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Selenium as a Potential Bait Active Ingredient Against the German Cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae)

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DEDICATION

I dedicate my thesis to my parents, Jung Hee and Soo Yong So, and my sister, Serah So, who have all been supportive of my entomological endeavors.

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Chapter I. Introduction

Pest Status and Economic Impact

Of the 4300 cockroach species found worldwide, only a few species have achieved pest status globally (Roth 2003). The German cockroach, *Blattella germanica* (L.), is a cosmopolitan pest species that has significant economic impacts and implications for human health. A survey of 389 pest management professionals found that cockroach control (23%) ranked the most important service revenue generator for the US pest management industry in 2018 (PCT 2019). Ant pest control followed closely second at 22%. Furthermore, German cockroach control accounted for 77% of the cockroach control service calls, more than three times that of all other cockroach species (PCT 2021). In addition, the German cockroach occupies the number 11 spot as the top resistant arthropod in agricultural and urban ecosystems (Zhu et al. 2016). Of the urban pest species, it ranks second to *Musca domestica*.

Chemical Control

At least 18 classes of insecticides have been used against the German cockroach (Lee & Rust 2021). Most conventional insecticides target the insect nervous system. Early control measures in the market included organochlorines, carbamates, organophosphates, and pyrethroids as they were cheap, effective, and had a broad-spectrum application (Wickham 1995). However, many of these early classes such as organophosphates and carbamates have been phased out for use as residual sprays in indoor environments due to

their potential health risks and the availability of safer alternatives like pyrethroids (Lee & Rust 2021).

Insect growth regulators (IGRs) have been considered as safer alternatives to conventional insecticides. They belong to three major groups: juvenile hormone analogs (JHAs), chitin synthesis inhibitors (CSIs), and ecdysteroid agonists (ESAs). As a group, IGRs disrupt the growth and development of immatures; however, they have also been shown to affect the reproductive capacity of adult females (Lim & Yap 1996, King 2005). An advantage to considering IGRs is that they have low mammalian toxicity, but insecticidal effects are delayed and selectively active (Bennet & Reid 1995). Early inorganic chemicals and dusts included boric acid, sodium fluoride, and phosphorous (Wickham 1995). Currently, the most widely used inorganic chemicals today are boric acid and desiccants like diatomaceous earth and silica aerogels (Lee & Rust 2021). These dust applications are effectively used to treat voids and eliminate harborage sites.

Resistance

Unfortunately, the success of early synthetic insects was short-lived. Cases of resistance were first reported in the early 1950s after the widespread use and reliance on the single active ingredient in the chlorinated hydrocarbon class, chlordane (Grayson 1966). Shortly after, a similar fate would fall upon the popular organophosphates and pyrethrins. Resistance is defined as the decrease in efficacy of a dosage of insecticide towards a cockroach population with which it once killed or controlled (Cochran 1995). Resistance is exacerbated by cases of cross- or multiple-resistant populations where resistance

towards one insecticide confers resistance to another (Cochran 1995). More than 279 cases of resistance have been reported worldwide to 42 different active ingredients (Arthropods Resistant to Pesticides Database, accessed on March 2022; Zhu et al. 2016). To date, boric acid, IGRs, and desiccant dusts do not have any reports of resistance to date in the German cockroach (Scharf & Gondhalekar 2021). Resistance mechanisms can be divided into four main categories: metabolic detoxification, target-site insensitivity, penetration, and behavioral (Cochran 1995).

Resistance Management and Novel Control Measures

There is a serious need for a practical German cockroach control approach to manage insecticide-resistant populations. Chemical approaches to resistance management remain the most cost-effective route for controlling cockroach infestations (Scharf & Gondhalekar 2021). Insecticide Resistance Action Committee (IRAC) was established in 1984 as an international association of crop protection companies and served to provide various entities with the mode of action (MoA) classification system (Sparks & Nauen 2015). As a result, informed decisions can be made for choosing insecticides that differ in MoA in techniques such as rotations. Furthermore, mixtures with conventional insecticides with IGRs or synergists have effectively reduced field populations (Scharf et al. 1997, Fardisi 2019). Integrated Pest Management (IPM) based approaches have been suggested to manage resistant populations many of which employ non-chemical methods like sanitation and habitat modification (Gold 1995). Although sanitation alone will not eliminate cockroaches, it can improve the efficacy of subsequent pesticide applications

(Schal 1988, Brenner et al. 2003, Rust 2021). Although they have limited application in field settings, alternatives to traditional insecticides such as biological control agents, household cleaners, heat, and cold have been explored (Rust 2021). Moreover, botanicals in essential oils (EOs) have been investigated since they have a wide range of effects, including insecticidal, repellent, antifeedant, and fumigant properties (Mossa 2016).

Selenium as an insecticide

Selenium, an essential trace element, was subject to early investigations as a systemic insecticide (Gnadinger 1933, Reed et al. 1962). Hurt-Karrer & Poos (1936) and Neiswander & Morris (1940) reported successful control of various crop pest species. However, as the knowledge of selenium toxicity and contamination grew, research began to focus on the area of environmental toxicology (National Research Council 1983, Maier & Knight 1993, Trumble et al. 1998, Hanson et al. 2004). These studies exploring the biotransfer of selenium through insect trophic interactions provided extensive evidence of toxicity to insects. After early studies on the insecticidal nature of selenium, the utility of selenium as an insecticide has been revisited recently. Hanson et al. (2004) suggested that selenium may be useful as a systemic due to causing deterrence and toxicity to the aphid, *Myzus persicae.* In addition, Mechora (2019) supported the notion that selenium may be effective as a repellent and a food fortifier on crops. Selenium and its effect on insects will be comprehensively reviewed in the following chapter.

Objective

This study aimed to explore the insecticidal property of selenium on the German cockroach, an examination that has been absent in the literature. There is an urgent need for novel active ingredients with different modes of action to use within rotational approaches. Expanding available treatment options will help to mitigate any future resistance problems. This research seeks to revisit the selenium as an insecticide in a more modern application, incorporation into a bait product. Using selenium as a toxicant in bait avoids the potential environmental contamination as it is contained in the matrix and is limited to the indoor environment. The goal of the study was to: 1) establish toxicological values for organic and inorganic forms of selenium, 2) compare the efficacy of selenium with commercially available bait products for susceptible and resistant strains, 3) explore any secondary transfer and behavioral effects, and 4) elucidate a potential mode of action.

Chapter II. Literature Review of the German Cockroach and Selenium

Distribution and Biology of the German cockroach

Despite being subject to much research, the German cockroach's native range is still unknown because it has never been found in the wild. Two key hypotheses have been proposed: the "out of Africa" and the "out of Asia" hypotheses (Tang et al. 2019). The latter hypothesis is currently the most accepted given that its four most closely related species all originate from Asia. It is likely that the transportation and movement of goods facilitated the invasion of an ancestor of *B. germanica* which adapted to living in indoor environments, leading to the current cosmopolitan distribution of the German cockroach (Lee & Wang 2021).

The German cockroach can be easily identified by the two distinct longitudinal bands running across the pronotum and its overall light brown coloration. Its closest relative is the Asian cockroach, *Blattella asanhinai* Mizukubo, which differs slightly in morphology on the male tergal glands (Roth 2003). The main difference is in their behavior in that they are attracted to light and can fly, characteristics that are absent in the German cockroach. In contrast to other common pest cockroach species such as *Periplaneta americana*, *B. germanica* is relatively small, measuring around 13-16 mm in length (Appel 2021). Although they are most commonly light brown colored, they may vary based on field and laboratory strains. Color mutations have been observed and identified such as the black and orange phenotypes (Ross & Cochran 1962, Smittle &

Burden 1963). Both sexes as adults are fully winged, but the males exhibit a narrow body morphology where the abdomen tapers making it easy to differentiate the two sexes.

The German cockroach, a hemimetabolous insect, undergoes incomplete metamorphosis with three life stages: egg, nymph, and adult. Depending on environmental conditions, the female holds the ootheca before hatching for around 20 to 30 days (Ross & Mullins 1995). Depending on sex and rearing conditions, nymphs undergo 5 to 6 instars until emergence as an adult; however, there have been reports of up to 7 instars in both males and females (Appel 2021, Willis et al. 1958). Nymphs make up most of the population's age class distribution (Ross et al. 1984). Adult males and females occur in a 1:1 ratio (Willis et al. 1958). Females live longer than males, with a lifespan of 140-280 days and 90-140 days, respectively (Ross & Mullins 1995). Under ideal conditions, the entire life cycle can be completed in as little as 100 days (Gould & Deay 1940). Furthermore, the German cockroach can exhibit exponential growth in laboratory and field settings (Ross 1976, Ross et al. 1984). One study on an inactive ship found a 24 to 28-fold increase in only three months (Ross et al. 1984). The short and overlapping generation times make the German cockroach a formidable pest to control in urban areas.

Medical and Veterinary Significance

The German cockroach is a synanthropic indoor pest species. Given its close association with humans, a German cockroach infestation can cause direct health risks by producing allergens and the transmitting microbes and antibiotic resistance genes (Schal & DeVries

2021). Allergens from the German cockroach, also known as *Blattella germanica* (*Bla g*) allergens, have been implicated as potential causative agents in bronchial asthma (Kang 1976, Kang et al. 1979). Asthmatic patients were sensitized to the CR (cockroach) antigens and exhibited immediate and late asthmatic reactions. The potent *Bla g* 1 allergen was produced exclusively in the midgut in both sexes and all life stages of the German cockroach (Gore & Schal 2005). In contrast, *Bla g* 4 was preferentially produced in the accessory reproductive glands of adult males and subsequently transferred to adult females through the spermatophore (Fan et al. 2005). These allergens are introduced into the indoor environment as dust to which humans can get exposed. This causes concern for at-risk groups such as children where cockroach allergens were found to be the most significant allergen associated with morbidity (Rosenstreich et al. 1997).

Cockroaches foraging through indoor spaces inevitably contact bacteria, protozoans, fungi, mold and even helminths present in the environment. Given their domiciliary nature, German cockroaches can serve as vectors and reservoirs for harmful pathogens to be transferred onto sensitive areas such as food preparation surfaces and hospitals. Menasria et al. (2014) found 174 bacterial isolates from German cockroaches collected from two public hospitals. These included *Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumoniae* which are all known to be pathogenic and can cause sickness. Furthermore, the German cockroach may also play a significant role in transmitting antibiotic-resistant bacteria in hospitals and animal production farms (Ahmad et al. 2011, Solomon et al. 2018). In addition, German cockroaches can harbor

protozoan and helminth human intestinal parasites in their gut and external surfaces (Hamu et al. 2014).

Not only does the German cockroach's health importance extend to humans, but also veterinary animals. When ingested, *B. germanica* has been shown to mechanically vector *T. canis* eggs when ingested based on experiments conducted in the laboratory (González-García et al. 2017). The infective stages were excreted after 6 days in the cockroaches and were successfully transferred to rats, a potential paratenic host. Although canids are definitive hosts, humans can also serve as paratenic hosts causing toxocariasis. In some settings, the German cockroach may serve as a vector to both animals and humans. In another example, a spiruid nematode, *Tetrameres americana*, uses *B. germanica* as an intermediate host to complete its life cycle in final avian hosts. Fink et al. (2005) demonstrated that *B. germanica* had a 100% infection rate when fed with female *T. americana*. After a maturation period of 32 days, Lohman brown chickens were fed with larvae from infected *B. germanica* and subsequently had a 100% infection rate.

Public Perception and Monitoring

The public perception of cockroaches is generally negative and associated with poor sanitary conditions and filth which are commonly found in low-income communities (Bradman et al. 2005, Wang et al. 2019a). In a survey examining attitudes of seven insects commonly found in households in the Netherlands, cockroaches ranked the least-liked animal based on aesthetic characteristics such as being scary or disgusting

(Schoelitsz et al. 2018). Tolerance thresholds are very low for cockroaches. A survey across four different cities revealed that 55% of the residents would take action if 0 to 2 cockroaches were seen within 24 hours (Zungoli & Robinson 1984).

Successful control strategies begin with an effective monitoring program, especially where cockroaches can be detected at low levels. The most basic detection technique would be a visual inspection of common aggregation areas such as the kitchen sink, stove, refrigerator, and bathroom (Wang 2021). Because German cockroaches are considered nocturnal, they are more likely to be found in cracks, crevices, and voids where they are not exposed to light during the photophase (Appel 2021). Other monitoring techniques include flushing, jar traps and sticky traps, although the former is generally not recommended. Flushing uses the repellent nature of pyrethrins and pyrethroids to displace cockroaches from their harborages. Jar traps are less effective than sticky traps despite requiring more time to prepare, place, and clean (Smith & Appel 2008). The effectiveness of traps can be increased by using food-based attractants such as bread with beer and apple plus blueberry oil, both highly attractive (Wang & Bennet 2006, Abbar & Wang 2021). All sampling techniques are subject to biases in the under or overestimating of the stages caught, especially the nymphs (Wang 2021). As a result, it is imperative for pest control technicians to have attention to detail with precise repetitions to make the most accurate interpretations of the infestation and implement the most effective control strategy (Owens 1995).

Baits

Baits have been a common and widely used method for controlling cockroach populations in infested areas since the 1990s and can be found in solids, powders, granules, and gels (Appel & Rust 2021). In their most basic form, baits are composed of an insecticide and a phagostimulant (Reierson 1995). Reports of baits for cockroaches date back to 1858 in London. During these times, pest control operators would mix inorganic toxicants with household food products that were thought to be attractive to cockroaches. Later in the 1950s, synthetic organic insecticides made their way into bait formulations, although some proved to be an ineffective control strategy in the field (Reierson 1995). Some classes of active ingredients are primarily or exclusively found in cockroach bait formulations: neonicotinoids, phenylpyrazole (fipronil), amidinohydrazone (hydramethylnon), oxadiazine (indoxacarb), and avermectins (Lee & Rust 2021). Due to the development of modern technologies, baits are available in complex formulations to increase attractants and feeding stimulants, many of which are proprietary information. Some baits can be found in combination with another active ingredient (AI) or an additional IGR. Although ingestion is the primary mode of exposure, contact toxicity may be another potential route for the kill (Bayer et al. 2012). Furthermore, the horizontal transfer through emetophagy, coprophagy, and/or necrophagy is an additional component that contributes to the efficacy of baits (Kopanic & Schal 1997; Buczowski et al. 2001, 2008).

In contrast to other formulations, baits have the advantages of being relatively safe, useful in sensitive areas, and long-lasting (Reierson 1995). Some baits are sold in

tamper-proof bait stations, and the small amount of AIs used can reduce the generated residues and any potential health risk (Appel & Rust 2021, Wang et al. 2019b). An advantage to baits is that they only require the necessary amount to treat the area. Paste or gel formulations can be applied in sensitive areas where exposure to humans or animals is a major concern, especially in areas such as hospitals and zoos. Baits are efficacious even after aging and may even be more attractive to cockroaches (Reierson 1995, Nalyanya 2001). However, control with baits can be a very complex issue making it difficult to predict a bait's performance in a field setting (Reierson 1995). To combat resistance, it is suggested to rotate baits every three months, and it has recently been shown to be a promising approach (Scharf & Gondhalekar 2021, Miller & Smith 2020).

Resistance

The main resistance mechanisms are metabolic detoxification, target-site insensitivity, penetration, and behavioral (Cochran 1995). Metabolic detoxification is the degradation of an insecticide mediated by Phase I and II enzymes that renders it ineffective (Scharf & Gondhalekar 2021). Important enzymes involved are cytochrome P450s, carboxylesterases and glutathione S-transferases. Increased susceptibility caused by using synergists like PBO and DEF is indicative of the presence of enzyme-based detoxification (Chai & Lee 2010). Target-site insensitivity involves the selection of mutations within the target site that interfere with insecticide binding (Scharf & Gondhalekar 2021). The *Rdl* (resistance to dieldrin) mutation, A302S, which confers resistance to cyclodienes and phenylpyrazole (fipronil), has been identified in the German

cockroach (Kaku & Matsumura 1994). In addition, knockdown resistance (*kdr*-type resistance) linked to the L993F substitution confers resistance to chlorinated hydrocarbons (DDT), and pyrethrin and pyrethroid classes exist in the German cockroach (Dong 1997). Penetration resistance refers to decreased insecticide absorption through the cuticle. This form of resistance is probably not an important mechanism in the German cockroach and usually occurs with other physiological resistance mechanisms (Scharf & Gondhalekar 2021, Chen et al. 2020). One of the documented behavioral resistance mechanisms in the German cockroach is the reduced or enhanced dispersal in response to insecticides (Ross 1992, Hostetler & Brenner 1994). In addition, the aversion to glucose in baits has been well studied (Silverman and Bieman 1993, Silverman and Selbach 1998). More recently, Wang et al. (2004) found a field strain significantly averse to other sugars: fructose, maltose, and sucrose.

Selenium

Selenium (Se) is a naturally occurring metalloid element that exists in several states; an amorphous liquid and three different crystalline forms (National Research Council 1983). It has an atomic number of 34 and an atomic mass of 78.6. Elemental Se (Se^0), heavy metal selenides (Se^{+2}), selenites (Se^{+4}) and selenates (Se^{+6}) are all common oxidation states of selenium. It is widely distributed in all earth materials at an average of 0.09 ppm, but black shales have concentrated amounts of up to 675 ppm (Lakin 1972). Other areas where selenium is concentrated are ore deposits of uranium and phosphate rocks. In

the western United States selenium is found in Cretaceous marine sedimentary rock (Presser et al. 1994).

Health Benefits of Selenium

Schwarz and Foltz (1957) first discovered the nutritional function of selenium in rats, but its role in humans remained unclear at the time. An interest in selenium peaked during the 1970s through breakthrough research that linked selenium as an essential component to glutathione peroxidase (Combs 1990). Research exponentially grew in the 1980s and new discoveries were made such as the relationship associated with selenium to reduced risks of cancer and heart disease. More recently, researchers found an association between people living in selenium-deficient areas and increased fatality rates from coronavirus disease 19 (Zhang et al. 2021). Selenium has become established as an essential trace element for humans and other animals. The vital role of selenium is attributed to its incorporation in selenoproteins as selenocysteine, most of which are redox enzymes (Burk & Hill 2005). Selenium-dependent glutathione peroxidases (Gpx) protect the body from oxidative injury by regulating hydroperoxides. In addition, selenium is also found in thyroid hormone metabolism (e.g., deiodinase enzymes) and redox control of intracellular reactions (e.g., thioredoxin reductase) (ATSDR 2003).

The primary route of selenium exposure for humans is through the ingestion of food products containing selenium (National Research Council 1983). The amount of selenium in food varies by location, but meat and cereal products tend to have the highest levels while fruit and vegetable products have the lowest. To benefit from selenium's

antioxidant properties, humans can take it as a supplement. The Institute of Medicine (2000) reported the Recommended Dietary Allowance (RDA) for selenium to be 55 μg (0.7 μmol)/day for both men and women. This value was generated based on the maximum glutathione peroxidase synthesis levels. Sodium selenite and sodium selenate have been used in the agricultural industry to supplement feed to prevent any disorders rising from selenium deficiency (National Research Council 1983). For example, Finnish soils naturally low in selenium are fertilized with selenium (Aspila 2005). Keshan disease, cardiomyopathy endemic to China, is the only human disease that is caused by selenium deficiency (Institute of Medicine 2000). In animals, selenium deficiency can lead to severe symptoms such as lipid peroxidation, liver necrosis and cardiac injury.

Selenium Toxicity

The duality of selenium presents a complex situation. On one hand, it is an essential trace element, but on the other, it can exhibit toxic effects on the consumer at slightly higher concentrations. The Tolerable Upper Intake Level (UL), the level at which there is no observed selenosis, is set at 400 μg (5.1 μmol)/day for adults (Institute of Medicine 2000). In human case studies, selenium toxicity can manifest itself as symptoms of diarrhea, fatigue, hair loss, nail discoloration or brittleness, and even death (See et al. 2006, MacFarquhur et al. 2010). In lab animals, acute toxicity of selenium resulted in vomiting, dyspnea, tetanic spasms, death from respiratory failure, and pathological changes to organs (National Research Council 1983). Furthermore, in livestock animals, similar severe effects are observed. The toxicity of selenium can be attributed to its

similarity to sulfur. In excess, selenium may substitute sulfur in proteins, ultimately leading to malformed, non-functioning proteins (Daniels 1996). Inorganic selenium tends to be the more toxic than organic selenides. In contrast, elemental selenium is relatively nontoxic (ATSDR 2003).

Historical Studies on Arthropods

Based on early reports, selenium may have been the first systemic insecticide explored for use on pest species (Gnadinger 1933, Reed et al. 1962). Hurd-Karrer & Poos (1936) provided various cereal plants with selenium concentrations ranging from 1 ppm to 12 ppm in the form of sodium selenate. The twospotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) and bird cherry-oat aphid, *Rhopalosiphum padi* (Hemiptera: Aphididae) were placed on two-month-old plants grown in concentrations greater than 3 ppm died within a few days. Similar results were observed using one-month-old plants. Complete inhibition of aphid infestations was achieved without negative effects on the plants, showing its potential utility as a systemic insecticide. However, the authors issued a precautionary statement due to the awareness of its toxicity to mammals. Shortly after, Mason & Phillis (1937) explored the selenium to protect cotton plants from the pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). Trelease & Trelease (1937) studied an interesting system of the two-grooved milkvetch plant, *Astragalus bisulcatus*, which accumulates selenium and its associated selenium-tolerant insects. Seeds were found to contain around 1475 ppm, an extremely high selenium content.

Nonetheless, chrysomelids (*Acanthoscelids fraterculus)* and seed-feeding chalcid wasps (*Bruchophagus* sp.) thrived on levels of selenium that could kill rats.

Shortly later, Neiswander & Morris (1940) sought to conduct a more comprehensive study into selenium as a systemic, building on earlier studies. The authors found a dose-dependent response in the decrease in *T. urticae* populations with 1 ppm of sodium selenate providing complete control on tomato plants grown in nutrient solutions. Other experiments conducted showed that higher concentrations of selenium might be required for adequate control of pests on ornamental plants due to the difference in selenium accumulation in the foliage. Morris et al. (1941) also reported on *T. urticae* control in corn plants. Similarly, they found that 1-2 ppm range resulted in no observed pest injury and complete pest eradication. Based on the reports provided by these earlier studies, the greenhouse industry started using selenium as a systemic by 1945 in granular and capsule forms to control mites, although not extensively (Reed et al. 1962).

Recent Studies on Selenium and Insects

Although selenium can be found naturally, anthropogenic activities can introduce it into areas at high levels thus concentrating it, potentially outweighing all-natural sources combined (Fairweather-Tait 2011). A shift from the insecticidal application of selenium towards environmental toxicology occurred in the 1990s as concern for selenium as an environmental toxin increased and warranted further investigation. In areas of contamination such as in the San Joaquin Valley of California, concern for aquatic fauna remained high (Presser et al. 1994, Lemley 1997). As a result, many studies examined the

effects of selenium on aquatic insects and herbivorous insects that could potentially feed on plants with bioaccumulated selenium in their tissues (Maier & Knight 1993, Trumble et al. 1998, Hanson et al. 2004, Franz et al. 2011). Furthermore, selenium has been subject to review within the context of other pollutants and metal contaminants (Jensen & Trumble 2003, Butler et al. 2009, Mogren & Trumble 2010). These studies expanded the knowledge of selenium in the field of environmental toxicology. Furthermore, bioaccumulating plants' phytoremediation efforts have also been investigated (Bañuelos et al. 2002).

Various inorganic and organic forms of selenium have been tested against many insect orders that can potentially be exposed to selenium in the environment, terrestrial or aquatic. Here we have reviewed relevant literature from the 1990s onward.

Impact of selenium on the survival of insects

In general, selenium compounds have been demonstrated to affect insects in a dose-dependent response. The high water-solubility of most selenium compounds makes them generally easy to work with in solutions and diet treatments. Commonly reported values to evaluate toxicity are LD_{50} (Lethal Dose) and LC_{50} (Lethal Concentration) values (Yu 2014). Studies investigating the toxicity of inorganic forms used selenium salts (strictly selenate and selenite), while studies evaluating organic forms commonly used selenomethionine and selenocysteine. Most studies have included selenate due to its environmental relevance, and thus represents the form we currently have the most information on.

All forms, organic and inorganic, have induced toxic effects on insects to varying degrees (Table 2.1). Several studies have comprehensively examined at least one of each inorganic and organic forms. Studies on the honey bee, *Apis mellifera* (Hymenoptera: Apidae), whether through single and chronic doses on foragers or through a larval artificial diet, have shown that selenate is the most toxic form (Hladun et al. 2012, 2013a). Mortality was observed in the pupation stage as only 9% of the larvae successfully pupated at 1 mg/L of spiked diet (Hladun et al. 2013a). Trumble et al. (1998) tested four different forms in spiked artificial diet against the beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), and reported that selenite had the lowest LC_{50} value of 9.14 µg/g. Another study looked at the effects of selenium on the tobacco budworm, *Heliothis virescens* (Lepidoptera: Noctuidae), through spiked artificial diet (Popham & Shelby 2007). Total mortality was observed with seleno-DL-methionine 100 µg/g, while selenite caused 60% larval mortality at the same concentration. In contrast to S. *exigua*, an inorganic form was reported as the most toxic to *H. virescens* even though both species are within the same family. The Argentine ant *Linepithema humile* (Hymenoptera: Formicidae), a cosmopolitan urban pest species, exhibited varying degrees of toxic effects on all forms of selenium (De La Riva et al. 2014, 2016). When selenium was added to 25% sucrose solutions, seleno-L-methionine was evaluated at an LC_{50} of 87.83 mg/L (De La Riva 2014). Selenate was the second most toxic, with a reported LC_{50} value of 131.6 mg/L at seven days. De La Riva et al. (2016) further supported the toxicity of selenate when queen mortality was observed, and 0% of larvae entered the pupal stage. The most toxic forms of selenium vary across insect orders.

