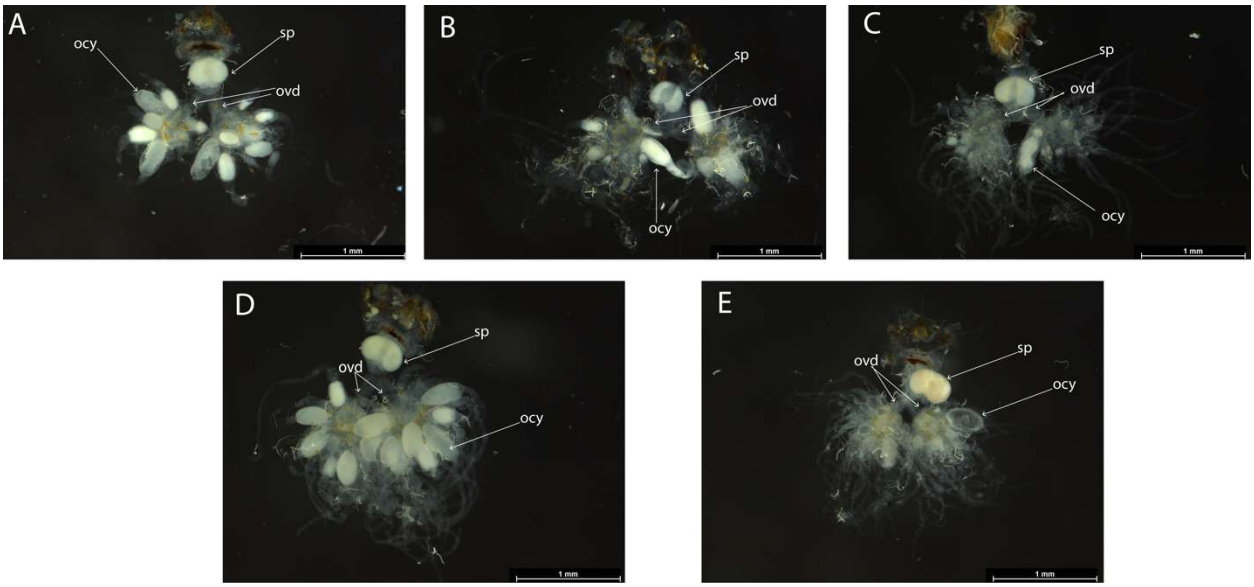


Fig. 4 Ovaries of queens from different treatments: (A) Control, (B) Advion Trio, (C) Maxforce FC Magnum, (D) Alpine Rotation 1, (E) Vendetta Nitro. Abbreviations: ocy=oocyte, ovd=oviduct, sp=spermatheca.