Selenium is known to have detrimental effects on aquatic fauna. As a result, many studies have investigated the toxicity of selenium on water-dwelling larval insects through waterborne exposure especially Dipterans (Debruyn & Chapman 2007). When *Chironomus decorus* (Diptera: Chironomidae) 4th instars were reared in toxicant solutions of selenate, selenite, and seleno-DL-methionine, the authors reported LC_{50} values of 23.7, 48.2, 194 mg/L at 48 hours, respectively (Maier & Knight 1993). *Chironomus riparius* (Diptera: Chironomidae) exposed to field-collected or laboratory water spiked with selenium exhibited higher mortality in seleno-L-methionine regardless of the water used in 40-hour acute tests (Ingersoll et al. 1990). Mixtures of both inorganic selenium forms (selenate: selenite, 6:1) were chronically exposed to larvae and resulted in reduced adult emergence. In addition, Beaty & Hendricks (2001) supported selenite-induced larval mortality through various experimental setups. Franz et al. (2001) found minimal effects of selenate, selenite, and seleno-DL-methionine (4 µg/L) on *Chironomus dilutus* (Diptera: Chironomidae) on larval survival after 10 days. Similarly, Gallego-Gallegos et al. (2013) exposed larvae to selenite in water, spiked fish food, and spiked algae at various concentrations without any significant effect on larval survival (>80%). A brine fly, *Ephydra cinerea* (Diptera: Ephydridae) 3rd instars evaluated to acute exposure of 24 and 48 hours (selenate, selenite, and seleno-DL-methionine) yielded no effect on larval survival of >92% and >88% for all forms, respectively (Rosetta & Knight 1995). Jensen et al. (2007) explored a medically relevant mosquito species, *Culex quinquefasciatus* (Diptera: Culicidae). When exposed to aqueous selenate solutions, an LC_{50} of 11 mg/L at 14 days was reported with 83% percent mortality at 16 mg/L. Based on larval mortality

of aquatic Dipterans, inorganic and organic selenium forms have been shown to exhibit a range of toxic effects.

Two other dipteran species subject to investigation were *Drosophila melanogaster* (Diptera: Drosophilidae) and *Megaselia scalaris* (Diptera: Phoridae). Newly emerged *D. melanogaster* flies were maintained on artificial media from 10^{-8} to 10^{-4} M (Martin-Romero et al. 2001). At the highest concentration, greater than 90% mortality was observed by 35 days; however, the lowest three concentrations did not affect mortality. Two studies examined *M. scalaris* on a laboratory diet. Selenocysteine was the most toxic form, followed by seleno-L-methionine and selenate based on lethal concentrations in *Drosophila* diet (Jensen et al. 2005). Jensen et al. (2006) reported a comparable LC_{50} value and decreased larval survival. However, there were no effects on the survival of puparia or eggs exposed to the treated diet.

Since plants bioaccumulate selenium into their tissues, studies involving Lepidopteran larvae potentially show the interaction between selenium hyperaccumulators and herbivorous insects in contaminated environments (Banuelos et al. 2002). *S. exigua* fed saltbush plants exposed to selenate for 30 days reduced the mean day of death and mortality on treated plants as seen in earlier reports with an artificial diet (Trumble et al. 1998). Vickerman & Trumble (2003) observed reduced survival to pupal and adult stages when alfalfa plants were irrigated at 0.20 g/ 60 L of water. Selenate had no effect on larval survival on the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), at an 80 mM dose on prince's plume. However, decreased larval survival was reported on a selenium tolerant strain (Freeman et al. 2006). In addition, the

imported cabbageworm *Pieris rapae* (Lepidoptera: Pieridae) was affected by two treated host plants (mustard and prince's plume) on larval survival (Hanson et al. 2003, Freeman et al. 2006). Selenite in an artificial diet, protected *Trichoplusia ni* (Lepidoptera: Noctuidae) from *Autographa californica* nucleopolyhedrovirus (Popham et al. 2005). Larvae that were fed their entire larval stage until pupation, and larvae that were fed on untreated diet and then transferred to treated diet increased the LC_{50} of the virus. This indicated that dietary selenium lowered the susceptibility of the larvae to the virus.

Due to the concerns of biotransfer across trophic levels, studies have sought to replicate the potential exposure of selenium on predators in the environment. When *Sympetrum corruptum* (Odonata: Libellulidae) were fed on selenium-exposed prey as *Culex quiquefasciatus* (Diptera: Culicidae), there were no differences in predator survival compared to that of untreated predators (Jensen 2006). Similarly, in another predator species, the spined soldier bug *Podisus maculiventris* (Hemiptera: Pentatomidae) survival was unaffected when fed *Megaselia scalaris* (Diptera: Phoridae) larvae reared on 125 µg/g selenate (Jensen 2006). In contrast, Vickerman & Trumble (2003) observed 59% and 58% mortality in *P. maculiventris* when fed *S. exigua* larvae reared on a diet of 109 µg/g and 135 µg/g, respectively. Parasitoids have also been subject to investigation. *Cotesia marginiventris* (Hymenoptera: Braconidae), a parasitoid of noctuids, were allowed to parasitize *S. exigua* larvae which were fed on alfalfa plants irrigated with selenate (Vickerman et al. 2004). The authors observed no effect on the survival of pupae and adults.

Studies on Coleoptera and Orthoptera have been limited. Hogan and Razniak (1991) investigated the effects of selenite on yellow mealworm, *Tenebrio molitor* (Coleoptera: Tenebrionidae) through nutrient media. The inorganic form decreased survivorship and had residual mortality effects on adults even when they were transferred onto control nutrient media after one week, although it progressively declined towards the latter part of the observation period. Audas et al. (1995) examined selenate on *T. molitor* and reported clear dose-dependent responses on survival when maintained on spiked nutrient media. Similar to Hogan and Razniak (1991) findings, insects later transferred to control media after exposure also caused decreased survival. The house cricket, *Acheta domesticus* (Orthoptera: Gryllidae) fed mustard leaves grown in selenate solution achieved 100% mortality by 11 days (Freeman et al. 2007). Various field-collected Orthopterans were fed desert princes' plume grown in two solutions of methyl-selenocysteine, 2 μM and 40 μM. The authors reported a 10% and 70% survival rate on the higher solution and lower solutions, respectively. Another study involving *A. domesticus* found that consumption of a selenite-spiked diet at 0.3 and 10 μmol Se/kg decreased survival when compared to a dose of 1 μmol Se/kg (Ralston et al. 2007).

Effects of selenium on the growth, development, and reproduction of insects

At sublethal doses, there is sufficient evidence to conclude that selenium can disrupt normal physiological development and impact reproduction through reduced fecundity (Table 2.2). Many toxicological studies are paired with observations on the growth and development of test insects.

Sublethal effects can be detrimental to social insects because colony-level effects can reduce overall health. *A. mellifera* has been subject to several studies as it is an important pollinator species present in many environments. Studies have documented sublethal developmental effects for inorganic and organic forms: selenate, selenite, methylselenocysteine, and selenocystine. Hladun et al. (2013a) found reduced RGI (relative growth index) for all forms when larvae were reared on a spiked artificial diet. However, there were no effects on prepupal weight for all treatments. De La Riva et al. (2016) further tested selenate and found injurious effects at the colony level in a 60-day exposure study. Brood surface area was reduced, and few capped cells and no pupae were observed. In addition, no brood were produced by the end of the experiments. *L. humile* fed selenate in a 25% sucrose solution produced fewer eggs, and the viability and development of the offspring were reduced as well (De La Riva et al. 2016).

Lepidopteran species have been extensively studied on the sublethal effects of selenium on artificial laboratory diet; *S. exigua* comprises the majority of the studies. In an artificial diet, both selenate and selenite reduced pupal weight at 12 μg/g (Trumble et al. 2018). In addition, time to pupation and adult emergence were both delayed, and decreased relative growth rate (RGR) and RGI were also observed. However, the organic forms seemed to have a limited effect on development. Seleno-DL-methionine did not affect any developmental parameters, while seleno-DL-cystine only affected RGR (Trumble et al. 2018). Bañuelos et al. (2002) observed reduced growth when 1st instars were exposed to selenate by feeding on saltbush leaves. Vickerman and Trumble (2003) found no effect on development of *S. exigua* on alfalfa plants irrigated at a lower dose of

0.0066 g/60 L, while 0.20 g/60L had a significant impact on the stage at death and RGI. Popham et al. (2005) reported a dose-dependent response of reduced larval weight of cabbage looper *Trichoplusia ni* (Lepidoptera: Noctuidae) from ranges 25 ppm - 100 ppm of selenite in an artificial diet. In other experiments in that tested ranges from 0 – 20 ppm, larval growth lagged at the two highest concentrations (10 and 20 ppm) regardless of feeding procedure: entire larval stage on a treated diet or treated diet until 4th instar then transferred to untreated diet and vice versa. There were no effects on pupal weight except in the second protocol (treated diet then moved to untreated diet), where the 10 ppm Se group had a statistically higher mean pupal weight suggesting a benefit to a Se supplemented diet (Popham et al. 2005). Lalitha et al. (1994) also documented a benefit of dietary Se in the rice moth *Corcyra cephalonica* (Lepidoptera: Pyralidae) reared on a wheat flour diet until the 4th instar. At 2 ppm selenite, larval weight increased by 100% and declined (30%) at a slightly higher concentration of 4 ppm, indicating a narrow range where selenium may be beneficial. Popham & Shelby (2007) subjected *H. virescens* to five different treatments in an artificial diet: selenate, selenite, seleno-DL-cysteine, seleno-DL-methionine, and a selenized wheat product. Selenate did not affect time to pupation or emergence at rates five times more than those tested with selenite. Test insects exhibited a wide range of sublethal effects from a decreased rate of development, pupation, emergence, and reduced pupal weight to the other forms of selenium.

Lepidopteran pest species exposed to selenium through host plant feeding had similar effects on development. *P. xylostella* decreased larval weight gain on princes' plume watered at 8 mM selenate for three days (Freeman et al. 2006). *P. rapae* exhibited

severe inhibition of larval growth on both mustard and princes' plume host plants watered with selenium. Newly hatched larvae did not grow within the nine days of feeding on mustard (20 μM Se), and all died. In addition, nine-day-old larvae introduced to mustard plants lost 20% of their weight within the first day, while control larvae gained 30% during the same period (Hanson et al. 2003). Freeman et al. (2006) also observed decreased larval weight when *P. rapae* was fed with treated princes' plume (80 mM Se).

An overwhelming amount of information is available with Chironomid species on the effects of organic and inorganic selenium forms. *C. decorus* larvae fed on selenium contaminated widgeon grass (*Ruppia maritima*) in 96 hour (4^{th} instar) and 14 day (egg to pupation) feeding times reported contradicting sublethal effects. For 96 hours, controls had the lowest mean weight while midges that accumulated the most Se had the highest mean weight. In contrast, in the 14-day experiment, higher selenium accumulation decreased mean midge weight. The authors suggest that the differing results may be due to the prolonged exposure time. Malchow et al. (1995) also investigated *C. decorus* on a non-artificial diet of algae (*Selenastrum capricornutum*) with selenate (0-40 μg/L) and selenite (0-40 μg/L). At higher concentrations, larval growth was reduced at 96 hours. In addition, larvae chronically maintained at 6:1 ratio of selenate and selenite increased the day of first emergence time (Ingersoll et al. 1990). Another chironomid species, *C. dilutus* was tested against selenate, selenite, and seleno-DL-methionine as larvae in aqueous solutions (4 μg Se/L). Both organic forms had no effect on growth rate, time of adult emergence, or adult weight. On the other hand, seleno-DL-methionine reduced the

growth rate during the first half of the experiment. Day 10 mean larval dry weight was 48% of the larval dry weight at day 20 versus 61% for control. In addition, adult emergence was only 56% for the organic Se compared to 82% emergence in the control group. Interestingly, male to female sex ratio was changed with a bias towards females with selenate, selenite, and seleno-DL-methionine. However, the effect was most drastic with selenite (0.35 Se vs. 1.41 control). This shift in sex ratio may suggest increased offspring in the next generation. Gallego-Gallegos et al. (2013) further examined the sublethal effects of selenite on *C. dilutus.* Nine to ten-day-old larvae exposed to waterborne Se, nanoparticles, or nanoparticles in fish food all had reduced growth at the highest concentrations. However, selenite in the form of spiked algae (*Scenedesmus* sp.) did not affect larval growth. In a series of oviposition bioassays, the spiked diet did not affect *M. scalaris* egg hatching when subject to selenate, selenite, or seleno-DL-methionine at an exposure time of 2 days (Jensen et al. 2005). However, Se-(methyl)-selenocysteine hydrochloride reduced egg hatchability at the highest concentration tested (100 μg/g). When observed from egg to adult on the same concentrations for each compound, larval development was delayed for all selenium forms. This delay was seen as low as 25, 50, 100, and 300 μg/g for selenocysteine, seleno-L-methionine, selenate, and selenite, respectively. No forms had any effect on the number of days to complete pupariation. There was an increase in the number of days for females to emerge compared to that of males in only both organic forms at 25 μg/g. Selenocysteine and seleno-L-methionine displayed the most negative sublethal effects compared to all other forms suggesting high toxicity with organic forms. Furthermore, Jensen et al. (2006) also

observed comparable effects on development and no effect on fecundity when diet flakes were rehydrated with selenium.

Various other Diptera were evaluated for sublethal effects. Male and female *D. melanogaster* were maintained separately on either chemically defined media with or without selenite 10^{-6} M or on complete media. After 14 days, they were combined and allowed to oviposit on untreated media for 36 hours. Although the viability of eggs was not affected, the number of eggs that were oviposited decreased by 50% when both sexes were maintained on a chemically defined medium without selenium versus all other treatments. This suggests that selenium may benefit egg production and/or fertilization. *C. quinquefasciatus* reared in solutions of selenate (2-32 mg/L) resulted in RGI as low as 2 mg/L from day 4 to experiment termination (Jensen et al. 2007). Rosetta & Knight (1995) found minimal effects of solutions of selenate, selenite, and seleno-DL-methionine on *E. cinerea.* No effect on larval weight changes and weight differences was observed at 10-20,000 µg/L for 24 and 48 hours. These results are similar to the minimal effects observed in *C. dilutus* (Franz et al. 2011).

Predatory insects exposed to selenium as their prey items have shown that sublethal effects can travel up trophic levels. Vickerman & Trumble (2003) provided *P. maculiventris* with treated *S. exigua* larvae as prey and observed a 20% reduction in weight compared to that of control predators and decreased developmental rate to reach various stadia. A parasitoid, *C. marginiventris*, was allowed to completely develop in *S. exigua* larvae that were fed alfalfa plants irrigated with 3.3 mg/L (Vickerman et al. 2004). Compared to controls, treated larval development and adult eclosion were delayed by two

days compared to wasps developed on untreated *S. exigua.* However, no significant effects were seen from time from pupation to adult. Cocoons also weighed 10% less. However, the authors could not conclude whether the increased developmental time was related to previous findings on the more extended development of the host. The biotransfer of selenium can cause a less suitable host or prey items for predators, thus resulting in negative consequences.

Minimal information on the development and reproduction of selenium on the orders of Ephemeroptera and Orthoptera. Conley et al. (2009) fed 4-6 day old larvae of *C. triangulifer* (Ephemeroptera: Baetidae) periphyton exposed to a mixture of selenious acid and selenite. At the two highest concentrations of 10 and 20 μg/L, fecundity was reduced. In addition, Se body burden was associated with reduced adult body mass; however, it was not linked to the decrease in fecundity. Freeman et al. (2007) found sublethal effects on *A. domesticus* and various Orthopteran species. The weight of *A. domesticus* was reduced by 10% while the various Orthopteran species produced no molts compared to 43 produced in the control group suggesting a delay in development. In contrast, Ralston et al. (2007) found no significant effect of selenite-spiked diet on growth when 1.0 μmol Se/kg was compared to 0.1, 1, 3, or 10 μmol Se/kg.

Impacts of selenium on the behavior and other effects of insects

Selenium may influence behavioral effects such as consumption, preference or avoidance of selenium detected in food or substrate (Table 2.3). The complex nature of selenium

toxicity has warranted investigations to determine other effects selenium may have on insects.

Hladun et al. (2012) conducted various experiments on *A. mellifera* to determine consumptions responses to selenate and selenomethionine. Selenate at ranges from 0.6-6000 μg/mL in 1M sucrose produced no significant effects on proboscis extension reflex (PER) in an antennal response assay and total consumption in a proboscis response assay. However, in the sucrose response threshold (SRT) assays, the authors observed a dose-dependent change in PER to increasing concentrations of sucrose which lowered the overall PER for all selenium treatments. In contrast, selenomethionine elicited more drastic behavioral changes. PER responses to antennal stimulation were significantly lower at 60 and 6000 μg/mL of Se in 1M sucrose. No differences in total consumption were noted in the proboscis response assays. Like selenate, there was a dose-dependent change in PER to increasing concentrations of sucrose where SRT occurred between 3 and 10% except for at 0.6 and 6 μg/mL where SRT was as high as 30%. Selenomethionine did not affect SRT. Burden (2016b) also observed no effect on PER in a sucrose responsiveness test for selenate and methylseleno-L-cysteine. Hladun et al. (2013a) were able to find evidence for reduced consumption of four selenium forms: selenate, selenite, methylselenocysteine, and selenocystine. Forager bees were treated with 19.2 μL of a 50% sucrose solution spiked with selenium. The bees were given untreated sucrose solutions in the following days, and consumption was calculated. In a field setting, *A. mellifera* did not discriminate between selenate-treated floral resources

suggesting that pollinators may be exposed to selenium in small amounts during foraging (Quinn et al. 2011, Hladun et al. 2013b).

Interestingly, selenium may impair the memory function of *A. mellifera* at sublethal doses of selenate and methylseleno-L-cysteine (Burden 2016b). An acute exposure as low as 18 ng reduced performance during a long-term recall test for both forms. These learning and memory impairments can severely impact colony success. Selenium may cause significant effects on the microbiome of Apidae based on a few recent studies (Rothman et al. 2019ab, 2020). When 0.6 mg/L of selenate was provided in 50% sucrose solutions, *A. mellifera* experienced an altered microbiome composition over a 7-day period (Rothman et al. 2019b). In *Bombus impatiens* (Hymenoptera: Apidae), selenate also altered microbiome composition and reduced core symbionts ESVs (Exact Sequence Variants) (Rothman et al. 2020). The microbiome may be a key component of bumble bee survival when exposed to selenium based on a study by Rothman et al. (2019a). Inoculated *B. impatiens* experienced a significant increase in mean survival when exposed to selenate in 40% sucrose. Interestingly, the authors found a significant increase in alpha diversity of the bee microbiome while finding that some core symbiont ESVs decreased in proportional abundance.

Behavioral effects of selenium were minimal in other Hymenoptera species. *L. humile* presented with selenate, selenite, seleno-L-methionine, or methylselenocysteine in 10 or 30% sucrose solutions had no effect on preference over untreated solutions (De La Riva et al. 2014). Competition assays with *L. humile* and *Dorymyrmex bicolor* (Hymenoptera: Formicidae) also revealed a minimal effect on behavior between these

two species (De La Riva et al. 2016). There was no effect on latency of bait discovery with the presence of a competitor or treatment for either species. However, *D. bicolor* did experience a slower time to bait discovery when these two factors were combined. In addition, competition behavior was not affected. The parasitoid, *C. marginiventris*, displayed no selenium volatile specific response in an olfactometer bioassay (Vickerman et al. 2004).

Selenium may have a repellent effect on Lepidopteran larvae through reduced palatability of the artificial diet. Vickerman et al. (1999) provided neonate and 3rd instars between a choice of a control diet and a diet spiked with selenate, selenite, seleno-DL-cystine, or seleno-DL-methionine. The inorganic forms had the most repellent effect as both neonates and 3rd instars preferred the control diet over the treated diet. Furthermore, consumption on the treated diet was reduced, showing evidence for antifeedant activity. On the other hand, the organic forms had variable responses where neonates displayed sensitivity to higher concentrations, but 3rd instars had no preference in choice studies. In addition, there was no effect on consumption. Vickerman et al. (2002) observed varied responses when neonates and 4th instars fed on alfalfa plants irrigated with selenate. At the higher concentration (0.20 g/ 60 L), neonates had no preference for either untreated or treated plants, and 4th instars preferred treated plants at 4 of 10 observed time points. In addition, consumption increased on treated plants indicating that larvae did not avoid lethal host plant material. Other studies on *T. ni*, *P. xylostella* and *P. rapae* revealed a similar preference for untreated plants over selenate treated plants (Vickerman et al. 1999, Bãnuelos et al. 2002, Hanson et al. 2003, Freeman et al. 2006).

Oviposition preference on contaminated host plant material can determine the outcome of potential selenium exposure. Extensive knowledge exists on the ovipositional effects of selenium across many species of Lepidoptera and Diptera. *S. exigua* females preferred to oviposit on low-Se alfalfa plants over control plants, but displayed no preference between high-Se and control plants (Vickerman et al. 2002). The authors state that these plants can potentially serve as a population "sink" where larvae will be exposed to a lethal dose of selenium. *P. xylostella* and *P. rapae* also oviposited preferentially on untreated plants (Freeman et al. 2006). Further observation to 30 days revealed that larvae fed less on the treated plants. In the Dipteran species, *M. scalaris*, no oviposition preference was shown with a diet spiked with selenate, selenite, seleno-methionine, or Se-(methyl)-selenocysteine hydrochloride (Jensen et al. 2005). Jensen et al. (2006) supported that *M. scalaris* does not have any oviposition preference at a maximum rate of 500 μg/g between selenate and control diets. *C. quinquefasciatus* also did not oviposit preferentially on control ponds not treated with selenate (Jensen et al. 2007). *D. radicum* showed mixed results in the field setting where there was an increase in the number of eggs laid on treated at only 2 dates (Mechora et al. 2007).

The order Odonata, Orthoptera, Coleoptera, and Blattodea have limited information regarding sublethal effects on behavior. *S. corruptum* increased feeding on *C. quinquefasciatus* when kept in a selenate solution compared to that in untreated solution (Jensen 2006). Five times more *A. domesticus* preferred to feed on untreated mustard leaves than treated leaves grown in 20 μM of selenate solution (Freeman et al. 2007). This preference for untreated leaves was observed for various Orthoptera spp. on princes'

plume grown in methyl-selenocysteine. In the field setting, herbivory by grasshoppers also dropped. Mechora et al. (2017) observed *Phyllotreta* spp. (Coleoptera: Chrysomelidae) feeding damage on selenate-treated broccoli in the field. One out of two growing seasons, there was increased damage on treated broccoli plants compared to control plants. Although fewer pupae were found on treated plants for both years, the number was only significant for only one (Mechora et al. 2017). There is evidence to suggest that aphid species can detect selenium in host plants and thus avoid them. *M. persicae* preferred feeding on untreated *Brassica* plants. Seven times more aphids were observed on plants grown in selenate solutions as low as 1 μM (Hanson et al. 2004). At higher concentrations ≥ 5 μM, few if any aphids were found. Similarly, *Brevicoryne brassicae* (Hemiptera: Aphididae) numbers decreased on selenate-treated radish plants (Hladun et al. 2013b). The only study on a cockroach species was conducted by Nakonieczny (1993) on *Gromphadorhina portentosa* (Blattodea: Blaberidae). The author found reduced activities of several enzymes when treated at a sublethal dose of sodium hydrogen selenite.

Conclusion

This chapter represents the most comprehensive literature review on the effects of selenium on insects to date. The literature encompasses a wide variety of insects that have different life histories and ecological niches. The various ways that selenium can affect survival, normal development and function, and behavior illustrate the complexity of selenium as a toxicant. Based on findings of repellency and toxicity to insects,

selenium has been reconsidered for use on crops as an insecticide (Hanson et al. 2004, Mechora 2019). These studies provide the basis for potential selenium toxicity to our test insect, *Blattella germanica* (Blattodea: Ectobiidae), warranting investigation of discovering a novel bait toxicant.

Table 2.1 Impact of selenium on the survival of insects

Order	Species	Se Form	Testing method	Observations	Reference
Odonata	*Sympetrum corruptum*	Selenate	Feeding *Culex quinquefasciatus* 2nd instars reared in 15 μg/g Grown in treated solution with treated prey and without	No differences in survival when in treatment or exposed to prey	Jensen 2006
Orthoptera	*Acheta domesticus*	Selenate	Feeding *Brassica juncea* leaf grown in 20 μM solution	100% mortality by day 11	Freeman et al. 2007
		Selenite	Two-week-old crickets fed torula yeast-based diets (0.1, 0.3, 1, 3, 10 μmol Se/kg) for 5 w	Decreased survival at 0.3 and 10 μmol vs 1 μmol Se/kg based on hazard ratios	Ralston et al. 2006
	Various spp.	Selenate	*Stanleya pinnata* grown in 40μM (high) or 2 μM (low) solution	High 10% survival rate vs low 70% survival rate (7 DAE)	Freeman et al. 2007
Hemiptera	*Podisus maculiventris*	Selenate	Feeding *Megaselia scalaris* larvae reared in 125 μg/g	No effect on predator survival	Jensen 2006
		Selenate	Feeding *Spodoptera exigua* larvae reared on diet 109 μg/g and 135 μg/g dry weight	59% (low) 58% (high) survival vs 89% (control) Stage at death and day of death not sig treated vs control	Vickerman & Trumble 2003
	Myzus persicae	Selenate	Feeding *Brassica juncea* plants 0, 10, 20 or 40 μM (nonchoice)	~0% aphids found on treated 7DAE indicate lethality	Hanson et al. 2004
			Detached leaves (0, 0.1, 1, 5, 10, or 20 μM Se for 7 d) (nonchoice)	Mortality observed from 1 μM and higher	Hanson et al. 2004
			Systemic 0, 0.1, 5 or 10 μM 7DAE	Decrease in aphid pop. as increase conc. up to 75% decrease in 10uM	Hanson et al. 2004
			Topical 20 μM sprayed every other day	20% decrease in pop. similar to 0.1 as systemic	Hanson et al. 2004
Hymenoptera	*Apis mellifera*	Selenate	Single dose mortality assay (0.6 to 6000 mg/mL) in 1M sucrose	Up to 67% mortality at 5 d	Hladun et al. 2012
			Chronic dose mortality assay 20 μl daily (0.6 to 6000 mg/mL) in 1M	Up to 89% mortality at 5 d	

			sucrose for 5 d		
		Seleno-methionine	Single dose mortality assay (0.6 to 6000 mg/mL) in 1M sucrose	Up to 59% mortality at 5 d	Hladun et al. 2012
			Chronic dose mortality assay 20 µl daily (0.6 to 6000 mg/mL) in 1M sucrose for 5d	Up to 81% mortality at 5 d	
		Selenate	Larva: artificial diet (0, 0.2, 0.4, 0.6, 1, 2 mg/L) Forager: 19.2µL of 50% sucrose solution (0, 30, 60, 120, 240, 480 mg/L)	Larval mortality; LC_{50} = 0.72 mg/L at 8 d Forager mortality; LC_{50} = 58 mg/L at 72 h Decreased prepupation; 9% pupation at 1mg/L	Hladun et al. 2013a
		Selenite	Larva: artificial diet (0, 0.2, 0.4, 0.6, 1, 2 mg/L) Forager: 19.2 µL of 50% sucrose solution (0, 30, 60, 120, 240, 480 mg/L)	Larval mortality; LC_{50} = 1.0 mg/L at 8 d Forager mortality; LC_{50} = 58 mg/L at 72 h Decreased prepupation; no effect on pupation %, no larvae pupated	Hladun et al. 2013a
		Methylseleno-cysteine	Larva: artificial diet (0, 4, 6, 7, 9, 10 mg/L) Forager: 19.2µL of 50% sucrose solution (0, 104, 125, 150, 200, 250 mg/L)	Larval mortality; LC_{50} = 4.7 mg/L at 8 d Forager mortality; LC_{50} = 161 mg/L at 72 h Decreased prepupation up to 95%; no effect on pupation	Hladun et al. 2013a
		Selenocystine	Larva: artificial diet (0, 2, 4, 6, 8, 10 mg/L) Forager: 19.2 µL of 50% sucrose solution (0, 104, 125, 150, 200, 250 mg/L)	Larval mortality; LC_{50} = 4.4 mg/L at 8d Forager mortality; LC_{50} = 148 mg/L at 72 h Decreased prepupation up to 68%; no effect on pupation	Hladun et al. 2013a
		Selenate	1 M sucrose solution (6 mg/kg) Pollen-sucrose patty (6 mg/kg) for 7 d	Increased worker mortality vs control	Burden et al. 2016a
		Methylseleno-L-cysteine	1 M sucrose solution (6 mg/kg) Pollen-sucrose patty (6 mg/kg) for 7 d	Increased worker mortality vs control	Burden et al. 2016a
	Bombus impatiens	Selenate	Microbiota-inoculated bees with 0.75 mg/L selenate in 40% sucrose for 10 d	Inoculated bumble bee microbiome significantly increased bee survival	Rothman et al. 2019a

				when exposed to selenate. In the preliminary experiment (exp. 1), the inoculated microbiome significantly increased bee survival 42% increased survival for no bees survived to 10 d	
		Selenate	10, 1, 0.1, 0.01, 0.001, and 0 mg/L spiked into 60% sucrose For 14 d	LC_{50}: 0.75 mg/L at 7 d LC_{50}: 0.09 mg/L at 14 d	Rothman et al. 2020
	Linepithema humile	Selenate	0, 0.5, 2.7, 5.4, 13.5, 27, and 54 µg Se/mL in 25% sucrose	LC_{50}: 131.57 mg/L at 7 d LC_{50}: 34.8 mg/L at 14 d	De La Riva et al. 2014
		Selenite	0, 2, 4, 10, 20, 30, 40 and 50 µg Se/mL in 25% sucrose	LC_{50}: 44 x 10^5 mg/L at 7 d LC_{50}: 709.89 mg/L at 14 d	De La Riva et al. 2014
		Seleno-L-methionine	0, 2, 4, 10, 20, 30, 40 and 50 µg Se/mL in 25% sucrose	LC_{50}: 87.83 mg/L at 7 d LC_{50}: 27.68 mg/L at 14 d	De La Riva et al. 2014
		Methylseleno-cysteine	0, 2, 4, 10, 20, 30, 40 and 50 µg Se/mL in 25% sucrose	LC_{50}: 29 x 10^3 mg/L at 7 d LC_{50}: 176.17 mg/L at 14 d	De La Riva et al. 2014
		Selenate	25% sucrose (0, 5 or 10 mg Se/mL)	Queen mortality 5 mg Se/mL died sooner than control 5mg–100% mortality 8 w 10 mg –100% mortality 11 w; Offspring did not develop beyond the larval stage	De La Riva et al. 2016
	Cotesia marginiventris	Selenate	*Spodoptera exigua* larvae feeding on *Medicago sativa* irrigated with 3.3 mg/L Se	No effect on the number surviving as pupae and adults; no effect on mortality during the pupal stage	Vickerman et al. 2004
Lepidoptera	*Spodoptera exigua*	Selenate	Larval artificial diet (5-7 conc)	LC_{50}: 21.41 µg g^{-1}	Trumble et al. 1998
		Selenite	Larval artificial diet (5-7 conc)	LC_{50}: 9.14 µg g^{-1}	Trumble et al. 1998
		Seleno-DL-methionine	Larval artificial diet (5-7 conc)	LC_{50}: 15.21 µg g^{-1}	Trumble et al. 1998
		Seleno-DL-cystine	Larval artificial diet (5-7 conc)	LC_{50}: 21.18 µg g^{-1}	Trumble et al. 1998
		Selenate	1st instar larvae fed leaves collected	Reduced mean day of death on	Bañuelos et al.

			from different *Atriplex* lines for 30 d (1 mg/L with sulfate and chloride salts 90 d exposure)	treated	2002
		Selenate	Feeding *Medicago sativa* irrigated with 0.0066 g / 60 L and 0.20g / 60 L of water	No effect on survival to pupal and adult stages (low); reduced survival to pupal and adult stages (high)	Vickerman et al. 2002
	Plutella xylostella Stanleyi	Selenate	Nonchoice bioassay: *Stanleya pinnata* leaves watered with 80 mM Se for 3 d	No effect on larval survival	Freeman et al. 2006
	Plutella xylostella G88	Selenate	Nonchoice bioassay: *Stanleya pinnata* leaves watered with 80 mM Se	Decreased larval survival	Freeman et al. 2006
	Pieris rapae	Selenate	Nonchoice bioassay: *Brassica juncea* watered with 20 µM Se 1) Newly hatched larvae allowed to feed for 9 d 2) 9 d old larvae allowed to feed for 2 d	1) 100% mortality by 9 d 2) 100% mortality by 2 d	Hanson et al. 2003
		Selenate	Nonchoice bioassay: *Stanleya pinnata* leaves watered with 80 mM Se	Decreased larval survival	Freeman et al. 2006
	Heliothis virescens	Selenate	Artificial diet (1, 100, 200, 500 µg/g)	Low larval mortality, 2% at 500 µg/g	Popham and Shelby 2007
		Selenite	Artificial diet (1, 5, 25, 50, 100 µg/g)	60% larval mortality at 100 µg/g	Popham and Shelby 2007
		Seleno-DL-cysteine	Artificial diet (1, 100, 200, 500 µg/g)	Low larval mortality, 12% at 500 µg/g	Popham and Shelby 2007
		Seleno-DL-methionine	Artificial diet (1, 5, 25, 50, 100 µg/g)	100% larval mortality at 100 µg/g	Popham and Shelby 2007
		Sel-Plex™ 2000[a]	Added into artificial diet (0, 1, 5, 25, and 50 µg/g)	100% larval mortality at 50 µg/g	Popham and Shelby 2007
Diptera	*Drosophila melanogaster*	Selenite	Newborn adult flies on artificial media (final concentration of 10^{-8}, 10^{-7}, 10^{-6} 10^{-5}, 10^{-4} M)	> 90% mortality at 10^{-4} M Se by 35 d; 2x mortality at 10^{-5} M Se vs control or 10^{-8}, 10^{-7}, 10^{-6} M Se; No effect on mortality at 10^{-8}, 10^{-7}, 10^{-6} M Se	Martin-Romero et al. 2001
	Chironomus decorus	Selenate	4th instar in toxicant solution	LC_{50}: 23.7 mg Se/L at 48 hr	Maier & Knight 1993
		Selenite	4th instar in toxicant solution	LC_{50}: 48.2 mg Se/L at 48 hr	Maier &

					Knight 1993
		Seleno-DL-methionine	4th instar in toxicant solution	LC_{50}: 194 mg Se/L at 48 hr	Maier & Knight 1993
	Chironomus riparius	Selenate	48 h acute toxicity test in San Joaquin River water (7 conc)	LC_{50}: 16.2 mg/L	Ingersoll et al. 1990
			48 h acute toxicity test in ASTM soft water (7 conc)	LC_{50}: 10.5 mg/L	
		Selenite	48 h acute toxicity test in San Joaquin River water (7 conc)	LC_{50}: 7.95 mg/L	Ingersoll et al. 1990
			48 h acute toxicity test in ASTM soft water (7 conc)	LC_{50}: 14.6 mg/L	
		Selenate/ selenite mixture (6:1)	48 h acute toxicity test in San Joaquin River water (7 conc)	LC_{50}: 9.34 mg/L	Ingersoll et al. 1990
			48 h acute toxicity test in ASTM soft water (7 conc)	LC_{50}: 14.3 mg/L	
		Selenate/selenite mixture (6:1)	30 d chronic testing (0, 750, 1500, 3000, 6000 µg/L Se)	Reduced % adult emergence at 6050 µg Se/L	
		Seleno-L-methionine	48 h acute toxicity test in San Joaquin River water (7 conc)	LC_{50}: 5.78 mg/L	Ingersoll et al. 1990
			48 h acute toxicity test in ASTM soft water (7 conc)	LC_{50}: 6.88 mg/L	
		Selenite	Spiking with aqueous Se (Various experimental designs)	Larval mortality	Vernon Beaty Jr. & Hendricks 2001
	Chironomus dilutus	Selenate	2nd instar (7-9 d old) in aqueous Se (4 µg/L Se) for 10 d followed by clean water for 10 d	Larval survival > 85%	Franz et al. 2011
		Selenite	2nd instar (7-9 d old) in aqueous Se (4 µg/L Se) for 10 d followed by clean water for 10 d	Larval survival > 85%	Franz et al. 2011
		Seleno-DL-methionine	2nd instar (7-9 d old) in aqueous Se (4 µg/L Se) for 10 d followed by clean water for 10 d	Larval survival > 85%	Franz et al. 2011

		Selenite	9-10 d old larvae exposed to waterborne SeNP[b] (5, 15, 50, 100, and 1000 µg Se/L) for 10 d	No effect on larval survival (> 80%)	Gallego-Gallegos et al. 2013
			9-10 d old larvae fed spiked fish food SeNP[b] (nominal concentrations of 5, 15, 50, and 150, 500 µg Se/g d.w.) for 10 d	No effect on larval survival (> 80%)	
			9-10 d old larvae spiked selenized algae (*Scenedesmus* sp.) (nominal concentrations of 5, 15, 50, and 150 µg Se/g d.w.) for 10 d	No effect on larval survival (> 80%)	
	Megaselia scalaris	Selenate	Drosophila diet (0, 100, 200, 300, 400, and 500 µg/g) egg to adult	LC_{50}: 258 µg/g; Decreased larval survival (28% at 100 µg/g and 79% at 500 µg/g)	Jensen et al. 2005
		Selenite	Drosophila diet (0, 100, 200, 300, 400, and 500 µg/g) egg to adult	LC_{50}: 392 µg/g; Decreased larval survival (19% at 100 µg/g and 58% at 500 µg/g)	Jensen et al. 2005
		Seleno-L-methionine	Drosophila diet (0, 0.5, 5, 25, 50, and 100 µg/g) egg to adult	LC_{50}: 130 µg/g; Decreased larval survival (22% at 100 µg/g and 97% at 400 µg/g)	Jensen et al. 2005
		Se-(methyl) selenocysteine hydrochloride	Drosophila diet (0, 0.5, 5, 25, 50, and 100, 200, 400, 800 µg/g) egg to adult	LC_{50}: 83 µg/g; Decreased larval survival (28% at 50 µg/g and 58% at 100 µg/g)	Jensen et al. 2005
		Selenate	Diet flakes rehydrated (0, 100, 200, 300, 400, 500 µg/g) egg to adult or 35 d	LC_{50} : 260 µg/g Decreased larval survival 27% (100 µg/g) and 79% (500 µg/g); Decreased overall survival 28% (100 µg/g) and 79% (500 µg/g) No effect on survival of puparia No effect on egg survival when exposed to treated diet	Jensen et al. 2006
	Culex quinquefasciatus	Selenate	Solutions (2, 4, 8, 16, 32 mg/L) 2nd instar to adult	LC_{50}: 11 mg/L at 14 d; Decreased larval survival at 8 mg/L (27%) and at 16 mg/L (83%)	Jensen et al. 2007

	Ephydra cinerea	Selenate	Solutions (10-20,000 µg/L) 3rd instar for 24 h and 48 h	No effect on larval survival (>92% at 24 h and >88% at 48 h)	Rosetta & Knight 1995
		Selenite	Solutions (10-20,000 µg/L) 3rd instar for 24 h and 48 h	No effect on larval survival (>92% at 24 h and >88% at 48 h)	Rosetta & Knight 1995
		Seleno-DL-methionine	Solutions (10-20,000 µg/L) 3rd instar for 24 h and 48 h	No effect on larval survival (>92% at 24 h and >88% at 48 h)	Rosetta & Knight 1995
Coleoptera	*Tenebrio molitor*	Selenite	Nutrient media (0.125, 0.25, 0.5, 1, 2% Se) 1 d old adults; 1 w in control then into treated until 28 d	0.125%: Progressive decreased survival from 7 – 28 d; survival percentages (72%) vs control (99%) at 7 d 0.25%: Decrease in survival; survival slope about 12 insects/d from 7-14 d 0.5%: Survival curve slope 13.1 insects/d from 7-14 d 1% and 2%: 100% mortality by 14 d	Hogan & Razniak 1991
			Nutrient media (0.125, 0.25, 0.5, 1, 2% Se) 1 d old adults; 1 w in treated then into control until 28 d	0.125%: Decreased survival through 14 d, then less mortality 14-28 d; survival curve slope 8.6 insects/d for 7- 14 d 0.25%: Survival curve slope 9.7 insects/d from 0-7 d then 3.3 insects/d from 7-14 d 0.5%: Survival curve slope 10.7 insects/d from 0-7 d and 0.85 insects/d from 7-14 d	
		Selenate	Nutrient media (0.0125, 0.025, 0.05, 0.1%) 1 w old adults on treated medium maintained at 4 and 25° C	4° C: Survival percentage curves steeper slopes; 100% mortality by 24 d 25° C: Clear dose-dependent survival response; mortality began ~12 d earlier than control; for 3 highest conc. kill was progressive and abrupt between 6 and 24 d; 0.025% final deaths protracted to 48 d	Audas et al. 1995

			Nutrient media (0.0125, 0.025, 0.05, 0.1%) 1 w old adults then transferred to control medium 12 d maintained at 4 and 25° C	4° C: No differences from groups that were maintained on treatment; slight shift to the right 25° C: Survival percentages for control, 0.0125, and 0.025% groups are comparable	

[a]Selenized yeast product containing 2000 µg/g Se, equal to or greater than 98% organic form, primarily as SeMet
[b]Selenium nanoparticles

Table 2.2 Effects of selenium on the growth, development, and reproduction of insects

Order	Species	Se Form	Testing method	Observations	Reference
Ephemeroptera	*Centroptilum triangulifer*	Selenious acid/Selenite	4-6 d old larvae (Periphyton exposed to 5, 10, 20 µg/L for 7 or 9 d) Fed for 4.5-6 w (until the emergence of subimagos)	Decrease in fecundity (most pronounced in 2 highest conc); Reduced adult body mass	Conley et al. 2009
Orthoptera	*Acheta domesticus*	Selenate	Feeding *Brassica juncea* leaf grown in 20 µM solution	Weight reduction ~10% (nonchoice)	Freeman et al. 2007
		Selenite	Two-week-old crickets fed torula yeast-based diets (0.1, 0.3, 1, 3, 10 µmol Se/kg) for 5 w	No effect on growth at any conc. vs 1 µmol Se/kg	Ralston et al. 2006
	Various spp.	Selenate	*Stanleya pinnata* grown in 40µM (high) or 2 µM (low) solution	No molts produced in high 7DAE	Freeman et al. 2007
Hemiptera	*Podisus maculiventris*	Selenate	Feeding *Spodoptera exigua* larvae reared on a diet 109 µg/g and 135 µg/g dry weight	Decreased developmental rate. Control nymphs achieved stadia 3,4,5, adult faster. No diff in developmental time for level ½ up to 3rd stadium, but level 2 decreased growth rate significantly 4, 5, and adult; About 20% less on both treatments vs fed control	Vickerman & Trumble 2003
Hymenoptera	*Apis mellifera*	Selenate	Larva: artificial diet (0, 0.2, 0.4, 0.6, 1, 2 mg/L)	No effect on prepupal weight at d 10 Reduced RGI[a]	Hladun et al. 2013a
		Selenite	Larva: artificial diet (0, 0.2, 0.4, 0.6, 1, 2 mg/L)	No effect on prepupal weight at d 10 Reduced RGI[a]	Hladun et al. 2013a
		Methylseleno-cysteine	Larva: artificial diet (0, 4, 6, 7, 9, 10 mg/L)	No effect on prepupal weight at d 10 Reduced RGI[a]	Hladun et al. 2013a
		Selenocystine	Larva: artificial diet (0, 2, 4, 6, 8, 10 mg/L)	No effect on prepupal weight at d 10	Hladun et al. 2013a

				Reduced RGI[a]	
		Selenate	0.6 mg/kg Se in sugar syrup 6 mg/kg Se in pollen patty For 60 d	Reduced brood surface area. No effect on whole-colony weight, forager activity. Reduced total worker weight. Very few capped cells and no pupae. Consumed 42% less pollen patty vs control. Produced no brood by the end of experiment.	Hladun et al. 2016
	Linepithema humile	Selenate	25% sucrose (0, 5 or 10 mg Se/mL)	Fewer eggs, viability and development of offspring affected	De La Riva et al. 2016
	Cotesia marginiventris	Selenate	*Spodoptera exigua* larvae feeding on *Medicago sativa* irrigated with 3.3 mg/L Se	Larval development and adult eclosion 2 days longer; no effects on time from pupation to adult; cocoons weighed 10% less	Vickerman et al. 2004
Lepidoptera	*Spodoptera exigua*	Selenate	Larval artificial diet (5 – 7 conc)	Reduced pupal weight at 12 µg/g, increased time to pupation and time to adult emergence; decreased RGR[b] and RGI[a]	Trumble et al. 1998
		Selenite	Larval artificial diet (5 – 7 conc)	Reduced pupal weight at 12 µg/g; increased time to pupation and time to adult emergence; decreased RGR[b] and RGI[a]	Trumble et al. 1998
		Seleno-DL-methionine	Larval artificial diet (5 – 7 conc)	No effect on pupal weight, developmental times, RGR[b] and RGI[a]	Trumble et al. 1998
		Seleno-DL-cystine	Larval artificial diet (5 – 7 conc)	No effect on pupal weight, developmental times, RGR[b]; decreased RGI[a]	Trumble et al. 1998
		Selenate	1st instar fed leaves collected from different *Atriplex* lines for 30 d	Reduced growth	Bañuelos et al. 2002

			(1 mg/L with sulfate and chloride salts 90 d exposure)		
		Selenate	Feeding *Medicago sativa* irrigated with 0.0066 g / 60 L and 0.20g / 60 L of water	Low: no effect on pupal weight, days to pupation, days to adult, stage at death, RGI[a] High: no effect on pupal weight, days to pupation, days to adult, but significant effect on stage at death and RGI[a]	Vickerman et al. 2002
	Trichoplusia ni	Selenite	Artificial diet (1-100 ppm)	No effect on larval weight (1-10 ppm), 40% reduction (25 ppm), 62% reduction (50 ppm), 75% reduction (100 ppm)	Popham et al. 2005
			Artificial diet (0, 1, 5, 10, 20 ppm) 1) Entire larval stage until pupation 2) Treated diet until early 4th instar then transferred to untreated diet 3) Untreated diet before the onset of 4th instar then transferred to treated diet	1) Lagged growth at 10 and 20 ppm; no effect on pupal weight 2) Lagged growth at 10 and 20 ppm; higher mean pupal weight at 10 ppm 3) Lagged growth at 10 and 20 ppm; no effect on pupal weight	Popham et al. 2005
	Plutella xylostella Stanleyi	Selenate	Nonchoice bioassay: *Stanleya pinnata* leaves watered with 80 mM Se for 3 d	No effect on larval weight gain	Freeman et al. 2006
	Plutella xylostella G88	Selenate	Nonchoice bioassay: *Stanleya pinnata* leaves watered with 80 mM Se for 3 d	Decrease in larval weight gain	Freeman et al. 2006
	Pieris rapae	Selenate	Nonchoice bioassay: *Brassica juncea* watered with 20 μM Se 1) Newly hatched larvae allowed to feed for 9 d 2) 9 d old larvae allowed to feed for 2 d	1) No larval growth 2) Lost 20% of f. wt in the 1st d vs 30% gain in untreated control	Hanson et al. 2003
		Selenate	Nonchoice bioassay: *Stanleya pinnata* leaves watered with 80 mM Se for 3 d	Decrease in larval weight gain	Freeman et al. 2006
	Corcyra	Selenite	Wheat flour diet (0.5, 1, 2, 4 ppm)	100% increase in larval	Lalitha et al.

	cephalonica		reared until 4th instar	weight (2 ppm) and 30% decrease in larval weight (4 ppm); increase in weight was directly proportional to Se added up to 2 ppm	1994
	Heliothis virescens	Selenate	Artificial diet (1, 100, 200, 500 μg/g)	No effect on the rate of development (5-100 μg/g), pupation, emergence and pupal weight	Popham and Shelby 2007
		Selenite	Artificial diet (1, 5, 25, 50, 100 μg/g)	Decreased rate of development, pupation (28% at 100 μg/g), emergence (7% at 100 μg/g); Reduced pupal weight	Popham and Shelby 2007
		Seleno-DL-cysteine	Artificial diet (1, 100, 200, 500 μg/g)	No effect on the rate of development (5-100 μg/g) and pupal weight; decreased rate of pupation and emergence	Popham and Shelby 2007
		Seleno-DL-methionine	Artificial diet (1, 5, 25, 50, 100 μg/g)	Decreased rate of development, pupation (15% at 50 μg/g) and emergence; No effect on pupal weight	Popham and Shelby 2007
		Sel-Plex™ 2000[c]	Added into artificial diet (0, 1, 5, 25, and 50 μg/g)	Decreased rate of development, pupation (8% at 25 μg/g) and emergence (0% at 25 μg/g); No effect on pupal weight	Popham and Shelby 2007
Diptera	*Drosophila melanogaster*	Selenite	Adult flies on artificial media for 14 d (combinations of 10^{-6} M Se or untreated) then transferred to untreated media to oviposit for 36 h	Viability of eggs >90% under all conditions; Decrease by 50% in the number of eggs when both sexes maintained on chemically defined medium without selenium vs when either males or females maintained on complete diet or diet supplemented with Se	Martin-Romero et al. 2001

	Chironomus decorus	Various[d]	96 h feeding with 12 d old 4th instar on *Ruppia maritima* substrate	Midges that bioaccumulated highest levels of Se had the greatest final mean weight than that of controls which had the lowest	Alaimo et al. 1994
			14 d feeding with egg to pupation on *Ruppia maritima* substrate	Decrease in mean midge weight as increase in Se	
		Selenate	4th instar (12-13 d) fed algal diet of *Selenastrum capricornutum* (0, 4, 10, 40 µg Se/L) for 4 d	No effect on growth rate; reduced larval growth at 96 h (>1 µg Se/L)	Malchow et al. 1995
		Selenite	4th instar (12-13 d) fed algal diet of *Selenastrum capricornutum* (0, 10, 40 µg Se/L) for 4 d	No effect on growth rate; reduced larval growth at 96 h (>1 µg Se/L)	Malchow et al. 1995
		Selenate/selenite mixture (6:1)	30 d chronic testing (0, 750, 1500, 3000, 6000 µg/L Se)	Increase in day of first emergence and emergence time ≥ 837 µg/L	Ingersoll et al. 1990
	Chironomus dilutus	Selenate	2nd instar (7-9 d old) in aqueous Se (4 µg/L Se) for 10 d followed by clean water for 10 d	No effect on the growth rate, time of adult emergence, adult weight; Sex ratio (M:F) 1.11 vs 1.41 control	Franz et al. 2011
		Selenite	2nd instar (7-9 d old) in aqueous Se (4 µg/L Se) for 10 d followed by clean water for 10 d	No effect on the growth rate, time of adult emergence, adult weight; Sex ratio (M:F) 0.35 vs 1.41 control	Franz et al. 2011
		Seleno-DL-methionine	2nd instar (7-9 d old) in aqueous Se (4 µg/L Se) for 10 d followed by clean water for 10 d	Reduced growth rate at 10 d, but no effect at 20 d; no effect on time of adult emergence but met only 56% criterion of adult emergence vs 82% control; no effect on adult weight; Sex ratio (M:F) 0.87 vs 1.41 control	Franz et al. 2011
		Selenite	9-10 d old larvae exposed to waterborne Se or SeNP[e] (5, 15, 50, 100, and 1000 µg Se/L) for 10 d	Reduced growth compared to control at highest conc. of nanoparticles SeNP[e] waterborne (overlying	Gallego-Gallegos et al. 2013

				water Se): LOEC = 592 µg/L NOEC = 60.2 µg/L IC_{50} = 281 µg/g d.w. IC_{25} = 130 µg/g d.w. (whole-body Se): LOEC = 63.6 µg/L NOEC = 45.7 µg/L IC_{50} = 57.0 µg/g d.w. IC_{25} = 51.1 µg/g d.w.	
			9-10 d old larvae fed spiked fish food SeNP[e] (nominal concentrations of 5, 15, 50, and 150, 500 µg Se/g d.w.) for 10 d	Reduced growth compared to control at highest conc. of SeNP[e] dietary (food Se): LOEC = 784 µg/L NOEC = 219 µg/L IC_{50} = 398 µg/g d.w. IC_{25} = 177 µg/g d.w. (whole-body Se): LOEC = 194 µg/L NOEC = 89.8 µg/L IC_{50} = 96.2 µg/g d.w. IC_{25} = 77.1 µg/g d.w.	
			9-10 d old larvae spiked selenized algae (*Scenedesmus* sp.) (nominal concentrations of 5, 15, 50, and 150 µg Se/g d.w.) for 10 d	No effect on larval growth	
Diptera	*Megaselia scalaris*	Selenate	Oviposition bioassay: Drosophila diet (0, 100, 200, 300, 400, and 500 µg/g) for 2 d	No effect on egg hatching	Jensen et al. 2005
			Drosophila diet (0, 100, 200, 300, 400, and 500 µg/g) egg to adult	Delay in larval development conc. as low as 100 µg/g; No effect on number of days to complete pupariation; No effect on number of days for females to emerge as compared to males; No females emerged at 500 µg/g	