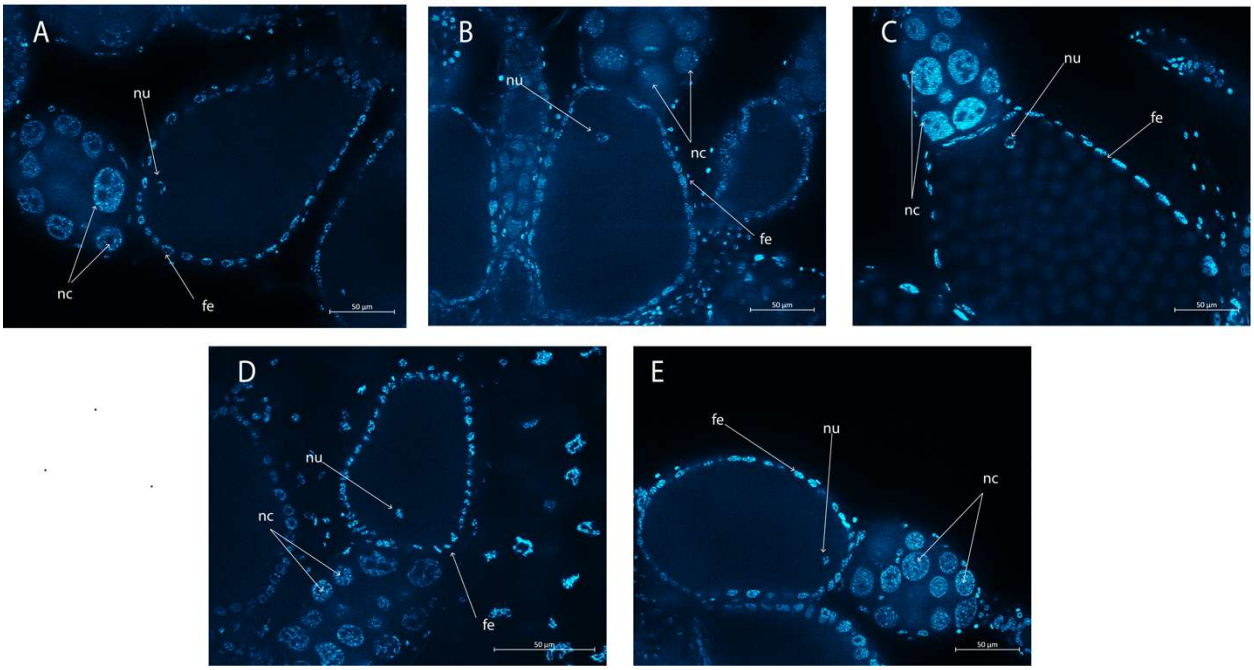


Fig. 5 Ovaries from different treatments stained with DAPI: (A) Control, (B) Advion Trio, (C) Maxforce FC Magnum, (D) Alpine Rotation 1, (E) Vendetta Nitro. Abbreviations: fe=follicular epithelium, nc=nurse cell, nu=oocyte nucleus.

Table. 1 Mean % reduction + SEM of worker numbers at 1st, 4th, 8th, and 12th week.

	Mean % reduction + SEM of worker numbers at n^{th} week[1]			
Treatment	**1**	**4**	**8**	**12**
Control	13.4±5.9 a	-62.6±12.9 a	-65.3±19.2 a	21.3±22.4 a
Advion Trio	58.6±7.9 b	-1.7±10.4 b	-20.9±7.3 ab	8.3±17 a
Maxforce FC Magnum	65.6±9 b	28.7±18.3 b	43.1±19.9 b	65.9±15.7 ab
Alpine Rotation 1	41.7±4.9 b	16.9±11.5 b	46.4±17.9 b	75.6±15.3 ab
Vendetta Nitro	25.9±9.8 b	-9±15.8 b	20.4±8.3 b	86.3±3 b

[1]Means followed by the same letters within the same column are not significantly different ($P > 0.05$; Tukey's HSD test).

Table. 2 Mean % reduction + SEM in total brood weight at 1st, 4th, 8th, and 12th week.

	Mean % reduction + SEM in brood weight (g) at nth week[1]			
Treatment	**1**	**4**	**8**	**12**
Control	-12.5±10.3 a	15.7±13.6 a	32.6±10 a	58±7.8 a
Advion Trio	32.3±5 b	50.4±6.1 ab	52.7±9.5 ab	63.8±10.8 ab
Maxforce FC Magnum	40±9 b	73.9±7.3 b	82.2±8.1 b	90.1±3.9 b
Alpine Rotation 1	40.1±10.6 b	53.2±8.5 b	70.2±9.4 b	85.7±8.8 ab
Vendetta Nitro	47.4±11.1 b	66±8.3 b	78.4±4.5 b	82.8±3.2 ab

[1]Means followed by the same letters within the same column are not significantly different ($P > 0.05$; Tukey's HSD test).

Table. 3 Effect of different bait treatments on the queen ovary development of the Pharaoh ant.

Treatment	Mean no. oocytes ± SEM[1]	Mean length of the largest oocyte ± SEM (µm)[1]
Control	9.3±1.7 a	418.4 ± 17.7 a
Advion Trio	8.6 ± 1.4 ab	384.2 ± 19.8 ab
MaxForce FC Magnum	2.8 ± 0.8 ab	332.0 ± 37.6 ab
Alpine Rotation 1	7.0 ± 1.9 ab	379.7 ± 16.4 ab
Vendetta Nitro	2.9 ± 0.6 b	327.8 ± 25.8 b

[1]Means followed by the same letters within the same column are not significantly different ($P > 0.05$; Tukey's HSD test).

Chapter 4: Summary and Conclusion

Effective management of Pharaoh ant infestations relies on a thorough understanding of their biology, behavior, and appropriate control strategies. This thesis focuses on colony movement patterns in response to over-the-counter aerosol-treated surfaces, and the impact of commercial cockroach gel baits on Pharaoh ant colonies after they fed on the cadavers of German cockroaches that were killed by the gel baits.