		Selenite	Oviposition bioassay: Drosophila diet (0, 100, 200, 300, 400, and 500 μg/g) for 2 d	No effect on egg hatching	Jensen et al. 2005
			Drosophila diet (0, 100, 200, 300, 400, and 500 μg/g) egg to adult	Delay in larval development conc. as low as 300 μg/g; No effect on number of days to complete pupariation; No effect in number of days for female to emerge as compared to males	
		Seleno-L-methionine	Oviposition bioassay: Drosophila diet (0, 0.5, 5, 25, 50, and 100 μg/g) for 2 d	No effect on egg hatching	Jensen et al. 2005
			Drosophila diet (0, 0.5, 5, 25, 50, and 100 μg/g) egg to adult	Delay in larval development conc. as low as 50 μg/g; No effect on number of days to complete pupariation; Increase in number of days for female to emerge as compared to males at 25 μg/g	
		Se-(methyl) selenocysteine hydrochloride	Oviposition bioassay: Drosophila diet (0, 0.5, 5, 25, 50, and 100 μg/g) for 2 d	Reduced egg hatchability only at highest conc. (100 μg/g)	Jensen et al. 2005
			Drosophila diet (0, 0.5, 5, 25, 50, and 100, 200, 400, 800 μg/g) egg to adult	Delay in larval development conc. as low as 25 μg/g; no effect on number of days to complete pupariation; Increase in number of days for female to emerge as compared to males at 25 μg/g	
		Selenate	Diet flakes rehydrated (0, 100, 200, 300, 400, 500 ug/g) egg to adult or 35 d	Delays in larval developmental time as low as 100 ug/g; no effect on number of days required to complete pupariation	Jensen et al. 2006

		Selenate	Diet flakes rehydrated (0, 100, 200, 300, 400, 500 ug/g) after 10 d number of larvae counted	No effect on fecundity (viable larvae per female)	
	Culex quinquefasciatus	Selenate	Solutions (2, 4, 8, 16, 32 mg/L) 2nd instar to adult	Relative growth index of all treatments was significantly different vs control from d 4 to experiment termination (as low as 2 mg/L)	Jensen et al. 2007
	Ephydra cinerea	Selenate	Solutions (10-20,000 µg/L) 3rd instar for 24 h and 48 h	No effect on larval weight changes and weight differences	Rosetta & Knight 1995
		Selenite	Solutions (10-20,000 µg/L) 3rd instar for 24 h and 48 h	No effect on larval weight changes and weight differences	Rosetta & Knight 1995
		Seleno-DL-methionine	Solutions (10-20,000 µg/L) 3rd instar for 24 h and 48 h	No effect on larval weight changes and weight differences	Rosetta & Knight 1995

[a] Relative growth index
[b] Relative growth rate
[c] Selenized yeast product containing 2000 µg/g Se, equal to or greater than 98% organic form, primarily as SeMet
[d] Analysis of waterborne selenium levels where the substrate was collected
[e] Selenium nanoparticles

Table 2.2 Impact of selenium on the behavior and other effects of insects

Order	Species	Se Form	Testing method	Observations	Reference
Odonata	*Sympetrum corruptum*	Selenate	Feeding *Culex quinquefasciatus* 2nd instars reared in 15 μg/g; Grown in treated solution with treated prey and without	Increased feeding when in the treatment solution	Jensen 2006
Blattodea	*Gromphadorhina portentosa*	Sodium hydrogen selenite	0.01 μmol Se solution (nonlethal dose)	Effect on enzymatic activity	Nakonieczny 1993
Orthoptera	*Acheta domesticus*	Selenate	Feeding *Brassica juncea* leaf grown in 20 μM solution	5x more crickets preferred to feed on the untreated leaf (choice)	Freeman et al. 2007
	Various spp.	Selenate	*Stanleya pinnata* grown in 40 μM (high) or 2 μM (low) solution	Significantly less feeding on treated plants (choice) <10% feed on high vs ~70% low (7DAE)	Freeman et al. 2007
			Field plot study *Stanleya pinnata* pretreated for 8 w with high Se concentration (40 μM), and four low-Se plants (two from each accession) pretreated for 8 w with 2 μM	Decreased feeding; final leaf areas was five-fold higher for high-Se vs low-Se	Freeman et al. 2007
Hemiptera	*Myzus persicae*	Selenate	Feeding *Brassica juncea* plants grown in 0, 1, 5, 10, or 20 μM solution (choice)	7 times more aphid on control vs 1μM Se, few if any at higher conc.	Hanson et al. 2004
			Plants 0, 20, or 40 μM (choice)	~100% aphids found on control 7DAE	Hanson et al. 2004
	Brevicoryne brassicae	Selenate	*R. sativus* (0, 0.51 mg, 1.53 Se/L)	Decrease aphid numbers and aphid mummies No effect on leaf number or avg leaf damage	Hladun et al. 2013b
Hymenoptera	*Apis mellifera*	Selenate	Antennal response assay, with PER[a] (0.6 to 6000 mg/mL) in 1M sucrose + or - extension	PER[a] to antennal stimulation not significantly different than 1M sucrose, but higher than water at all conc.	Hladun et al. 2012

			Proboscis response assay (0.6 µl) (0.6 to 6000 mg/mL) in 1M sucrose + or – total consumption	No significant difference in consumption of droplet between 1M sucrose and any of 5 selenate conc.	
			Sucrose response threshold assay (24 hrs later fed 20 µl of treatment, then antennae stimulated with increasing sucrose solutions) (0.6 to 6000 mg/mL) in 1M sucrose + or - extension	Dose-dependent change in PER[a] to increasing conc. of sucrose (SRT at 3-10% except 60ug) at 2 h; Decrease in PER low as 17%; No interaction between sucrose antennal treatment and selenate feeding treatment; selenate feeding did not alter the sucrose response threshold of 3 to 10%	
			Total consumption (0.6 to 6000 mg/mL) in 1M sucrose	No effect at 24 h	
		Seleno-methionine	Antennal response assay, with PER[a] (0.6 to 6000 mg/mL) in 1M sucrose + or - extension	PER[a] responses to antennal stimulation were significantly lower than 1M sucrose at 60, 6000 µg, but higher than water at 4 lowest conc.	Hladun et al. 2012
			Proboscis response assay (0.6 µl) (0.6 to 6000 mg/mL) in 1M sucrose + or – total consump.	No significant difference in consumption of droplet between 1M sucrose and any of the 5 SeMet conc.	
			Sucrose response threshold assay (24 hrs later fed 20 µl of treatment, then antennae stimulated with increasing sucrose solutions) (0.6 to 6000 mg/mL) in 1M sucrose + or - extension	Dose-dependent change in PER[a] to increasing conc. of sucrose (3 and 10% except for 0.6 and 6 µg) at 2 h SeMet feeding treatment did not have significant effect on SRT	

				Interaction of treatment feeding and sucrose antennal treatment was not significant	
			Total consumption (0.6 to 6000 mg/mL) in 1M sucrose	No effect at 24 h	
		Selenate	Forager: 19.2 µL of 50% sucrose solution (0, 30, 60, 120, 240, 480 mg/L) then 50% every d	Reduced volume consumed	Hladun et al. 2013a
		Selenite	Forager: 19.2 µL of 50% sucrose solution (0, 30, 60, 120, 240, 480 mg/L) then 50% every d	Reduced volume consumed	Hladun et al. 2013a
		Methylseleno-cysteine	Forager: 19.2 µL of 50% sucrose solution (0, 104, 125, 150, 200, 250 mg/L) then 50% every d	Reduced volume consumed	Hladun et al. 2013a
		Selenocystine	Forager: 19.2 µL of 50% sucrose solution (0, 104, 125, 150, 200, 250 mg/L) then 50% every d	Reduced volume consumed	Hladun et al. 2013a
		Selenate	High vs low *B. juncea* plants (20 or 0 µM)	No effect on floral visitation	Quinn et al. 2011
			High vs low *S. pinnata* plants (80 and 0 µM)	No effect on flora visitation	
		Selenate	*R. sativus* (0, 0.51 mg, 1.53 Se /L)	No effect on pollinator visitation	Hladun et al. 2013b
		Selenate	Fed 3 µl of 0.5 M sucrose + Se (0.6, 6, 60 mg/L) 3 h prior to conditioning	No effect on PER[a] during sucrose responsiveness test; 1.8 ng before conditioning caused reduction in behavioral performance during conditioning; 18 ng caused reduction in performance during long-term recall test; No effect on short term recall tests	Burden 2016b
			Fed 3 µl of 0.5 M sucrose + 6 mg/L Se beginning of long-term recall test	No effect on either recall tests	

		Methylseleno-L-cysteine	Fed 3 µl of 0.5 M sucrose + Se (0.6, 6, 60 mg/L) 3 h prior to conditioning	No effect on PER[a] during sucrose responsiveness test; 18 ng caused reduction in performance during long-term recall test; no effect on short term recall tests	Burden 2016b
			Fed 3 µl of 0.5 M sucrose + 6 mg/L Se beginning of long-term recall test	No effect on either recall tests	
		Selenate	50% sucrose spiked with 0.6 mg/L for 7 d	Altered microbiome	Rothman et al. 2019b
	Bombus impatiens	Selenate	Microbiota-inoculated bees with 0.75 mg/L selenate in 40% sucrose for 10 d	Increased bee survival (42%) for inoculated bees when exposed to Se	Rothman et al. 2019a
			60% sucrose spiked with 0.5 mg/L for 4 d	Significant increase in the alpha diversity (as measured by the Shannon Diversity Index) off microbiome; ESVs[b] of some gut symbionts lower proportional abundance	Rothman et al. 2019a
		Selenate	0.5 mg/L into 60% sucrose for 4 d	Altered the composition of non-core bacteria; core symbiont ESVs[b] less abundant	Rothman et al. 2020
	Linepithema humile	Selenate	Choice test 50 µg Se/mL in 10 or 30% sucrose (0,1, 2, 30, 60, 90, and 120 min)	No effect on choice	De La Riva et al. 2014
		Selenite	Choice test 50 µg Se/mL in 10 or 30% sucrose (0,1, 2, 30, 60, 90, and 120 min)	No effect on choice	De La Riva et al. 2014
		Seleno-L-methionine	Choice test 50 µg Se/mL in 10 or 30% sucrose (0,1, 2, 30, 60, 90, and 120 min)	No effect on choice	De La Riva et al. 2014
		Methylseleno-cysteine	Choice test 50 µg Se/mL in 10 or 30% sucrose (0,1, 2, 30, 60, 90, and 120 min)	No effect on choice	De La Riva et al. 2014

		Selenate	Competition assay with *D. bicolor* 25% sucrose (0 or 5 mg Se/mL) 1 w exposure Competition assay with D. bicolor	No effect on latency of bait discovery No effect on competition behavior	De La Riva et al. 2016
	Dorymyrmex bicolor	Selenate	Competition assay with *L. humile* 25% sucrose (0 or 5 mg Se/mL) 1 w exposure	No effect on latency of bait discovery with Se alone, but slower time with Se and competitor; No effect on competition behavior	De La Riva et al. 2016
	Cotesia marginiventris	Selenate	Olfactometer bioassay (Se irrigated *Medicago sativa* 3.3 mg/L leaves *S. exigua* feeding damage and frass)	No Se-volatile-specific behavioral response	Vickerman et al. 2004
Lepidoptera	*Spodoptera exigua*	Selenate	Choice test: Larval artificial diet (14.9, 18.5, 21.4, 24.8 μg/g) neonates or 3rd instar	Neonates and 3rd instars preferred control diet; decreased consumption	Vickerman & Trumble 1999
		Selenite	Choice test: Larval artificial diet (4.8, 7.0, 9.1, 11.9 μg/g)	Neonates and 3rd instars preferred control diet; decreased consumption	Vickerman & Trumble 1999
		Seleno-DL-cystine	Choice test: Larval artificial diet (9.0, 12.3, 15.2, 18.9 μg/g)	Neonates preferred the control diet, but 3rd instars had no preference; no effect on consumption	Vickerman & Trumble 1999
		Seleno-DL-methionine	Choice test: Larval artificial diet (13.9, 17.8, 21.2, 25.1 μg/g)	Neonates had no preference except at 25.1 μg/g; 3rd instar had no preference; no effect on consumption	Vickerman & Trumble 1999
		Selenate	Food preference bioassay: *Medicago sativa* irrigated with 0.0066 g / 60 L and 0.20g / 60 L of water (every half h for 5 h)	High: Neonates: no preference; 4th instars: preference for control only at time interval 3, no effect on consumption Low: Neonates: preference for control only at time interval 4; 4th instars: Preference for treated plants	Vickerman et al. 2002

				at time intervals 3, 5, 6, and 9; more consumption	
			Oviposition preference bioassay: *Medicago sativa* irrigated with 0.0066 g / 60 L and 0.20g / 60 L of water	Females preferred to oviposit on low Se treated plants over control; no preference between control and high Se plants	
	Trichoplusia ni	Selenate	Neonate larvae on *Brassica juncea* (1 mg/L Se) for 14 d	Fewer pupae found on treated plants (14 vs 38 control)	Bañuelos et al. 2002
		Selenite	Artificial diet (0, 5, 10 ppm) then infected with *Ac*MNPV[c] 1) Entire larval stage until pupation 2) Treated diet until early 4th instar then transferred to untreated diet 3) Untreated diet before the onset of 4th instar then transferred to treated diet	1) Increase in LC_{50} at 96 h of virus 2) Little effect on LC_{50} of virus 3) Increase in LC_{50} at 96 h of virus *LT_{50} decreased at higher viral concentrations in 5 ppm fed insects (averaged by dose for all Se regimes)	Popham et al. 2005
	Plutella xylostella Stanleyi	Selenate	Choice bioassay: *Stanleya pinnata* leaves watered with 80 mM Se vs untreated	No effect on larval preference	Freeman et al. 2006
			Oviposition choice bioassay: *Stanleya pinnata* plants watered with 80 mM Se vs untreated at 7 d	No effect on oviposition preference; 30 days after oviposition larvae completely eaten all plants	
	Plutella xylostella G88	Selenate	Choice bioassay: *Stanleya pinnata* leaves watered with 80 mM Se vs untreated	Larval preference for untreated leaves	Freeman et al. 2006
			Oviposition choice bioassay: *Stanleya pinnata* plants watered with 80 mM Se vs untreated at 7 d	Oviposition preference for untreated leaves; 30 days after oviposition larvae fed less on treated plants	

	Pieris rapae	Selenate	Choice bioassay: *Brassica juncea* 20 μM selenate at 6 h	Larval preference for untreated leaves (*c*. 15-fold higher rate)	Hanson et al. 2003
			Choice bioassay: *Stanleya pinnata* leaves watered with 80 mM Se vs untreated	Larval preference for untreated leaves	Freeman et al. 2006
			Oviposition choice bioassay: *Stanleya pinnata* plants watered with 80 mM Se vs untreated at 7 d	Oviposition preference for untreated leaves; 30 days after oviposition larvae fed less on treated plants	
			Oviposition choice bioassay: *Stanleya pinnata* plants watered with 80 mM Se vs untreated at 7 d	Oviposition preference for untreated leaves; 30 days after oviposition larvae fed less on treated plants	
Diptera	*Megaselia scalaris*	Selenate	Oviposition bioassay: Drosophila diet (0, 100, 200, 300, 400, and 500 μg/g) for 2 d	No oviposition preference	Jensen et al. 2005
		Selenite	Oviposition bioassay: Drosophila diet (0, 100, 200, 300, 400, and 500 μg/g) for 2 d	No oviposition preference	Jensen et al. 2005
		Seleno-L-methionine	Oviposition bioassay: Drosophila diet (0, 0.5, 5, 25, 50, and 100 μg/g) for 2 d	No oviposition preference	Jensen et al. 2005
		Se-(methyl) selenocysteine hydrochloride	Oviposition bioassay: Drosophila diet (0, 0.5, 5, 25, 50, and 100 μg/g) for 2 d	No oviposition preference	Jensen et al. 2005
		Selenate	Oviposition preference: diet (0, 100, 200, 300, 400, 500 μg/g) for 2 d	No oviposition preference between 50 μg/g vs control	Jensen et al. 2006
	Culex quinquefasciatus	Selenate	Oviposition bioassay using experimental ponds (30 mg/L Se) for 4 w	No effect on the number of egg rafts	Jensen et al. 2007
	Delia radicum	Selenate	*Brassica oleracea* var *italica* watered with 0.5 mL of 50 mg/L in the field (2 growing seasons)	Increase in number of eggs laid on treated vs control for a single monitoring periods/dates during 1st	Mechora et al. 2017

				generation (significant only at 2 dates)	
Coleoptera	*Phyllotreta* spp.	Selenate	*Brassica oleracea* var *italica* watered with 0.5 mL of 50 mg/L in field (2 growing seasons)	Increased damage on treated plants vs control (only 1 year); Reduced number of pupae vs control both years (significant only 1 year)	Mechora et al. 2017

[a]Proboscis extension response
[b]Exact Sequence Variants
[c]*Autographa californica* multiple nucleopolyhedrovirus

Chapter III: Toxicity of selenium against insecticide-susceptible and resistant German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae)

Introduction

The German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae), is a synanthropic cosmopolitan pest species that poses a serious public health risk through the transmission of pathogens, allergens, antibiotic-resistant bacteria, and parasites (Schal & DeVries 2021). The first case of insecticide resistance in the German cockroach dates back to 1952 when an overreliance on a few active ingredients resulted in the selection for resistant populations (Heal et al. 1953, Cochran 1995). Recently, studies have evaluated cases of resistance in German cockroach field populations globally (Wu & Appel 2017, Liang et al. 2017, Hu et al. 2020, Lee et al. 2021). Complete control of these resistance populations remains a challenge due to the German cockroach's short generation time and ability of populations to grow exponentially (Gould & Deay 1940, Ross et al. 1984). These characteristics allow for the rapid development of resistance toward new active ingredients. The German cockroach was ranked as the 2nd most important resistant urban pest species behind *Musca domestica* with reported cases of resistance to 42 different active ingredients (Zhu et al. 2016).

Baits have remained the most common control measure for German cockroach infestations since the 1990s and have shown to be efficacious in the field setting (Miller & Smith 2020, Appel & Rust 2021). Reports of homemade baits were documented as early as 1858, when household food products would be mixed with inorganic bait

toxicants such as boric acid, sodium fluoride, and phosphorous (Reierson 1995). Later in the 1950s, synthetic organic insecticides were incorporated into bait formulations; however, field performance was variable. Compared to other insecticide formulations, baits are relatively safe, useful in sensitive areas, and long-lasting (Reierson 1995). The minimal amount of bait material needed to treat an area can reduce the amount of insecticide residues and any associated health risks (Appel & Rust 2021, Wang et al. 2019b).

To combat resistance, insecticides are recommended to be rotated based on the mode of action. Unfortunately, the active ingredients that are found in baits are often limited (Lee & Rust 2021). Classes of active ingredients commonly found in cockroach bait formulations are neonicotinoids, phenylpyrazole (fipronil), amidinohydrazone (hydramethylnon), oxadiazine (indoxacarb), and avermectins. Furthermore, given that there are reports of cross- and multiple-resistance in the German cockroach, the currently available chemistry may no longer be effective, furthering the necessity to discovering novel active ingredients (Liang et al. 2017, Hu et al. 2020, Lee et al. 2022). Alternatives to conventional insecticides including biological control agents, household cleaners, and botanicals in essential oils have been explored (Mossa 2016, Rust 2021). Unfortunately, many of these safer alternatives have limited application in field settings.

Selenium is a trace element that is essential for humans and other animals at low doses. Its role as an antioxidant is attributed to the fact that it is a component of glutathione peroxidase (National Research Council 1983). There are four major well-characterized glutathione peroxidases (GPxs) that assist in the reduction of hydrogen

peroxide and hydroperoxides (Fairweather-Tait 2011). The Institute of Medicine (2000) reported the Recommended Dietary Allowance (RDA) for selenium to be 55 µg (0.7 µmol)/day for both men and women based on the maximum glutathione peroxidase synthesis levels. However, at amounts slightly higher than necessary (400 µg or 5.1 µmol/day), it can induce toxic effects in humans. Historically, selenium has exhibited toxicity to plant arthropod pests and may have been the first systemic insecticide tested (Hurt-Karrer & Poos 1936, Neiswander & Morris 1940, Reed et al. 1962). A shift towards investigating selenium as an environmental toxicant occurred in the late 1980s due to concern of the biotransfer of selenium from contaminated water and soil to living organisms (Ingersoll et al. 1990, Rosetta & Knight 1995, DeBruyn & Chapman 2007). Several studies have recently implicated selenium as a protective agent against pest herbivory through toxicity and repellency on bioaccumulated plants (Hanson et al. 2004, Freeman et al. 2007, Mechora 2019).

This study aimed to determine the insecticidal effects of several selenium compounds on *B. germanica*. We used a liquid sucrose solution as a simple phagostimulant to test the different compounds. We chose two organic and two inorganic compounds to evaluate their efficacy against adult male cockroaches of the UCR susceptible strain and three additional insecticide-resistant strains. Besides, we also tested five different commercial bait products against all strains in choice bioassays to characterize their susceptibility profiles. Choice tests with a sodium selenate-based bait allowed us to compare the performance of commercial bait products against resistant

strains. We also demonstrated a method to determine lethal doses of an oral toxicant in a water droplet.

Methods and Materials

Insects

All strains were kept in 121-liter garbage bins equipped with electrical barriers maintained at 24 ± 2°C, 30–50% RH, and a 12-hour photoperiod. Cockroach cultures were provided with harborages (rolled corrugated cardboard), dog food (Purina Dog Chow, Nestlé Purina Petcare, St. Louis, MO), and a water source (dH_2O), *ad libitum.* The UCR strain is a laboratory susceptible strain that has not been exposed to any insecticides for ≥ 40 years. It was originally a subculture of the Orlando susceptible strain. WM, RG386, and Ryan strains were established from populations that were collected across California (Lee et al. 2022). Prior treatment history is summarized in Table 3.1. Healthy adult male German cockroaches were collected from mass culture through brief anesthetization with CO_2 for choice and nonchoice bioassays. Cockroaches used for lethal dose and feeding preference tests were gently collected with a glass vial without carbon dioxide anesthetization.

Treatments

The two inorganic selenium compounds evaluated were sodium selenate (98%, Acros Organics, Fair Lawn, NJ) and sodium selenite (≥98%, Sigma Chemical Company, St. Louis, MO). The two organic selenium compounds evaluated were DL-selenomethionine

(99+%, Acros Organics, Fair Lawn, NJ) and L-selenocystine (98%, Acros Organics, Fair Lawn, NJ). The five commercial bait formulations evaluated were Advion Evolution (0.6% indoxacarb, Syngenta Corporation, Wilmington, DE), Alpine Rotation Reservoir 1 (0.5% dinotefuran, BASF Corporation, Research Triangle Park, NC), Maxforce FC Magnum (0.05% fipronil, Bayer Environmental Science, Cary, NC), Siege (2% hydramethylnon, BASF Corporation, Research Triangle Park, NC), and Vendetta (0.05% abamectin, MGK, Minneapolis, MN) (Table 3.2).

Nonchoice Bioassays

Arenas (29.2 x 15.2 x 10.8 cm) were composed of 4.7 L plastic latching boxes (IRIS USA, Inc.; Sunny, Arizona) with a sheet of filter paper taped down to the bottom using masking tape (Figure 3.1). A thin layer of petroleum jelly was applied on the inner wall of the arena to prevent escape. A pleated rectangle (24 x 6 cm) of corrugated paper was placed at one end of the arena as harborage. Glass vials (8 mL) were filled with DI water and plugged with a piece of dental cotton roll. The vial was placed opposite the harborage. A few pieces of dog food (Purina Dog Chow, Nestlé Purina Petcare, St. Louis, MO) were provided on either side of the water vial. Ten adult male German cockroaches were introduced into each arena within ±1 hour of scotophase and acclimatized for 48 hours. Water vials were removed after 24 hours to encourage feeding on the selenium solutions.

All selenium compounds were serially diluted to 0.025, 0.05, 0.1, 0.25, 0.5, and 1% concentrations (m/v) in a 3% sucrose solution. Glass vials (8 mL) were filled with the

solutions plugged with a piece of dental cotton roll. At the end of the acclimatization period, they were placed opposite the harborage where the water vials were previously. Control arenas were provided with 3% sucrose as a water source. All treatments were replicated three times. Data was collected at selected time intervals until all individuals in the treatments achieved complete mortality or up to 14 days, whichever came first. Mortality was defined as the inability of a moribund cockroach to right itself within 2 minutes of being turned over. Dead cockroaches were removed daily from the test arenas.

Choice Bioassays

Choice bioassays were conducted based on a similar setup as previously described for the nonchoice bioassays (Figure 3.2, 3.3). However, during the 48-hour acclimatization period, water vials were not removed. Choice experiments with commercial bait + laboratory dog chow were conducted to characterize the susceptibility profiles of the field strains. At the end of the acclimatization period, bait products (~2.5 g) in a plastic weigh boat and laboratory dog chow were placed on either side of the water vial, equidistant from the sides of the arena. The original laboratory dog chow was removed. The location of food choices was randomized to eliminate any positional effects. Control arenas were not provided with a bait product. All treatments were replicated three times.

For water + selenium experiments, 0.5% (w/v) sodium selenate diluted in a 3% or 6% sucrose solution was used as our liquid bait. A higher concentration of sucrose was additionally tested to determine whether it had any effect on the bait's performance. Glass vials (8 mL) were filled with either DI water or sodium selenate solution and then

plugged with a piece of dental cotton roll. The original water vial was removed, and new vials (DI water and sodium selenate solution) were introduced after the 48-hour acclimatization period. The two vials were placed opposite of the harborage and equidistant from the sides of the arena. The laboratory dog chow was then moved to the center where the original water vial had been. The location of the two vial choices was randomized to eliminate any positional effects. Control arenas were provided with a 3% or 6% sucrose solution. All treatment concentrations were replicated four times.

Data was collected at selected time intervals up until all individuals in the treatments achieved complete mortality or at 21 days post-treatment, whichever came first. Mortality was defined as the inability of a moribund cockroach to right itself within 2 minutes of being turned over. Dead cockroaches were removed daily from the test arenas.

Table 3.1. The German cockroach strains used in this study[a]

Strain	Collection Location	Type of building	Collection Date	Treatment History
UCR	-	-	In culture for over 40 years	Laboratory susceptible strain; no insecticide exposure
WM	Los Angeles, CA	Public housing	September 2018	Products containing deltamethrin, imidacloprid, beta-cyfluthrin, and lamda-cyhalothrin.
RG386	Los Angeles, CA	Public housing	August 2019	Products containing indoxacarb and chlorfenapyr.
Ryan	San Jose, CA	Apartment	2020	Products containing fipronil, dinotefuran, methoprene, pyriproxyfen, novaluron, and pyrethroids. Received from Dr. Ryan Neff of MGK.

[a]Information published in Lee et al. (2022)

Table 3.2. The commercial bait products evaluated in this study

Product	Manufacturer	Active Ingredient	Insecticide Class	IRAC MoA[a]
Advion Evolution	Syngenta	0.6% indoxacarb	Oxadiazine	22A
Alpine Rotation Reservoir 1	BASF	0.5% dinotefuran	Neonicotinoid	4A
Maxforce FC Magnum	Bayer	0.05% fipronil	Phenylpyrazole	2B
Siege	BASF	2% hydramethylnon	Amidinohydrazone	20A
Vendetta	MGK	0.05% abamectin	Avermectin	6

[a]International Resistance Action Committee (IRAC) Mode of Action (MoA) classification

Figure 3.1 Nonchoice bioassay setup

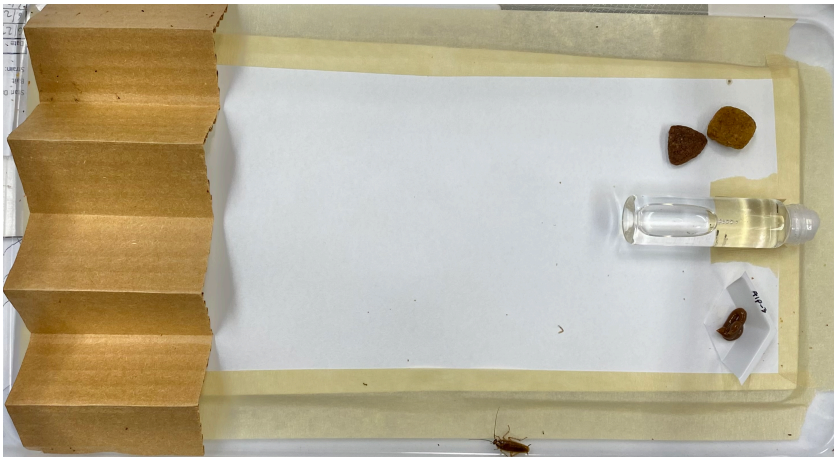

Figure 3.2 Choice bioassay setup to test commercial bait products

Figure 3.3 Choice bioassay setup to test selenium liquid bait

Oral dose test

An oral dose method was developed to determine the amount of selenium required to kill adult male German cockroaches. One adult male *B. germanica* was placed in a 30 mL plastic cup with four small holes for air on the lid. Only a small piece of dog chow was provided for 24 hours before the treatment to encourage the males to drink the toxicant solutions. A 0.5-10 μL ErgoOne® single-channel pipette (USA Scientific Inc., Ocala, FL) was used to dispense a 1 μL droplet of sodium selenate (containing 2.5–17.5 μg). The lid of the cup was opened, and the pipette tip was carefully inserted and placed in front of the mouthparts of the cockroach. The droplet was suspended on the first stop of the plunger until the cockroach displayed stimulation as indicated by the movement of its palps. As the cockroach started feeding, the plunger was completely depressed to the second stop. The pipette was held steady until the cockroach completely consumed the droplet. If the cockroach did not completely consume the droplet or if the droplet contacted its body, the replicate was excluded from the study. A small piece of cotton with DI water was placed in the cup after the cockroaches drank, and cockroaches were scored for mortality at 4 and 5 days post-treatment.

Feeding Preference Test

One adult male German cockroach was introduced in a 100 x 15 mm polystyrene petri dish (Fisherbrand® Fisher Scientific, Waltham, MA) with a piece of laboratory dog chow placed at one end. The cockroaches were deprived of water for 48 hours. At the end of this period, the lid was opened, and the cockroach was held in place by an upside-down

glass vial with the inside coated with fluon (BioQuip Products Inc., Rancho Dominguez, CA). The cockroach was gently moved to the area opposite the piece of dog chow. A 5 μL droplet of sodium selenate solution (0.5% w/v) and DI water were placed on a cut piece (5 x 2.5 cm) of Parafilm™ M (Bemis Company, Inc., Neenah, WI) equidistant from both ends of the strip. The location of the selenate and water droplets was alternated between every trial to cancel any positional effects. The parafilm strip was then placed in the center of the petri dish. The glass vial holding the cockroach was removed, and the lid was replaced. At the discovery of either droplet, a 10-minute observation period commenced. The first choice of the cockroach was recorded. The experiment was concluded if the cockroach did not feed within 10 minutes of introducing the solutions.

Data Analyses

Data for nonchoice/choice bioassays and lethal dose tests were pooled and subjected to probit analysis using PoloPlus Version 2.0 (LeOra Software LLC, Petaluma, CA) for probit analysis to obtain the LC and LD values. Control mortality was used to correct observed mortality in the treatments. Kaplan-Meier survivorship analysis was done using SPSS Statistics version 27.0 (IBM Corporation, Armonk, NY) to obtain mean survival times. Log-rank tests (Mantel-Cox) were also conducted for pairwise comparisons between treatments or strains in the bait evaluations. A binomial test was run in SPSS Statistics version 27.0 (IBM Corporation, Armonk, NY) for feeding preference test data to determine whether the cockroaches preferred to feed on the water droplet over the

sodium selenate droplet. The null hypothesis was either accepted or rejected based on the Clopper-Pearson 95% CI.

Figure 3.4 The oral dose test on the German cockroach

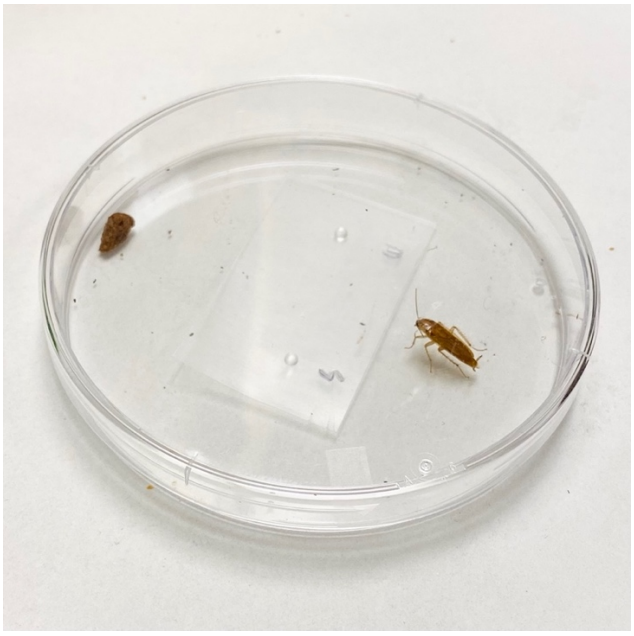

Figure 3.5 The feeding preference test setup

Results

Nonchoice bioassays

We tested two organic and two inorganic compounds in a nonchoice/force-feeding bioassay with our UCR susceptible strain to identify the most effective compound. In contrast to both inorganic forms, selenomethionine and selenocystine failed to show a clear dose-dependent response suggesting possible repellency or feeding deterrence at higher concentrations. Mean survival times were reported for the concentrations tested for both organic forms (Table 3.3). Overall, selenocystine (4.74 – 7.29 days) had the highest mean survival times followed by sodium selenite (1.78 – 6.57 days). Sodium selenate (0.66 – 3.61 days) had the lowest mean survival times followed by selenomethionine (1.58 – 5.23 days). Based on LC_{50} values, we found that sodium selenate exhibited higher toxicity than did sodium selenite, which had an LC_{50} of 0.11% at 72 hours (Table 3.4). At the same time point 0.03%, sodium selenate's performance was significantly different from that of sodium selenite. The LC_{50} of sodium selenate at 48 hours overlapped with that of sodium selenite at 72 hours based on 95% CI.

We tested our resistant field strains using sodium selenate (Table 3.5, 3.6). Of the other three field strains, WM had the highest LC_{50} value of 0.24, but only differed significantly with UCR and not with RG386 based on the 95% CI. At 48 hours, we could not generate LC values for Ryan due to low mortality. At 72 hours, WM and RG386 overlapped with UCR with LC_{50} in their 95% CI at 72 hours. At 72 hours, Ryan significantly differed from all other strains with an LC_{50} of 0.20%.

Choice bioassays

Our bait evaluations to characterize the susceptibility profiles of our test strains revealed that the UCR strain was the most susceptible to all five bait products, differing from all field strains based on both mean survival times and log-rank tests. Ryan exhibited the highest resistance against all active ingredients tested (Figure 3.4-3.8, Table 3.7). However, when evaluated with Alpine and Vendetta baits, Ryan did not significantly differ from RG386, and both RG386 and WM, respectively. WM and RG386 were comparable in all baits tested except for Alpine (Figure 3.7). At the end of 14 days, mortality ranged from 63.3-100%, 56.7-100%, and 30-100% for WM, RG386, and Ryan, respectively (Table 3.7). Furthermore, at the end of 21 days, mortality ranged from 80-100%, 63.3-100%, and 40-100% for WM, RG386, and Ryan, respectively. UCR consistently had the lowest mean survival time and achieved 100% mortality by day 10 for all baits.

To examine how a sodium selenate liquid bait would perform with other commercially available baits, we conducted choice bioassays with two solutions of 0.5% sodium selenate in either 3% or 6% sucrose. Cockroaches were given the choice between feeding on the liquid bait or water. For the UCR susceptible strain, Vendetta and both sodium selenate baits were comparable in mean survival times (1.7–2.2 days) and log-rank pairwise comparisons (Figure 3.9, Table 3.7). Sodium selenate baits outperformed Siege, which had a mean survival time of 3.4 days. In addition, 100% mortality was achieved by day 6 for both sodium selenate baits. Mean survival times for sodium selenate baits against WM were comparable to Alpine, Maxforce, and Vendetta and

outperformed Siege, which had the longest mean survival time. Sodium selenate baits achieved 100% mortality by 14 days compared to Alpine and Maxforce, which only completed 76.7% and 86.7%, respectively. Furthermore, Alpine and Maxforce failed to achieve >95% mortality by 21 days. Similar to WM, RG386's mean survival times for sodium selenate baits (3.6–3.8 days) were comparable to Vendetta (3.8 days) but outperformed Alpine, Maxforce, and Siege. Sodium selenate baits achieved 100% mortality by day 14, unlike Alpine, Maxforce, and Siege, which achieved only 56.7%, 66.7%, and 73.3% mortality, respectively. These commercials baits failed to achieve >95% mortality by 21 days. For Ryan, Vendetta was the best performing bait with a mean survival time of 17.4 days. It was also the only bait that achieved 100% mortality at either 14 or 21 days. Both sodium selenate baits failed to kill 100% of Ryan by the end of 21 days, but achieved 97.5% and 95% mortality for sodium selenate in 3% or 6% sucrose, respectively. These values exceeded those of all other baits excluding Vendetta. Ryan was the only strain where sodium selenate in 3% or 6% sucrose significantly differed in mean survival times of 10.4 and 6.8 days, respectively. Sodium selenate in 3% sucrose did not significantly differ from Alpine and Maxforce based on mean survival times and log-rank tests. In comparison, sodium selenate in 6% sucrose did not significantly differ from Advion or Alpine based on mean survival times and log-rank tests.

Lethal dose

The lethal dose values of sodium selenate on adult male UCR registered 8.33 μg (95%

CI: 6.71 - 9.70 μg) and 25.44 μg (95% CI: 19.65 - 41.52 μg) for LD_{50} and LD_{95} at four days, respectively (Table 3.8). At five days, lethal dose values registered 7.19 (95% CI: 5.71 – 8.42 μg) and 21.10 (95% CI: 16.80 - 31.57 μg) for LD_{50} and LD_{95}, respectively. The 5% control mortality was used to adjust treatment mortality values in probit analysis.

Feeding Preference Test

Of the 38 individuals that chose to drink either droplet in the 10-minute observation period, 42% chose to drink from the water droplet as a first choice, while 58% chose to drink from the sodium selenate droplet. Based on our binomial test (n = 38, $p = 0.417$), we accepted the null hypothesis that there was no preference for water over sodium selenate as the 1st choice. The Clopper Pearson 95% CI ranged from 26.3% to 59.2%.

Table 3.3 Mean survival times of tested selenium compounds against *B. germanica* (UCR)

Concentration	Selenium Compound	Mean Survival Time (Days)	95% CI	SE	Mortality 2d	Mortality 3d
0.025%	Sodium selenate	3.614	3.148 - 4.08	0.238	13.3	46.7
	Sodium selenite	6.565	5.461 - 7.669	0.563	3.3	6.7
	Selenomethionine	5.225	4.57 - 5.88	0.334	3.3	10.0
	Selenocystine	7.289	6.224 - 8.353	0.543	6.7	16.7
0.05%	Sodium selenate	3.233	2.895 - 3.571	0.173	16.7	56.7
	Sodium selenite	5.063	4.407 - 5.718	0.335	3.3	26.7
	Selenomethionine	3.778	3.267 - 4.288	0.26	13.3	36.7
	Selenocystine	4.857	3.912 - 5.802	0.482	30.0	46.7
0.1%	Sodium selenate	2.343	1.948 - 2.738	0.201	46.7	80.0
	Sodium selenite	3.501	3.008 - 3.994	0.251	6.7	50.0
	Selenomethionine	2.544	2.06 - 3.029	0.247	50.0	70.0
	Selenocystine	4.953	4.309 - 5.596	0.328	13.3	40.0
0.25%	Sodium selenate	1.463	1.075 - 1.85	0.198	76.7	90.0
	Sodium selenite	2.796	2.315 - 3.277	0.245	40.0	80.0
	Selenomethionine	1.578	1.139 - 2.016	0.224	76.7	86.7
	Selenocystine	4.982	4.246 - 5.718	0.375	16.7	40.0
0.5%	Sodium selenate	1.044	0.736 - 1.353	0.157	86.7	96.7
	Sodium selenite	2.335	1.894 - 2.775	0.225	58.6	82.8
	Selenomethionine	1.739	1.18 - 2.297	0.285	63.3	73.3
	Selenocystine	5.281	4.357 - 6.205	0.471	23.3	36.7
1%	Sodium selenate	0.66	0.43 - 0.889	0.117	93.3	100
	Sodium selenite	1.778	1.441 - 2.114	0.172	76.7	93.3
	Selenomethionine	1.789	1.3 - 2.278	0.25	63.3	73.3
	Selenocystine	4.743	4.172 - 5.314	0.291	13.3	43.3

Table 3.4 Lethal concentrations of inorganic selenium compounds against adult male *B. germanica* (UCR)

Selenium form	n	LC_{50} (95% CI) (%)	LC_{95} (95% CI) (%)	Slope ± SE	χ^2 (df)
Sodium selenate[a]	180	0.12 (0.09 - 0.16)	0.98 (0.61 - 2.00)	1.81 ± 0.23	1.855 (4)
Sodium selenite[b]	179	0.11 (0.08 - 0.15)	0.80 (0.48 - 1.89)	1.91 ± 0.27	2.081 (3)

[a]Reported at 48 h
[b]Reported at 72 h

Table 3.5 Lethal concentration of sodium selenate against male *B. germanica* susceptible and resistant field strains at 48 h

Strain	n	LC_{50} (95% CI) (%)	LC_{95} (95% CI) (%)	Slope ± SE	χ^2 (df)
UCR	180	0.12 (0.09 - 0.16)	0.98 (0.61 - 2.00)	1.81 ± 0.23	1.855 (4)
WM	180	0.24 (0.18 - 0.35)	3.39 (1.69 - 11.20)	1.44 ± 0.21	3.273 (4)
RG386	180	0.18 (0.13 - 0.27)	1.88 (0.93 - 7.04)	1.61 ± 0.26	0.190 (3)
Ryan[a]	-	-	-	-	-

[a]Unable to generate values

Table 3.6 Lethal concentration of sodium selenate against male *B. germanica* susceptible and resistant field strains at 72 h

Strain	n	LC_{50} (95% CI) (%)	LC_{95} (95% CI) (%)	Slope ± SE	χ^2 (df)
UCR	180	0.03 (0.02 - 0.05)	0.41 (0.23 - 1.33)	1.48 ± 0.29	0.583 (3)
WM	180	0.05 (0.02 - 0.08)	1.88 (0.82 - 10.25)	1.05 ± 0.20	2.759 (4)
RG386	180	0.04 (0.02 - 0.06)	0.83 (0.39 - 4.47)	1.24 ± 0.26	0.925 (3)
Ryan	180	0.20 (0.12 - 0.43)	14.52 (3.25 - 590.79)	0.89 ± 0.20	2.208 (3)

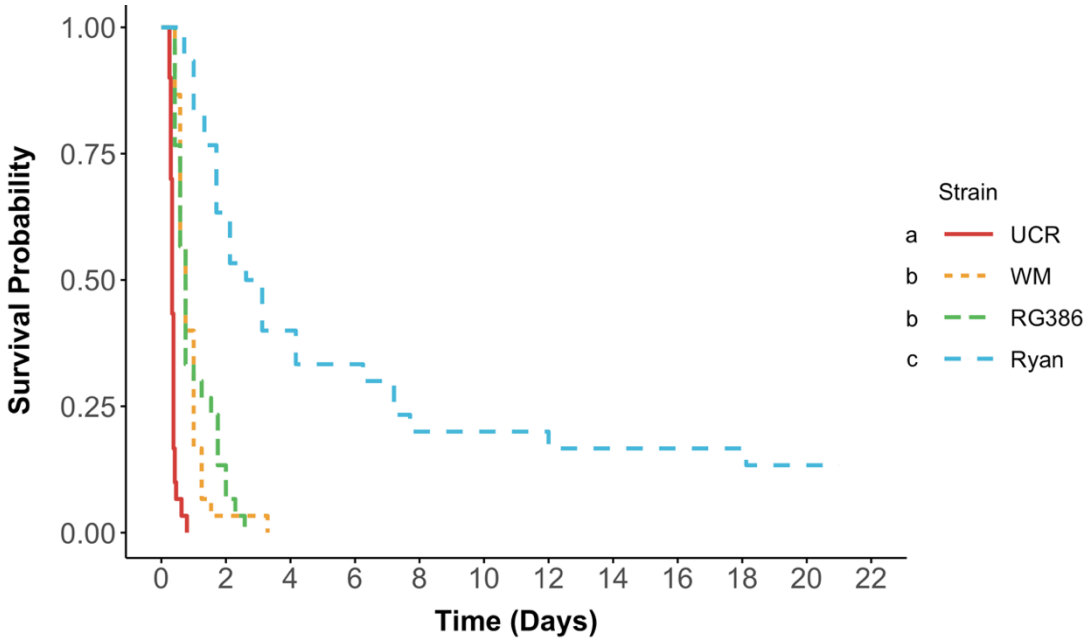

Figure 3.6 Survivorship curves of Advion Evolution against adult male *B. germanica*. Lowercase letters represent significant difference between strains based on log-rank tests (p<0.05).

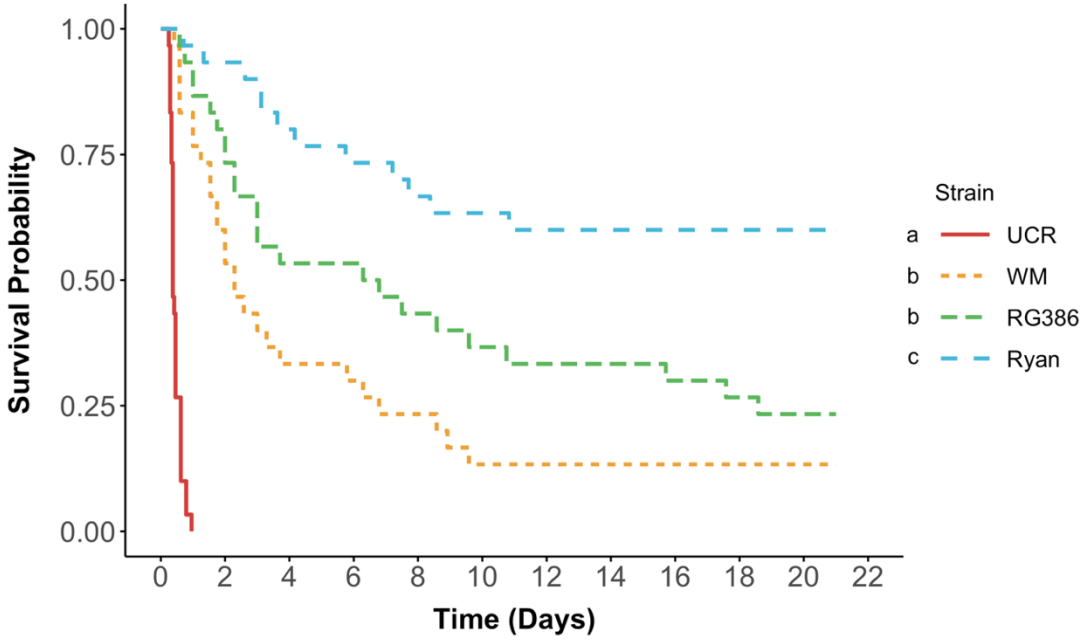

Figure 3.7 Survivorship curves of Maxforce FC Magnum against adult male *B. germanica*. Lowercase letters represent significant difference between strains based on log-rank tests (p<0.05).

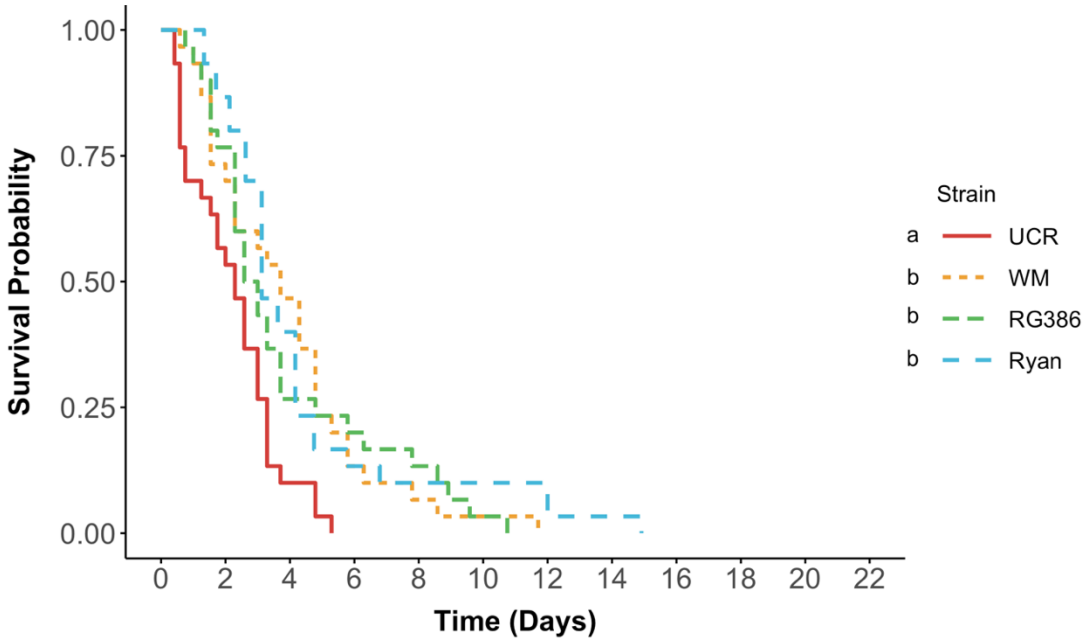

Figure 3.8 Survivorship curves of Vendetta against adult male *B. germanica.* Lowercase letters represent significant difference between strains based on log-rank tests (p<0.05).