Findings from Chapter 2 revealed that Zevo-treated surfaces had a higher probability of inducing budding at higher insecticide application rates. In contrast, Raid- and Black Flag-treated surfaces triggered budding more frequently at lower application rates. Notably, fewer ant cadavers were observed on Zevo-treated surfaces, while more were found on Raid- and Black Flag-treated surfaces. This suggests that workers exposed to Raid and Black Flag were rapidly knocked down and killed, preventing contaminated individuals from delivering chemical cues to the main nest. Conversely, Zevo exhibited spatial repellency, leading to colony budding. This finding aligns with previous studies on German cockroaches, where pyrethroids act as irritants, while essential oils function as true spatial repellents (Yoon et al. 2009, Gaire et al. 2024).

As the insecticide application rate increased, the presence of workers outside the main nest site decreased in Zevo-treated groups, whereas it increased in Raid- and Black Flag-treated groups. Additionally, Pharaoh ants were more likely to budding into potential nest sites with previously more ants visited in Raid-treated groups. A similar pattern was observed in Black Flag-treated groups, though the influence of the number of workers present on the final nest location was not statistically significant. In contrast,

worker presence did not influence the final nest location in Zevo-treated groups, possibly due to the immediate budding, while delayed buddings were observed in Raid- and Black Flag-treated groups.

In Chapter 3, when Pharaoh ant colonies were provided with bait-contaminated German cockroach cadavers alongside an untreated clean diet, all tested cockroach gel baits significantly reduced queen survivorship, and Maxforce FC Magnum (0.05% fipronil) induced queen mortality significantly faster than the other treatments. In addition, Maxforce FC Magnum was the only treatment that resulted in significant brood reduction, which could significantly impact colony survival, as all Pharaoh ant workers are sterile. Advion Trio (0.6% indoxacarb, 0.1% pyriproxyfen, 0.1% novaluron) initially also caused a significant rapid reduction in all castes, but the ants abandoned the treated cockroaches and fully recovered from the treatment, likely due to aversive pheromone and memory (Robinson et al. 2005, Zanola et al. 2025). Vendetta Nitro (0.5% clothianidin, 0.5% pyriproxyfen) significantly reduced the worker population due to the fast-acting nature of clothianidin, combined with worker attrition caused by pyriproxyfen, which disrupts larval development. This finding reveals cockroach gel bait toxicants could be horizontally transferred to Pharaoh ant colonies and suppress the colony growth if ants were exposed to treated German cockroach cadavers.

By examining the morphological and developmental changes in the reproductive organs of Pharaoh ant queens, we found that, as expected, Vendetta Nitro treatment resulted in significantly fewer and smaller oocytes because of pyriproxyfen. Surprisingly, a similar reduction in oocyte size and number was also observed in queens treated with

Maxforce FC Magnum. This effect may be attributed to the critical role of larval secretions in Pharaoh ant queen ovary development, while most broods were killed.

Although morphological changes were observed in Vendetta Nitro- and Maxforce FC Magnum-treated groups, no developmental abnormalities were observed in all treatments as all oocytes present a visible nucleus, nurse cells, and well-developed follicular epithelium. This phenomenon indicated that the pyriproxyfen delivered to the queens was insufficient to induce oocyte developmental abnormality.

This thesis highlighted the limitations of OTC aerosols and revealed a cross-species mechanism of toxicant transfer in insects. Chapter 2 demonstrated that Pharaoh ant colony would bud to nest sites with previously more worker visited, yet we don't know how accurate this prediction would be in the field situation where the number of potential budding sites are not limited. Therefore, additional field studies on Pharaoh ant colony movement pattern after OTC aerosol insecticide treatment are warranted.

Co-infestations of Pharaoh ants with German cockroaches have been documented (Burden and Smittle 1975, Gliniewicz et al. 2003, Gliniewicz et al. 2006). In Chapter 3, MaxForce FC Magnum demonstrated promising control outcomes when Pharaoh ants were exposed to treated German cockroach cadavers. While laboratory experiments provide valuable insights into the horizontal transfer of cockroach bait toxicants as Pharaoh ants were exposed to contaminated cockroach cadavers, whether this cross-species mechanism of toxicant transfer between Pharaoh ants and German cockroaches occurs in the field remains unknown. Future studies should simulate field conditions by infesting an enclosed space, such as a cargo cabinet, with both German cockroaches and

Pharaoh ants, and evaluate Pharaoh ant colony growth after cockroach gel bait treatment, as Pharaoh ants are exposed to bait-killed cockroach cadavers.

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