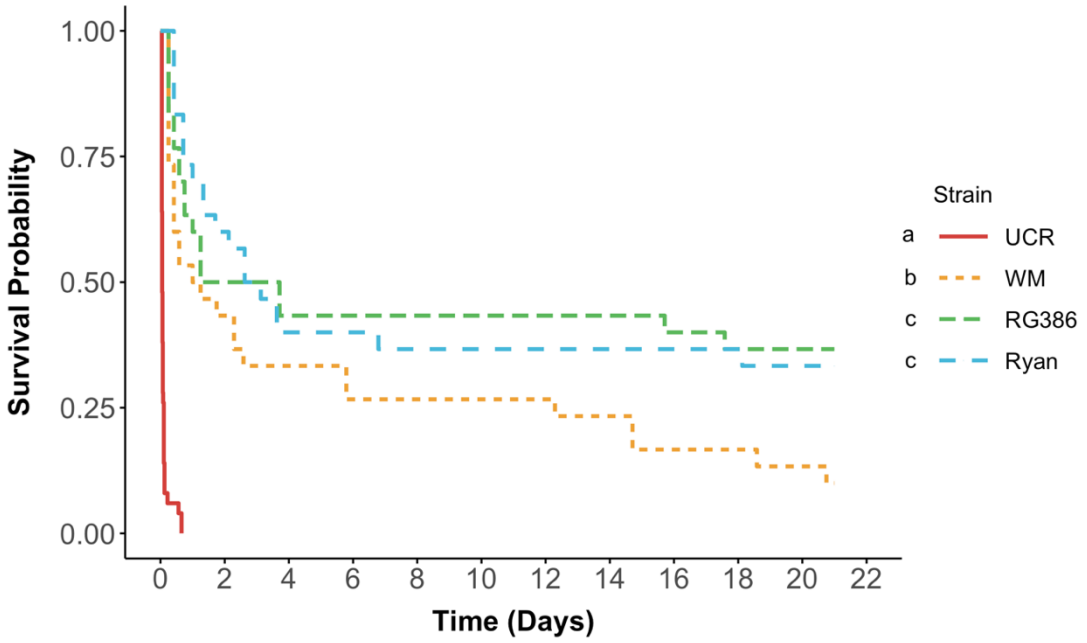

Figure 3.9 Survivorship curves of Alpine Rotation Reservoir 1 against adult male *B. germanica.* Lowercase letters represent significant difference between strains based on log-rank tests (p<0.05).

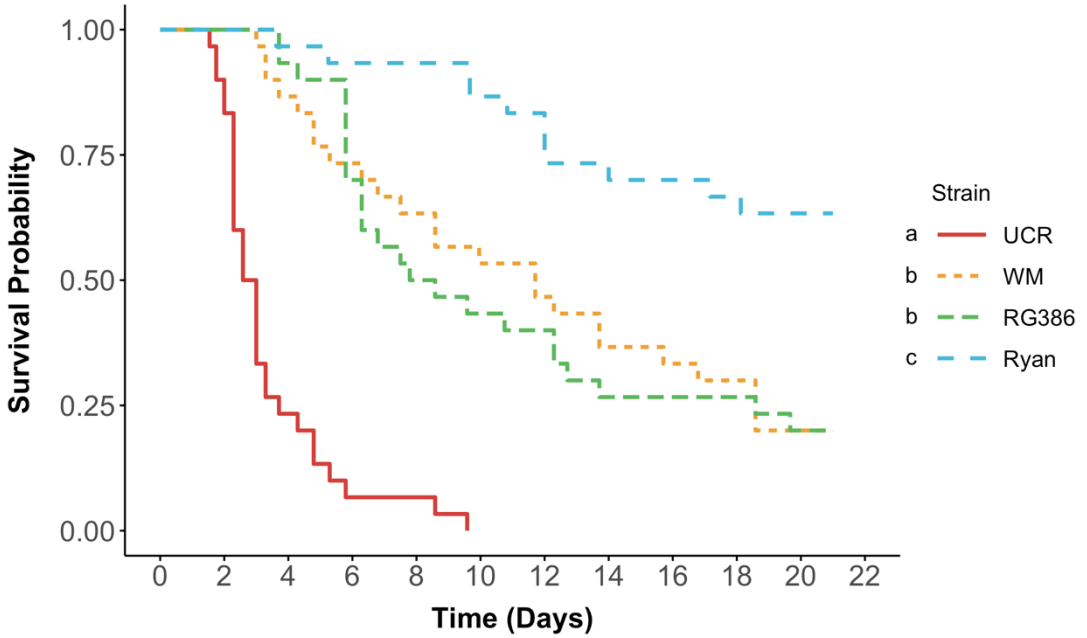

Figure 3.10 Survivorship curves of Siege against adult male *B. germanica*. Lowercase letters represent significant difference between strains based on log-rank tests (p<0.05).

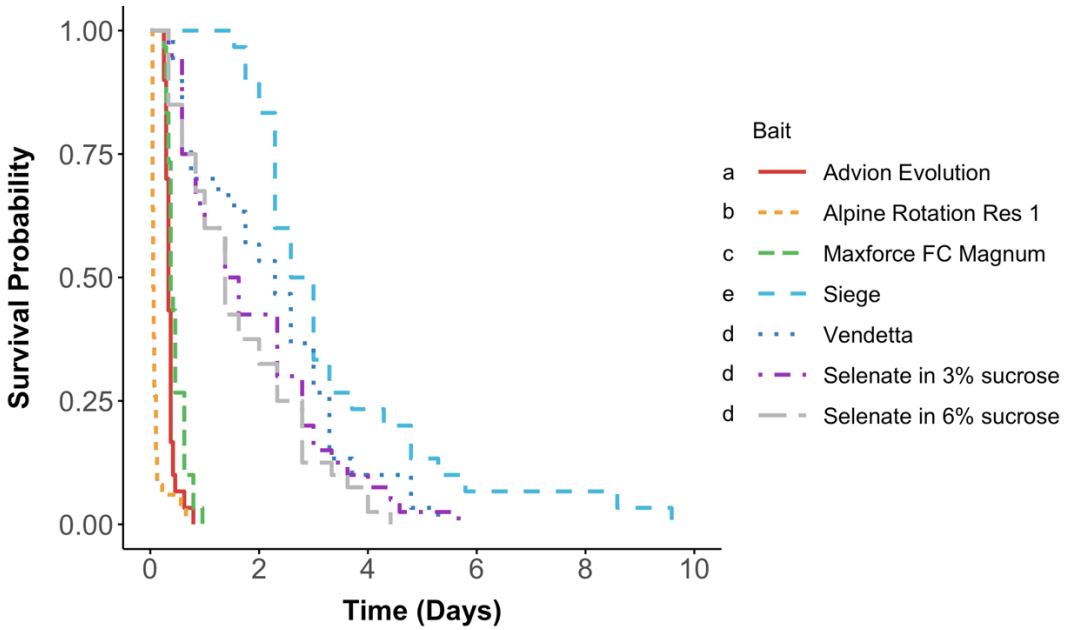

Figure 3.11 Survivorship curves of baits against adult male *B. germanica* (UCR). Lowercase letters represent significant difference between strains based on log-rank tests (p<0.05).

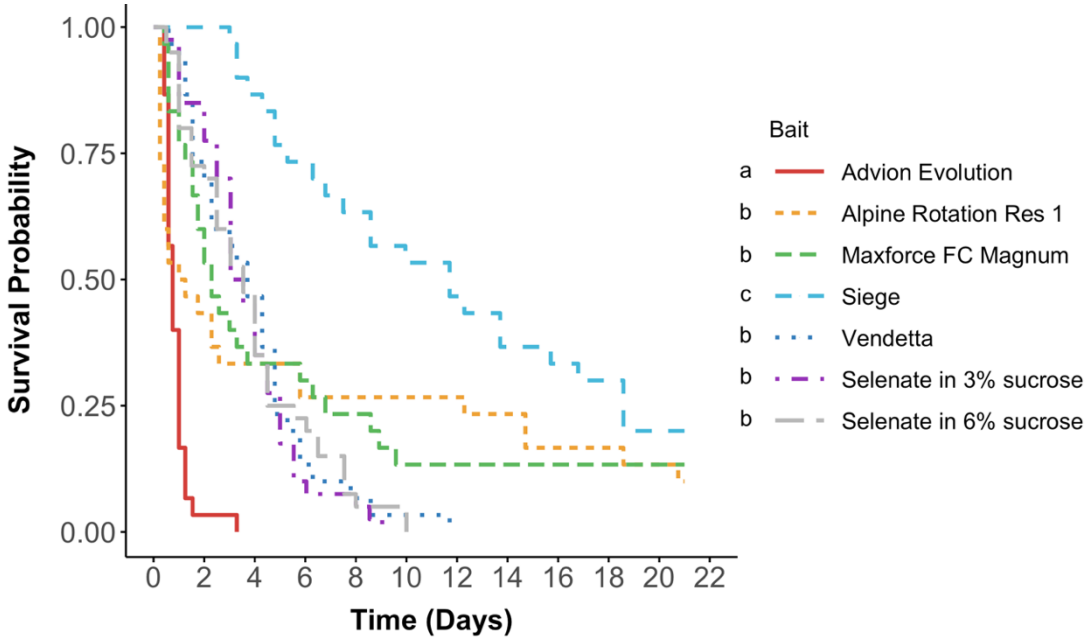

Figure 3.12 Survivorship curves of baits against adult male *B. germanica* (WM)**.** Lowercase letters represent significant difference between strains based on log-rank tests (p<0.05).

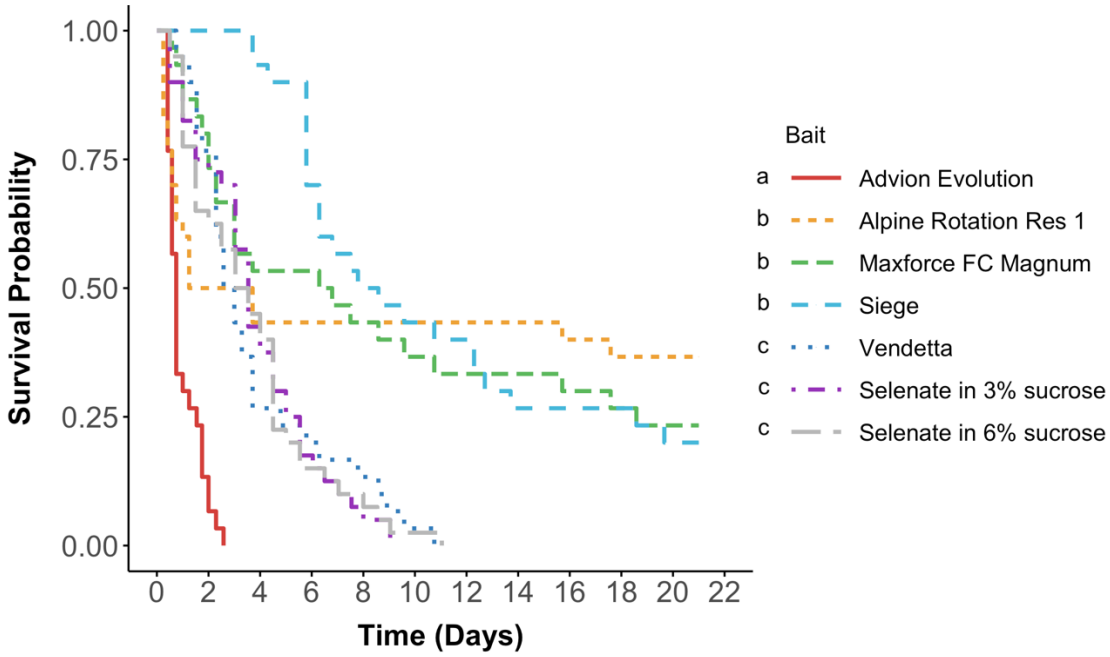

Figure 3.13 Survivorship curves of baits against adult male *B. germanica* (RG386)**.** Lowercase letters represent significant difference between strains based on log-rank tests (p<0.05).

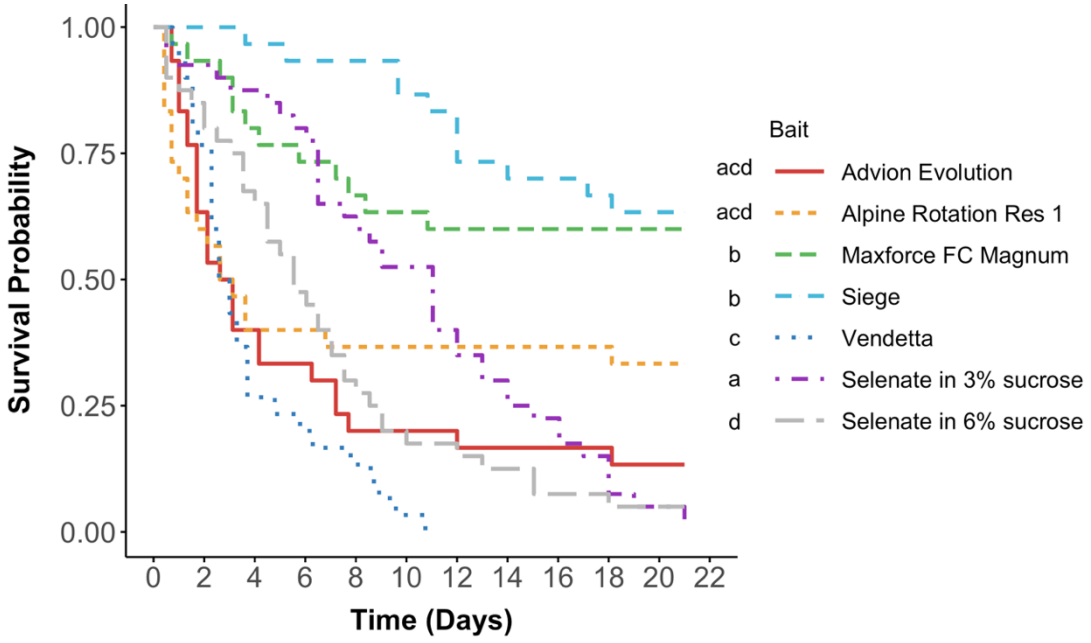

Figure 3.14 Survivorship curves of baits against adult male *B. germanica* (Ryan)**.** Lowercase letters represent significant difference between strains based on log-rank tests (p<0.05).

Table 3.7 Mean survival times of tested baits against strains of *B. germanica*

Strain	Bait	Mean Survival Time (Days)	95% CI	SE	Mortality 14d	Mortality 21d
UCR	Advion Evolution	0.363	0.324 - 0.401	0.02	100	100
	Alpine Rotation Reservoir 1	0.1	0.062 - 0.139	0.02	100	100
	Maxforce FC Magnum	0.46	0.398 - 0.522	0.032	100	100
	Siege	3.375	2.699 - 4.051	0.345	100	100
	Vendetta	2.221	1.716 - 2.726	0.258	100	100
	Sodium selenate in 3% sucrose	1.888	1.472 - 2.303	0.212	100	100
	Sodium selenate in 6% sucrose	1.676	1.317 - 2.035	0.183	100	100
WM	Advion Evolution	0.875	0.681 - 1.069	0.099	100	100
	Alpine Rotation Reservoir 1	5.721	2.955 - 8.487	1.411	76.7	90.0
	Maxforce FC Magnum	5.457	3.093 - 7.821	1.206	86.7	86.7
	Siege	11.918	9.584 - 14.252	1.191	63.3	80.0
	Vendetta	3.871	2.967 - 4.774	0.461	100	100
	Sodium selenate in 3% sucrose	3.704	3.08 - 4.329	0.319	100	100
	Sodium selenate in 6% sucrose	3.823	3.026 - 4.62	0.407	100	100
RG386	Advion Evolution	0.986	0.755 - 1.217	0.118	100	100
	Alpine Rotation Reservoir 1	9.374	5.912 - 12.835	1.766	56.7	63.3
	Maxforce FC Magnum	9.21	6.336 - 12.083	1.466	66.7	76.7
	Siege	11.053	8.813 - 13.292	1.143	73.3	80
	Vendetta	3.826	2.844 - 4.809	0.501	100	100
	Sodium selenate in 3% sucrose	3.795	3.069 - 4.521	0.37	100	100
	Sodium selenate in 6% sucrose	3.611	2.813 - 4.41	0.407	100	100
Ryan	Advion Evolution	6.104	3.638 - 8.57	1.258	83.3	86.7
	Alpine Rotation Reservoir 1	8.742	5.438 - 12.045	1.685	63.3	66.7
	Maxforce FC Magnum	14.553	11.649 - 17.457	1.482	40	40
	Siege	17.444	15.544 - 19.345	0.969	30	36.7
	Vendetta	3.826	2.844 - 4.809	0.501	100	100
	Sodium selenate in 3% sucrose	10.378	8.615 - 12.141	0.9	75	97.5
	Sodium selenate in 6% sucrose	6.793	5.153 - 8.432	0.836	87.5	95

Table 3.8 Lethal dose of sodium selenate against *B. germanica* 96 h post-treatment

Time (days)	n	LD_{50} (95% CI) (µg)[a]	LD_{95} (95% CI) (µg)[a]	Slope ± SE	χ^2 (df)
4	180	8.33 (6.71 - 9.70)	25.44 (19.65 - 41.52)	3.39 ± 0.59	2.306 (5)
5	180	7.19 (5.71 – 8.42)	21.10 (16.80 – 31.57)	3.52 ± 0.58	3.930 (5)

[a]Lethal dose values are expressed as µg of sodium selenate per adult male cockroach

Discussion

This is the first report on toxicity values of selenium on any cockroach species. Previously, Nakonieczny (1993) tested sodium hydrogen selenite on *Gromphadorhina portentosa* (Blattodea: Blaberidae) to examine effects on enzymatic activity after exposure to a sublethal dose; however, this dose was based on a prior study on *Musca domestica* by Simmons et al. (1988). The present study found that sodium selenate was the most toxic compound when evaluated against our UCR susceptible strain in both nonchoice and choice bioassays. The toxicity of sodium selenate has been shown in various other insect species, given its environmental relevance. Sodium selenate is the most bioavailable selenium form and is most commonly up taken by plants in the environment (Terry et al. 2000). As a result, sodium selenate has been subjected to numerous studies on insects based on environmental toxicology (Vickerman & Trumble 2003, Freeman et al. 2007, Jensen et al. 2007, De La Riva et al. 2016). Of the studies that investigated multiple selenium forms, sodium selenate ranked the most active against several insects. *Apis mellifera* (Hymenoptera: Apidae) larvae that were fed sodium selenate treated artificial diet had the lowest LC_{50} value compared to that of sodium selenite, methylselenocysteine, and selenocystine (Hladun et al. 2013a). The high toxicity

of sodium selenate was further shown through a severely reduced percentage of larvae reaching the pupation stage (9%) (Hladun et al. 2013a). In contrast, an organic form was most toxic to *Linepithema humile* (Hymenoptera: Formicidae) (De La Riva et al. 2014). Seleno-L-methionine reported the lowest LC_{50} of 87.83 mg/L, while sodium selenate followed second with an LC_{50} value of 131.6 mg/L. The variability of toxicity demonstrates the complex effects of selenium on insects.

Based on our mean survival time results, there was evidence to show that selenomethionine and selenocystine may have some repellency or deterrent properties to *B. germanica*, making them unsuitable for further testing as a potential bait toxicant. In addition to low mortality at higher concentrations, some cockroaches removed from the arena subsequently regained mobility even after exhibiting severe symptoms of toxicity. The ability for insects to detect and thus be deterred or repelled from selenium-treated food material has been shown in various insects, including *Acheta domesticus*, *Myzus persicae* , and *Spodoptera exigua* (Vickerman et al. 1999, Hanson et al. 2004, Freeman et al. 2007). In the context of a bait toxicant, it is highly disadvantageous for an active ingredient to exhibit any repellent or deterrent properties since baits rely on ingestion to deliver a lethal dose (Reierson 1995). In our feeding preference test, cockroaches had no preference when drinking from a droplet of either water or 0.5% sodium selenate. Similarly, other insects have shown no preference when exposed to both untreated and treated material (Jensen et al. 2005, Quinn et al. 2011, De La Riva et al. 2014). It is also important to note that a phagostimulant was not used in our feeding preference

experiment, which further supports the fact that German cockroaches are not deterred from feeding on sodium selenate.

The field strains used in this study exhibited broad-spectrum resistance toward the five active ingredients tested. Lee et al. (2022) previously reported multiple resistance of these field strains when tested against five commercial bait products containing either fipronil, clothianidin, indoxacarb, emamectin benzoate, or hydramethylnon, in addition to topical bioassays with diagnostic doses. In our study, choice bioassays revealed that WM and RG386 were moderately resistant, while Ryan was highly resistant towards the five baits tested. Although LC_{50} values in nonchoice bioassays for sodium selenate were comparable at 72 hours for UCR, WM, and RG386, sodium selenate was less efficacious in choice bioassays. This reduced performance was more profound in Ryan, which suggests that there may be a correlation to its high resistance level. We suspect that preexisting detoxification mechanisms, a common resistance mechanism for many insecticides, may be involved in increased selenium tolerance within the resistant strains (Yu 2014). Moreover, Ryan was the only strain where the two sodium selenate baits differed from one another. The better performance of the 6% sucrose solution suggests that Ryan may prefer higher levels of sugar to induce feeding. Another possibility is that Ryan is averse to sodium selenate and more sucrose is required to overcome this effect, in contrast to observations seen with the feeding preference bioassay with the UCR strain. Although there is some evidence that resistant strains have tolerance toward sodium selenate, our liquid baits were comparable to several commercial bait products and outperformed others.

The presence of survivors after treatment may indicate control failure as even a small number of cockroaches can repopulate an environment. Sodium selenate achieved 100% mortality in WM and RG386 strains by day 14, in contrast to Alpine, Maxforce, and Siege, which failed to provide 100% mortality even by day 21. Further observation of Ryan concluded that 100% mortality was achieved by both sodium selenate baits achieved by day 23, a time point outside of our set observation period. Since our liquid bait only utilized sucrose, the potential for increased performance is excellent. The attractiveness of selenium may increase if sodium selenate is combined with other known attractants and feeding stimulants, as seen in the complex matrix formulation of commercial bait products (Appel & Rust 2021).

The lethal dose of an ingested toxicant required to kill a cockroach is rarely reported in studies due to the difficulty in determining the precise amount the test organism has consumed (Appel & Rust 2021). We successfully demonstrated a working protocol of an oral dose method to determine the lethal dose. Reid & Bennet (1989) previously reported lethal dose values of abamectin (LD_{50}: 5.00, LD_{95}: 15.05), chlorpyrifos (LD_{50}: 10.62, LD_{95}: 20.17), and chlordecone (LD_{50}: 62.62, LD_{95}: 155.84) at μg/g of body weight 48 hours post treatment. Delayed toxicants, abamectin (LD_{50}: 2.10, LD_{95}: 9.33), sulfuramid (LD_{50}: 4.73, LD_{95}: 22.35), dechlorane (LD_{50}: 22.24, LD_{95}: 69.10), and hydramethylnon (LD_{50}: 59.24, LD_{95}: 155.38), were analyzed around 14 days post treatment at μg/g of body weight. Gondhalekar et al. (2011) reported LD values for indoxacarb (LD_{50}: 0.12, LD_{95}: 1.11 μg per insect) at 72 hours post treatment using pellets of a blank matrix to deliver the toxicant rather than a liquid. Compared to these other oral

toxicants, sodium selenate represents higher LD values given that adult males weigh about 0.0472 g (Wu & Appel 2017). Since we did not have access to a blank matrix, we developed a method for determining lethal doses for an ingested toxicant. However, our protocol would be limited to water-soluble insecticides that are not repellent.

In conclusion, selenium in the form of sodium selenate has the potential to be an effective bait toxicant against the German cockroach. In addition, it may prove efficacious against field collected German cockroaches. Given that these experiments took place only within the laboratory setting, this may not provide a full representation of the performance of a selenium-based bait when applied in the field. Further investigation is warranted to elucidate a potential mode of action and other characteristics that can contribute to its performance.

Chapter IV: Horizontal transfer, behavioral, and histopathological effects of sodium selenate on the German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae)

Introduction

The German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae), is regarded as the most important indoor cockroach pest species globally, causing significant economic impacts as well as mediating the transfer of pathogens, allergens, antibiotic-resistant bacteria, and parasites (Ahmad et al. 2011, Menasria et al. 2014, Hamu et al. 2014, Lee & Wang 2021, Schal & DeVries 2021). Control of the German cockroach has relied heavily on insecticides resulting in the rapid development of resistance to many different toxicants (Cochran 1995, Wickham et al. 1995, Zhu et al. 2016). Although baits have been used to control cockroaches for 200 years, it was only after the 1990s that baiting became increasingly commonplace; previously, liquid sprays were the industry standard (Appel & Rust 2021). Baits are generally safer than other insecticide formulations since application in sensitive areas is possible and precise application minimizes residues and health risks from pesticide exposure (Reierson 1995, Appel & Rust 2021, Wang et al. 2019b).

Selenium is an essential trace element for humans and animals at low doses as it is a component of selenoproteins that is incorporated into enzymes protecting from oxidative stress (ATSDR 2003). The Recommended Dietary Allowance (RDA) for selenium is 55 µg (0.7 µmol)/day for both men and women; however, selenium can be toxic at slightly higher concentrations (The Institute of Medicine 2000). Insects have

been the subject of many studies on the impact of selenium as an environmental toxicant (Chapter II). Mechora (2019) implicated selenium as a protective agent for plants based on evidence of toxicity and repellency. The utility of selenium as a bait toxicant was demonstrated against *B. germanica* of both susceptible and resistant strains in liquid bait bioassays (Chapter III). Sodium selenate was found to be the most active among the four selenium compounds evaluated, making it the most suitable bait toxicant candidate.

Some baits have been shown to secondarily kill insects by transferring the AI through emetophagy, coprophagy, and/or necrophagy, contributing to the efficacy of baits (Reierson 1995, Buczkowski & Schal 2001b). As a result, baits can target stages of *B. germanica* (females and nymphs) that are less inclined to forage outside of harborage areas (Silverman et al. 1991, Metzger 1995). A number of studies have demonstrated the horizontal transfer in laboratory experiments (Kopanic & Schal 1997, 1999; Buczkowski & Schal 2001a; Buczowski et al. 2001, 2008). Kopanic & Schal (1997, 1999) observed coprophagy mediated horizontal transfer of hydramethylnon, a slow-acting toxicant, resulting in high mortality among 1st instars. Other toxicants, including fipronil and indoxacarb, are translocated by donor adult males, causing mortality to recipients (Buczkowski & Schal 2001a, Buczowski et al. 2008). Horizontal transfer serves as an additional component that may contribute to the efficacy of baits. Secondary transfer of bait toxicants has not been established in the field and it may be insignificant under field conditions (Reierson 1995).

Besides direct toxicity-induced mortality, insecticides can have marked effects at sublethal doses, especially on parameters such as longevity and fecundity (Abd-Elghafar

& Appel 1992, Lee et al. 1998, Lee 2000). Furthermore, sublethal insecticidal effects can affect insect behavior, a complex topic that has not been well studied (Haynes 1988). In addition, an evaluation of sublethal effects can reveal modes of action previously unknown. The interference of insecticides on normal locomotor behavior may subsequently affect critical areas such as reproduction, host-finding, dispersal and feeding (Haynes 1988). With modern video tracking software, effects on locomotor behavior may be more precisely studied and analyzed. Agrafioti et al. (2021) evaluated the effects of the fumigant phosphine on the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) and the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrychidae) after 2 or 24 hours after exposure. The authors observed significant changes in total distance moved and velocity for both susceptible populations of the two species.

The cause of selenium toxicity has been hypothesized to be due to its chemical similarity to sulfur (Daniels 1996). The definitive cause of mortality in insects remains unknown and has not been a common focus of the research. Several studies have investigated the accumulation of selenium in insect tissues. In yellow mealworm, *Tenebrio molitor* (Coleoptera: Tenebrionidae), selenium was preferentially localized in the Malpighian tubules, followed by the digestive tract and then the reproductive tissues (Hogan & Razniak 1991). Hladun et al. (2013a) hypothesized that selenium toxicity in the honey bee, *Apis mellifera* (Hymenoptera: Apidae) may be caused by oxidative stress from reducing organic forms since they lack detoxification proteins. In addition, excessive toxicant levels may damage vital organs. Imaging diamondback moth, *Plutella*

xylostella (Lepidoptera: Pyralidae), larvae fed treated princes' plume suggested the loss of integrity of internal organs (Freeman et al. 2006).

To study the various effects of sodium selenate as a toxicant, several studies were conducted to further understand the potential of a selenium-based bait product. First, I investigated whether selenate has the potential to be horizontally transferred through fresh residues of male donor cockroaches and subsequently kill conspecifics. Secondly, I studied the locomotor behavior of cockroaches acutely exposed to selenium using video monitoring software. Lastly, I conducted histological examination on cockroach alimentary tract after subjecting the insect to acute or chronic exposure to discover pathological anomalies to elucidate a potential mode of action.

Methods and Materials

Insects

All strains were kept in 121-liter garbage bins equipped with electrical barriers maintained at 24 ± 2°C, 30–50% RH, and a 12-hour photoperiod. Cockroach cultures were provided with harborages (rolled corrugated cardboard), dog food (Purina Dog Chow, Nestlé Purina Petcare, St. Louis, MO), and a water source (dH_2O), *ad libitum.* The UCR strain is a laboratory susceptible strain that has not been exposed to any insecticides for ≥ 40 years. It was originally a subculture of the Orlando susceptible strain. Healthy adult male and nymphs (2^{nd} and 3^{rd} instars) of German cockroaches were gently collected using a glass vial without carbon dioxide anesthetization.

Horizontal Transfer to Nymphs

Ten adult male cockroaches (UCR strain) serving as donor roaches were introduced into 472 mL deli cups (Edris Plastics Mfg., Inc, Vernon, CA) with a rectangle corrugated paper (4 cm x 8.5 cm) folded in half as harborage and laboratory dog chow as a food source. Five holes were made in the lid for aeration. Cockroaches were acclimatized without a water source for 24 hours. At the end of the acclimatization period and 2 hours before scotophase, sodium selenate solutions of 0.125%, 0.25%, 0.5%, and 1% (w/v) were introduced in microfuge tubes (1.7 mL) plugged with cotton roll. The cockroaches were allowed to drink for 2 hours, and then the vials were removed. Control donor cockroaches were provided with deionized water instead of sodium selenate solutions. Ten nymphs (2^{nd} and 3^{rd} instar) were introduced into each area and a water tube. Mortality of adults and nymphs were recorded every 24 hours until 7 days. Dead nymphs or adults were not removed to facilitate the horizontal transfer.

Effect of acute toxicity on locomotor behavior

One adult male German cockroach was introduced in a 100 x 15 mm polystyrene Petri dishes flipped upside down (Fisherbrand® Fisher Scientific, Waltham, MA) with a piece of laboratory dog chow. The inner sides of the Petri dishes were coated with fluon (BioQuip Products Inc., Rancho Dominguez, CA) to limit climbing of the cockroaches. The cockroaches were acclimatized for 24 hours without water. At the end of this period, the droplet feeding method from Chapter II was employed using 10 μg of sodium selenate which is equivalent to 1 μL of 1% sodium selenate solution. Control

cockroaches were given DI water. The piece of dog chow was removed before tracking to aid in better detection by the system.

The movement was tracked using a GigE camera (acA1300-60gc, Basler, Ahrensburg, Germany) and processed through the Ethovision XT 15 Software (Noldus Information Technology, Leesburg, VA) at 25 frames s^{-1}. The camera was positioned about 30 cm above two Petri dish arenas placed on top of white filter paper. The movement was captured immediately after feeding, 1 day, and 2 days after exposure of the same individuals. Tracking was set to begin after 1 minute of detection by the system to allow the cockroaches to settle after potential disturbance from movement. Then, the 30-minute tracking period commenced. The piece of dog chow was replaced, and a small piece of dental cotton roll soaked with DI water was provided after the first detection. At later tracking periods when retesting the same cockroaches, both dog chow and dental cotton roll were removed and immediately replaced after tracking was over. Since I wanted to observe the effects of toxicity, only cockroaches that exhibited symptoms of intoxication were captured for tracking at day 1. Furthermore, dead cockroaches at day 2 were automatically given a 0 for both 'distance traveled' and 'velocity' parameters.

Histological Procedure

Acute and chronic exposure

Deli cup setups were as described previously for the horizontal transfer study. Ten to fifteen cockroaches were introduced into each cup, and sodium selenate solutions of 0.5% and 0.005% (w/v) were provided for acute and chronic exposure, respectively.

Dissection

To avoid any changes after death, only severely moribund cockroaches were collected for dissection at the selected times: 24 h (acute toxicity) and 10-12 d (chronic toxicity). Cockroaches were briefly anesthetized by placing them in the freezer (-20°C). Vannas spring scissors (angled 2.5 mm cutting edge, Fine Science Tools Inc., Foster City, CA) were used to cut the last two abdominal segments. Then, an extra-fine tip featherweight pair of forceps was used to grab behind the pronotum and gently pull to extract the entirety of the alimentary tract.

Fixation, Dehydration and Embedding

Excised guts (Figure 4.3) were fixed in Bouin's fixative (Electron Microscopy Sciences, Hatfield, PA) for 24 hours. Fixed guts were dehydrated in serial ethanol washes beginning with 70%, 80%, and 90% solution for 10 minutes each. Then the samples were passed through three 100% ethanol washes of 10 minutes, 10 minutes, and 40 minutes followed by three xylene washes of 10 minutes, 10 minutes, and 40 minutes. Dehydrated samples were embedded in paraffin wax Histoplast PE (Epredia™, Kalamazoo, MI) at 60°C for 24 hours and placed in embedding molds.

Sectioning

The paraffin blocks were cut with a rotary microtome (American Optical 820, Buffalo, NY) at 7 µm thick sections. Cut sections were floated in a water bath at 38°C until wrinkle-free, then placed on a charged microscope slide (Research Products International Corp, Mount Prospect, Illinois). Slides were dried at 35°C for 12-24 hours.

Deparaffinization, Hydration, and Mounting

Slides were then deparaffinized and hydrated in the following solutions: xylene (3 min), xylene (3 min), 95% ethanol (3 min), 70% ethanol (3 min), and DI water (3 min). According to the manufacturer's protocol, samples were stained with hematoxylin and eosin (Scytek Laboratories, West Logan, UT). Slides were mounted with glass coverslips and then dried in the fume hood. At least eight midguts from adult males were examined for any histopathological effects for each treatment: untreated, acute, and chronic. Slides were examined under a fluorescent microscope (Zeiss Axioskop 2, Carl Zeiss, Jena, Germany) for any abnormalities, and photos were captured with a digital camera and stacked using CombineZP (Alan Hadley).

Data Analyses

Survivorship of nymphs was analyzed with Kaplan-Meier analysis, and survivorship curves were compared with that of the control group using log-rank (Mantel-Cox) tests in SPSS Statistics version 27.0 (IBM Corporation, Armonk, NY). Locomotor behavior was analyzed using the nonparametric Friedman test with each group for 'distance traveled' and 'velocity.' Differences among time points within groups were analyzed through *post hoc* Wilcoxon signed-rank test with an adjusted Bonferroni-adjusted significance level.

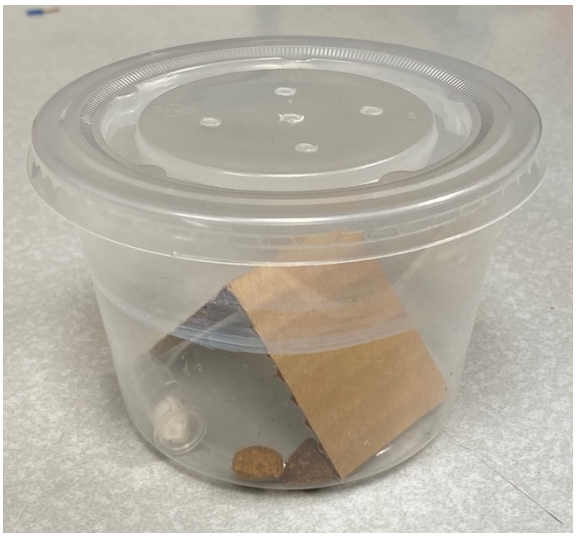

Figure 4.1 Deli cup setup used for horizontal transfer and histopathology study

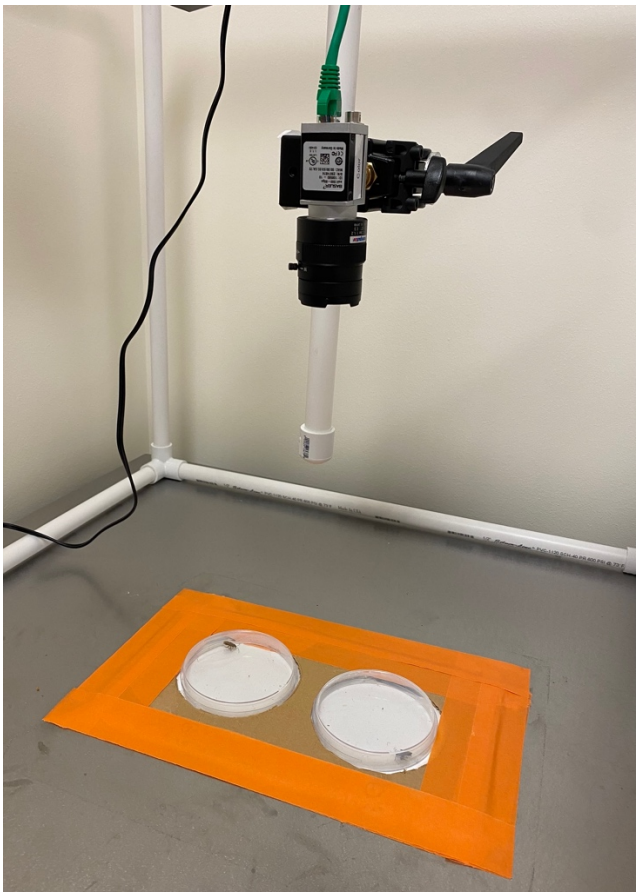

Figure 4.2 Locomotor behavior tracking setup

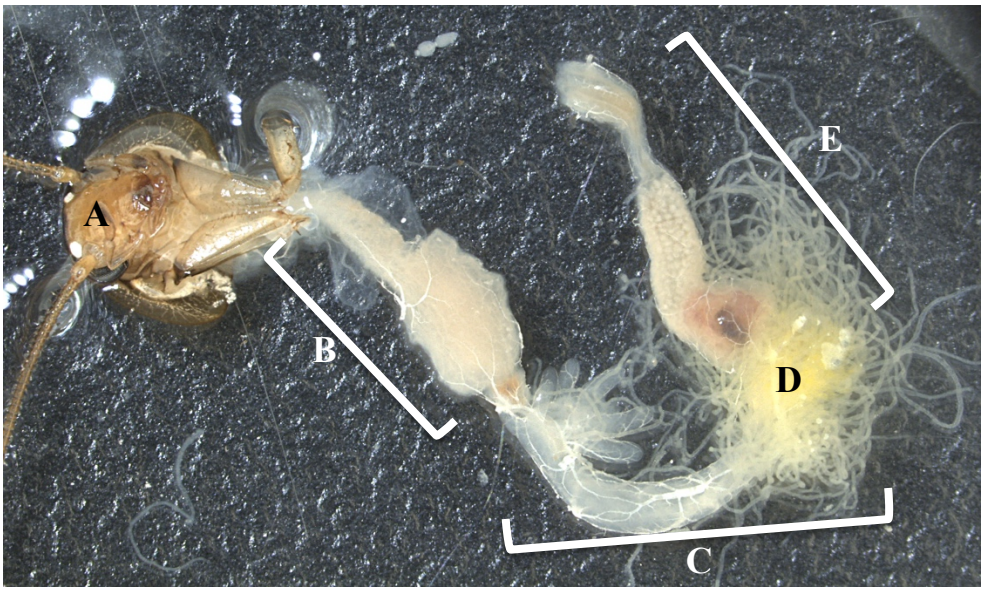

Figure 4.3 Excised alimentary tract of a male *B. germanica*. A) head, B) foregut, C) midgut, D) malpighian tubules, E) hindgut

Results

Horizontal Transfer to Nymphs

Both 0.125% and 0.25% concentrations of sodium selenate resulted in 10% nymphal mortality. Additionally, both 0.5% and 1% concentration resulted in 22.5% mortality. Nymphal control mortality did not exceed 5% by the end of the seven days. Based on the Kaplan-Meier survivorship analysis and log-rank tests, there was a significant difference between the control and the two higher concentrations (Figure 4.4). The survivorship at the lower concentrations of 0.125% and 0.25% was not significantly different from that of the control. In addition, mortality for donor adult male cockroaches was 3.7%, 15%, 52.5%, 75%, and 82.5% for control, 0.125%, 0.25%, 0.5%, and 1% sodium selenate, respectively.

Effect of acute toxicity on locomotor behavior

Distance traveled by the control group averaged 595.0 cm, 548.9 cm, and 650.2 cm on day 0, day 1, and day 2, respectively (Table 4.1). The velocity of the control group averaged 0.323 cm/s, 0.305 cm/s, and 0.361 cm/s on days 0, day 1, and day 2, respectively (Table 4.2). Distance traveled by the treated group averaged 710.7 cm, 138.9 cm, and 68.0 cm on day 0, day 1, and day 2, respectively (Table 4.1). Velocity of the treated group averaged 0.395 cm/s, 0.077 cm/s, and 0.038 cm/s on day 0, day 1, and day 2, respectively (Table 4.2). There was no significant difference registered for both distance traveled (Friedman Test, X^2=1.867, df=2, p=0.393) and velocity (Friedman Test, X^2=1.867, df=2, p=0.393) at the three time points in the control groups. Pairwise

comparisons between the time points for both distance traveled, and velocity also revealed no significant difference based on Wilcoxon signed-rank tests: day 0 vs day 1 (Z= -0.895, p=0.371), day 0 vs day 2 (Z= -0.298, p=0.766), and day 1 vs day 2 (Z= -1.039, p=0.299) (Figure 4.6, 4.7). In contrast, the treated groups registered high overall significance for distance traveled (X^2=42.296, df=2, p<0.001) and velocity (X^2=42.296, df=2, p<0.001) based on the Friedman test. Pairwise comparisons between time points distance traveled also revealed significance based on Wilcoxon rank signed-rank tests day 0 vs day 1 (Z= -4.517, p<0.001), day 0 vs day 2 (Z= -4.541, p<0.001), and day 1 vs day 2 (Z= -3.772, p<0.001) (Figure 4.6). Furthermore, pairwise comparisons between time points for velocity also revealed significance based on Wilcoxon rank signed-rank tests day 0 vs day 1 (Z= -4.517, p<0.001), day 0 vs day 2 (Z= -4.541, p<0.001), and day 1 vs day 2 (Z= -3.724, p<0.001) (Figure 4.7). About half (48.1%) of treated cockroaches that were alive on day 1 died by day 2. Based on visual of the paths recorded with Ethovision XT, untreated cockroaches tended to walk the inner circumference of the petri dish regardless of the time period (Figure 4.5). However, some variation did exist in some individuals as a preference for one side of the arena was evident. Paths of treated cockroaches that were alive by day 2 are shown in Figure 4.6. Visually, paths of treated cockroaches at day 0 were similar to those of the control group. However, on day 1 and 2 after treatment, paths were drastically shorter and irregular.

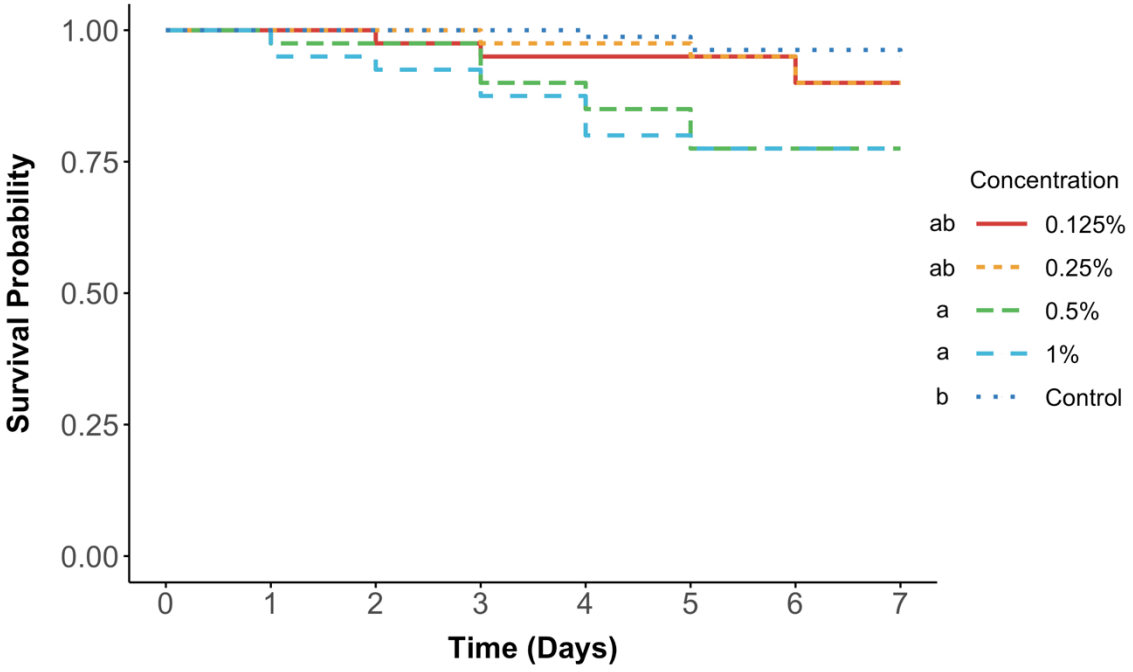

Figure 4.4 Survivorship curves of 2nd and 3rd instar after exposure to treated adult male *B. germanica*. Letters represent significance based on log-rank tests (p<0.05).

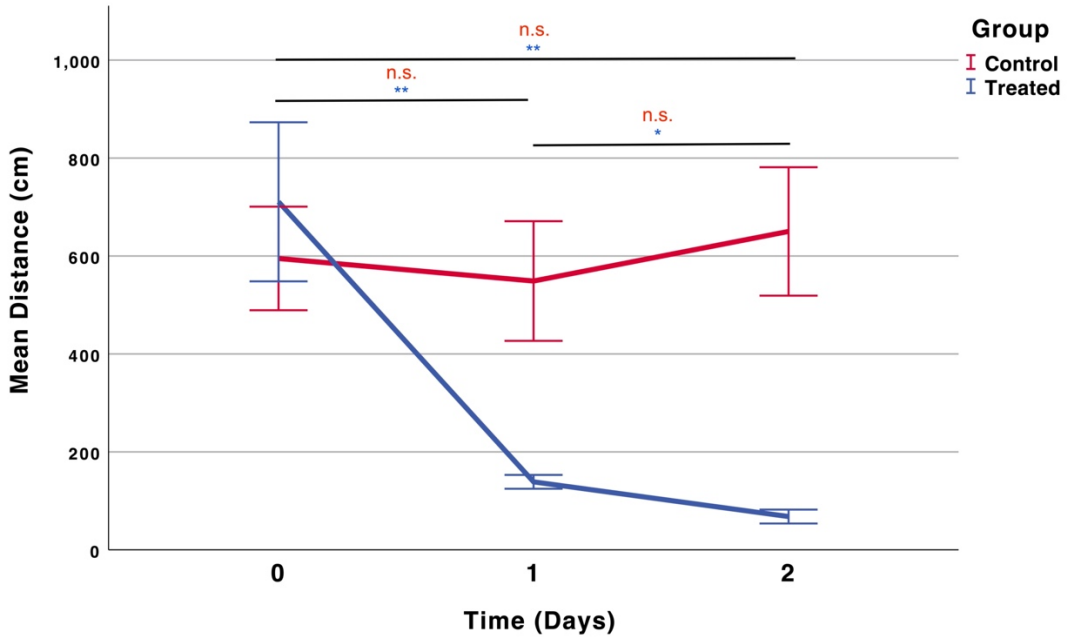

Figure 4.5 Mean distance traveled by untreated and acutely treated adult male *B. germanica*. Bars represent ±SE. Asterisks denote significance within a group between the time points: **(p<0.00001), *(p<0.0005), n.s. (not significant).

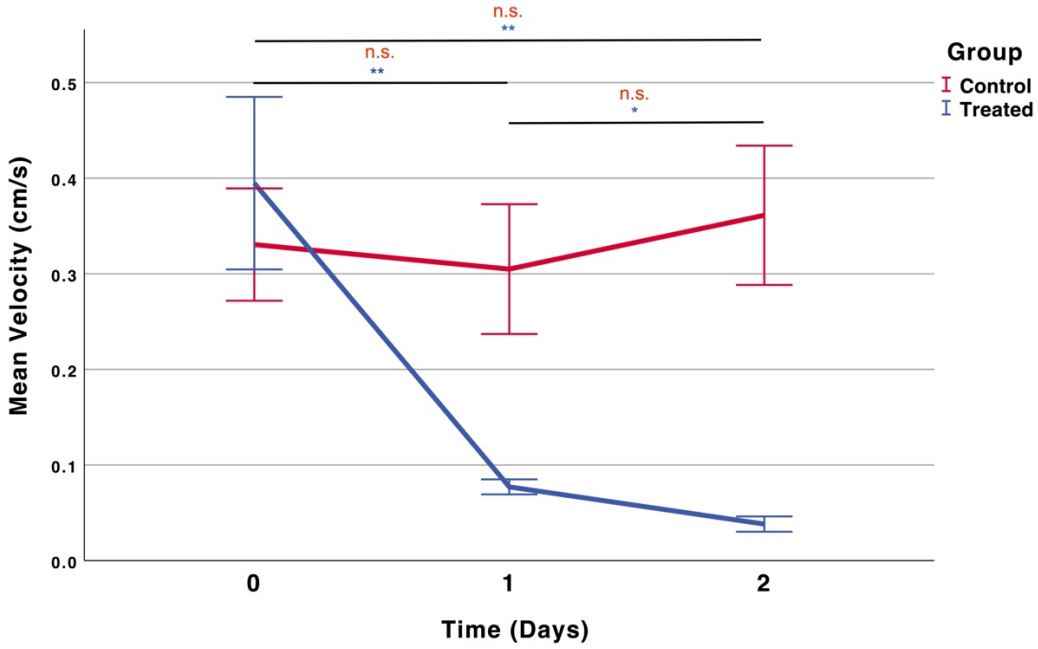

Figure 4.6 Mean velocity traveled by untreated and acutely treated adult male *B. germanica*. Bars represent ± SE. Asterisks denote significance within a group between the time points: **(p<0.00001), *(p<0.005), n.s. (not significant).

Table 4.1 Mean distance traveled for control (n=30) and acutely treated (10 µg sodium selenate) (n=27) *B. germanica*

	Distance (cm) ± SE		
	0 days	**1 day**	**2 days**
Control	595.0 ± 105.7	548.9 ± 122.2	650.2 ± 131.1
Treated	710.7 ± 162.4	138.9 ± 14.1	68.0 ± 14.2

Table 4.2 Mean velocity for control (n=30) and acutely treated (10 µg sodium selenate) (n=27) *B. germanica*

	Velocity (cm/s) ± SE		
	0 days	**1 day**	**2 days**
Control	0.323 ± 0.059	0.305 ± 0.068	0.361 ± 0.073
Treated	0.395 ± 0.090	0.077 ± 0.008	0.038 ± 0.008

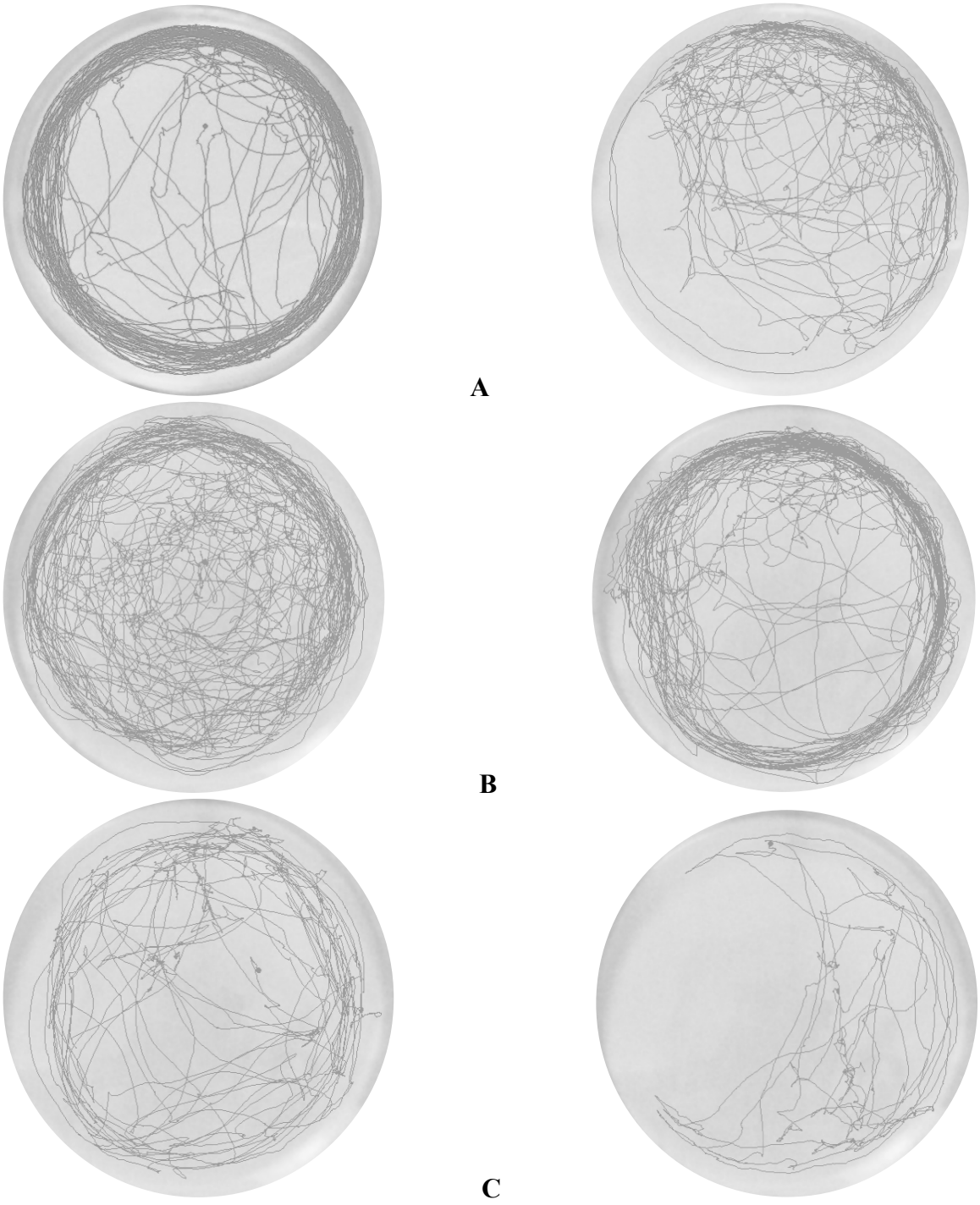

Figure 4.7 Representative tracks of control cockroaches at A) 0 days, B) 1 day, and C) 2 days. Tracks are not indicative of an individual's progression through time periods.

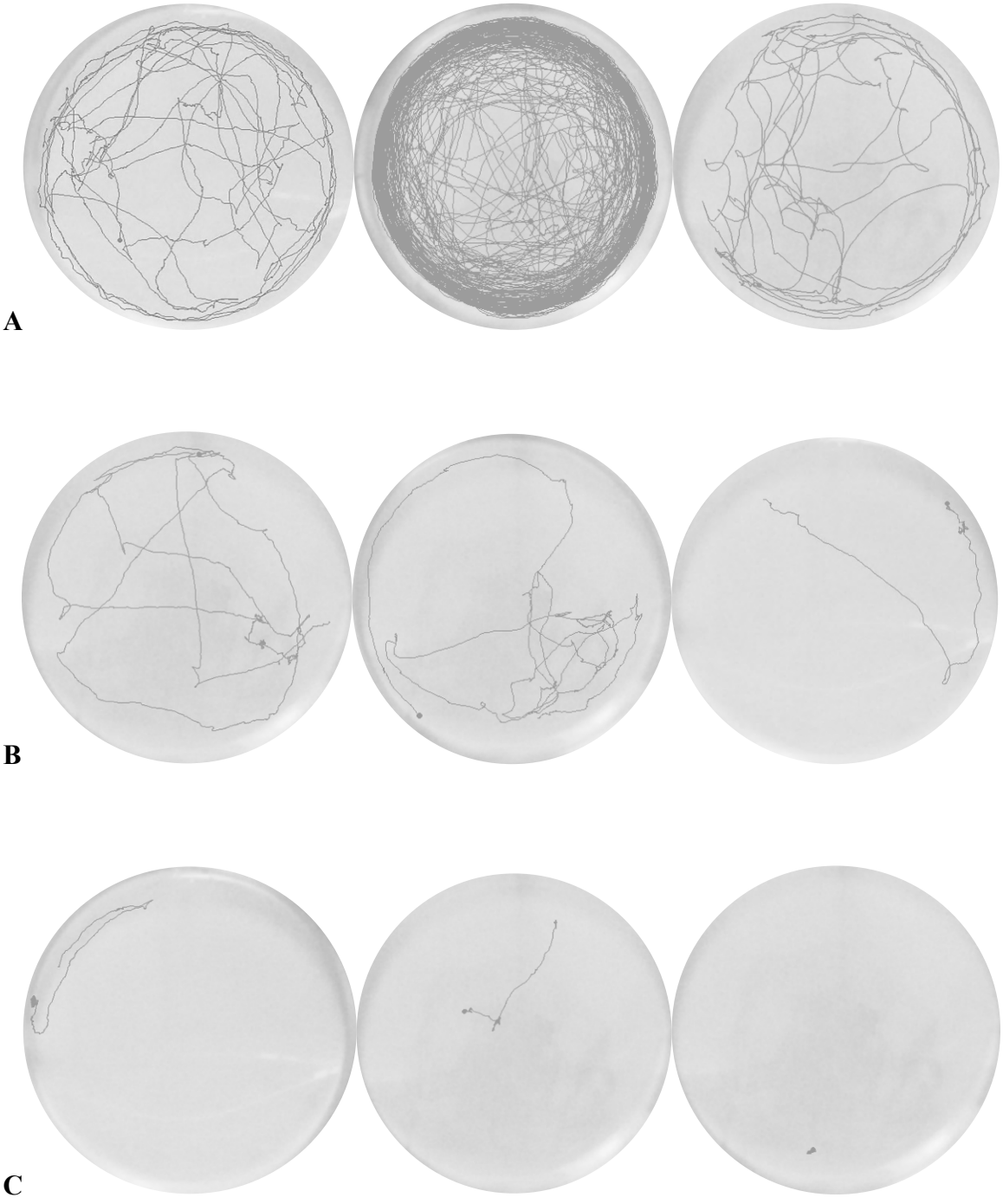

Figure 4.8 Representative tracks of sodium selenate treated cockroaches at A) 0 days, B) 1 day, and C) 2 days. Columns represent the same individuals through the different time periods.

Histopathological effects of acute and chronic toxicity

The cross-sections of control cockroaches revealed normal midgut characteristics. Untreated cockroaches had a well-developed brush border lining the midgut and a continuous and intact peritrophic membrane (Figure 4.7A, 4.8A). The basement membrane and epithelium were also distinct. The histopathological examination found minimal effects on acutely treated individuals and severe effects on chronically treated individuals. The midgut epithelium of acutely treated cockroaches did not exhibit any obvious abnormalities; however, the peritrophic membrane did exhibit some damage (Figure 4.7B). Chronically (0.005% sodium selenate) treated cockroaches had severe histopathological effects. There was evidence that the peritrophic membrane had degenerated (Figure 4.7C). In addition, the brush border was not clearly defined and was broken up across the surface (Figure 4.7C, 4.8C). There was cytoplasmic vacuolization in several individuals (Figure 4.8B, C). In contrast to vacuolization seen in the control midgut (4.7A), the vacuolization in the chronically treated cockroaches was significantly larger, creating large pockets in between the epithelial cells.

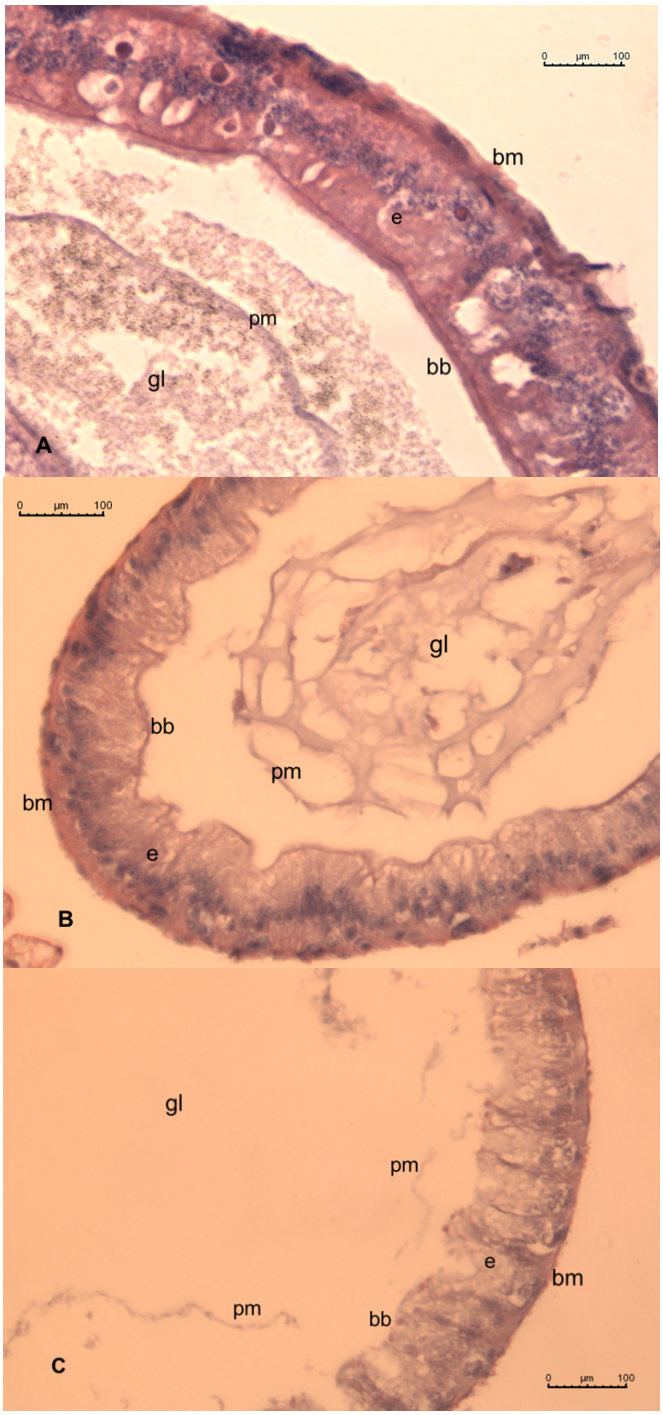

Figure 4.9 Midgut (7 µm) cross-sections of A) control, B) 24 h acutely treated and C) 10-12 d chronically treated adult male *B. germanica* with sodium selenate. Abbreviations: peritrophic membrane (pm), gut lumen (gl), the epithelium (e), brush border (bb), basement membrane (bm).

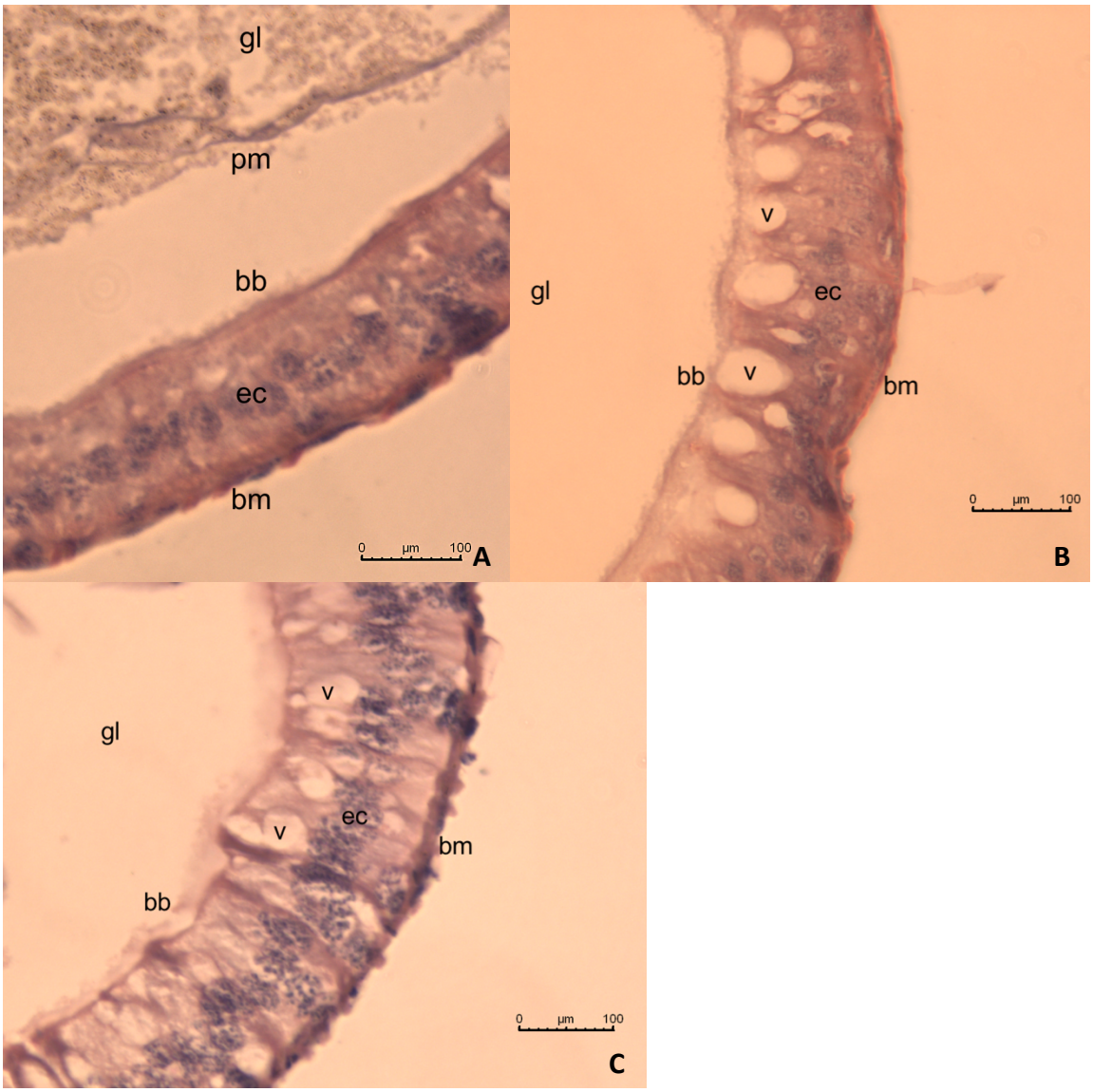

Figure 4.10 Midgut (7 µm) cross-sections of A) control, B/C) 10-12 d chronically treated adult male *B. germanica* with sodium selenate. Abbreviations: gut lumen (gl), peritrophic membrane (pm), epithelial cells (ec), brush border (bb), basement membrane (bm), nuclei (n), vacuole (v).

Discussion

Horizontal transfer of a toxicant through emetophagy, coprophagy, and/or necrophagy may contribute as another route of exposure for an active ingredient in baits (Reierson 1995, Buczkowski & Schal 2001b). This indirect mode of transfer is particularly useful in eusocial pest insects such as ants and termites, where a toxicant is disseminated to colony members through trophallaxis by foragers (Rust & Su 2012). In the German cockroach, this transfer mechanism may prove to be effective against stages that do not readily venture out of harborages such as gravid females and early instar nymphs (Cochran 1983, Demark et al. 1993). Deli cups were used as arenas to facilitate the transfer of sodium selenate more effectively through fresh residues produced by the donor males. Feeding of the male donor roaches on the sodium selenate solution was almost immediate upon introduction, and the onset of toxic symptoms were very quick for higher concentrations. Because of this short time window where the donor cockroaches exhibited normal behavior, fresh residues from regurgitate produced by the cockroaches were most likely the cause of nymphal mortality. Furthermore, the fact that nymphs fed on excreted residues from adults over the provided water source also supports the lack of feeding preference of sodium selenate as seen previously (Chapter III). Donor cockroaches were either moribund or dead by 24 hours (63-70%) for the two higher concentrations tested. In contrast, only 5–22.5% were either moribund or dead by 24 hours for the two lower concentrations. Coprophagy was eliminated as a cause of mortality because the cockroaches did not live long enough to produce frass in the higher concentrations. To a lesser extent, surviving roaches in lower concentrations may have excreted selenium-

containing feces on surfaces during the 7-day observation period but most likely did not contain a lethal dose. Because 0.125% or 0.25% did not result in significant mortality, fresh residues were probably not produced or regurgitate did not contain a lethal dose required to kill nymphs. Although necrophagy of donor cockroaches by nymphs was not observed, there was some evidence that surviving donor cockroaches consumed dead nymphs. Any future studies on coprophagy mediated horizontal transfer, as demonstrated by Kopanic and Schal (1997), would have to use a very low concentration so that donor roaches could live long enough to produce toxicant-laced frass. A concentration around 0.005% as used for chronic exposure tests in Chapter III may be suitable.

Sublethal effects of a toxicant on locomotor behavior may be exhibited in either stimulation or depression (Haynes 1988). When males drank sodium selenate, the distance traveled and velocity was severely impaired in intoxicated cockroaches within 24 hours. The decrease in both parameters may significantly affect normal behavior such as foraging, dispersal, and mating in treated German cockroaches. The placement of bait products is important for their efficacy (Reierson 1995). It is generally recommended to apply bait products in close proximity to harborage sites. Although toxicity symptoms occur very quickly, there may still be enough to return to harborage sites after consuming a sodium selenate-based bait. Cockroaches can travel some distance even after toxicant consumption, as seen with ‘distance traveled’ captured immediately after treatment (0 days). After returning to harborage sites, the cockroach may succumb to the toxicant, as observed with the tracks of both treated cockroaches at 1 and 2 days, where locomotor behavior was highly variable. This scenario allows the horizontal transfer through fresh

residues or necrophagic behavior. The period between moribund to death seemed like a very narrow range of time as at the end of the 48 hours as death occurred in 50% of individuals by the 1st day after treatment.

As with all ingested toxicants, sodium selenate must pass through the alimentary tract of the cockroach. To investigate any histopathological effects, alterations to the midgut and Malpighian tubules were examined based on evidence provided by past studies that selenium may accumulate in these structures and subsequently cause mortality (Hogan & Razniak 1991, Freeman et al. 2006). In addition, other toxicants such as heavy metals, synthetic insecticides, and IGRs are known to cause histological alternations to vital organs in various insect species (Zhang et al. 2001, Habes et al. 2006, Gutiérrez et al. 2016, Martínez et al. 2018, Fiaz et al. 2019). A commonly used inorganic bait toxicant, boric acid, has detrimental effects on the midgut of *B. germanica* as the epithelial tissues and cells of treated cockroaches are destroyed (Habes et al. 2006). The midgut holds a vital role for the digestion of food through the transport of nutrients, production of signaling molecules, and the protection from pathogens (Caccia et al. 2019). As a result, any alterations to the midgut tissues will most likely negatively alter the proper functioning of digestion and nutrient absorption.

The observations of alterations to the midgut were significant for chronically treated cockroaches. However, not all individuals examined exhibited histopathological signs in the midgut. Thus, it is unclear whether these effects on the organs are the main cause of death or if another primary or secondary mechanism is involved. Oxidative stress leading to the damage to the peritrophic membrane and other organs may be a

potential mode of action in *B. germanica* as hypothesized by Hladun et al. (2013a) for *Apis mellifera*. The minimal histopathological effect in acutely treated cockroaches suggests that sodium selenate may act differently when presented in a single high dose. Observations of toxicity symptoms while conducting experiments revealed some characteristics of a neurotoxic nature. As reported in Chapter III, it is evident that the highly resistant Ryan could not overcome the toxicity of sodium selenate. This suggests that sodium selenate has a different mode of action than that of other insecticides tested or that sodium selenate can somehow disarm these resistance mechanisms. A Se-tolerant strain of *Plutella xylostella*, accumulated methylselenocysteine whereas the Se-sensitive strain accumulated selenocysteine (Freeman et al. 2006). As a result, selenium was less incorporated into proteins in the Se-tolerant strain, protecting them from toxic effects. Since sodium selenate, an inorganic form, was used in this study, tolerance of selenium may be attributed to the transformation into a less toxic form as seen in *P. xylostella.* However, this mechanism may not fully protect *B. germanica* from sodium selenate toxicity as we have observed in Chapter III.

In conclusion, this study revealed some characteristics of sodium selenate that may be relevant to its use as a bait toxicant. Evidence that selenate toxicity can be mediated through the horizontal transfer of fresh residues was observed. Although sodium selenate was only used in a liquid bait, a gel bait formulation may have broader and more effective secondary transfer effects. Furthermore, the fast-acting nature of sodium selenate on locomotor behavior was quantified and illustrated. Histopathological effects were observed from both acute and chronic feeding of sodium selenate, although

changes were more drastic in the latter. Alterations to the gut contribute to mortality, but the presence of other mechanisms cannot be eliminated because acutely treated cockroaches exhibited minimal alterations. More organs may have been affected that were not within the scope of this study such as other parts of the alimentary tract. Overall, this study provides new insights on sodium selenate as a novel bait toxicant.

Chapter V: Conclusions

Insecticide resistance has been a major challenge in German cockroach management (Scharf & Gondhalekar 2021). The development of resistance to currently available insecticides has warranted the need for novel chemistry with new modes of action and management strategies. Based on an extensive literature review, selenium has been shown to have insecticidal and repellent properties in previous studies (Chapter II). However, there existed a lack of knowledge with pests considered to be of urban importance such as the German cockroach. Since cockroach baits remain the most widely used and reliable control technique, I decided to explore selenium's utility as an ingested bait toxicant, hoping to discover a previously unused active ingredient.

This study provided strong support and evidence for selenium toxicity in our test species *Blattella germanica.* In Chapter III, I conducted various laboratory bioassays and determined that the inorganic form sodium selenate was the most toxic form against the German cockroach. Furthermore, sodium selenate was also toxic to the three field-collected strains that have shown some resistance to several conventional insecticides. Based on survivorship, I found that sodium selenate was comparable to or outperformed some of the commercial gel baits included in the tests. This is particularly important if sodium selenate were to be used against resistant populations in the field. In addition, the lack of complete mortality of cockroaches when exposed to gel baits suggests that their use in the field may lead to control failure. The fact that sodium selenate killed 100% of two resistant strains (WM and RG386) within the observation period supports that

sodium selenate represents a different mode of action compared to those of the commercial baits tested.

In Chapter IV, I sought to shed some additional insight into other unknown characteristics of sodium selenate as a bait toxicant. Based on laboratory bioassays, I provided evidence that adult male cockroaches could deliver a lethal dose to early instars. This was most likely mediated through fresh residues from regurgitate. In addition, I was able to quantify the effects of selenium consumption on locomotor behavior. The fast-acting toxicity of sodium selenate most likely suggests that cockroaches foraging cockroaches would be exposed to a lethal dose and return to harborages. Then the cockroaches would exhibit signs of toxicity and would be unable to feed again and carry out regular interactions with conspecifics. Lastly, I investigated any histopathological effects on the alimentary tract of adult male cockroaches. I found that chronically exposed cockroaches had severe alterations while acutely exposed cockroaches had a minimal impact. These alterations may be the cause of death in test cockroaches; however, we could not eliminate other modes of action that we did not explore. This result, coupled with toxicity tests on resistant strains in Chapter III, we can hypothesize that sodium selenate represents a different mode of action than current bait toxicants on the market.

Although I have reported previously unknown areas about selenium on the German cockroach, there is still much more that can still be investigated. Future research would extend sodium selenate toxicity to females and nymphs to better understand population level effects. Furthermore, experiments may involve other sublethal effects on

developmental time, longevity, fecundity, and even the microbiome. In addition, I can explore the accumulation of selenium in different parts of the body and see if there are any differences between our susceptible and resistant strains. This may provide insight into the mechanism of selenium tolerance of resistant strains. In horizontal transfer, I can conduct studies to determine if selenium is excreted in feces and whether coprophagy could contribute to nymphal mortality. Future directions to further the understanding of selenium may provide additional support for its use as a bait toxicant.

Through this current study, I have provided evidence for the efficacy of a sodium selenate-based bait product. However, insecticides are highly regulated in the United States through various governing bodies, and a novel bait toxicant would have to undergo rigorous and extensive investigations. As a result, the approval of a future sodium selenate-based bait product is currently unknown and was not the scope of this research. I know that selenium has a narrow range between being beneficial and toxic to a potential consumer. Thus, a dose that reduces potential health risks but still effectively targets pests would have to be determined. Nonetheless, sodium selenate has exhibited potential to be used as a bait toxicant against the cosmopolitan urban pest species, *Blattella germanica*.

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