Management of two dermestid species and four other select stored product beetles in food storage facilities

by

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B.S., University of Nebraska - Lincoln, 1992
M.S., Kansas State University, 1996

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Entomology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2026

Abstract

Stored product pests in food processing facilities can be challenging with the varied environments in which they can occur, hide and reproduce. Cracks and crevices prove to be some of the more challenging areas to keep free of food debris. Studies in this dissertation focus on the adult and larval stages of three primary species: *Trogoderma variable*, *Trogoderma inclusum*, and *Tribolium castaneum*. One study explored the effects of a gel bait with the initial three species and three additional species which occur on the label which are *Tribolium confusum*, *Lasioderma serricorne*, and *Oryzaephilus surinamensis*.

A series of experiments were conducted testing various pest management controls including chlorfenapyr contact insecticide, pyrethrin aerosol treatment, several bait and pheromone traps, and fipronil gel bait. All are commercially available and labeled products for the uses and insects they were tested on. The various studies were designed to mimic real issues in food warehousing and processing facilities. While these can be very different environments, the stored product pests listed above could survive and thrive in a wide variety of places and food sources.

In the chlorfenapyr crevice arena study, chlorfenapyr PI and SC formulations had the lowest adult survival rate on *T. castaneum*, followed by *T. variabile*, these were both lab reared populations. *T. inclusum* adults had a higher survival rate overall. In general, the crevice treatment had the lowest survival of all treatments and all populations. When treating the surface only or having food in the crevice, had higher survival rates. Chlorfenapyr PI had lower survival rates in adults at 96-hours than chlorfenapyr at 96-hours post-treatment. Larvae of these four populations exposed to chlorfenapyr treated arenas had much higher survival to both formulations at 96-hours post exposure. *T. castaneum* still had the lowest survival to both

formulations, all three dermestid populations had 89.4% survival and higher for all treatments at 96-hours post survival. When larvae were held for an additional two weeks, the dermestid survival rates fell to 45% to 92% for the chlorfenapyr PI formulation and 28% to 100% for the chlorfenapyr SC formulation.

In the study with a barrier to food and partial arena chlorfenapyr PI and SC treatments, adults had lower survival when compared to larvae at 96-hours post exposure. In general, the chlorfenapyr PI formulation had less survival than the chlorfenapyr SC formulation. Due to the high number of surviving larvae, larval plates were held for one month post exposure. Even after one month the larval survival was above 90%. Food was available for the duration of this study.

The first experiment was conducted using an effective rate of chlorfenapyr SC. When comparing the effective rate to the label rate in larvae, there was no significant difference in any of the three dermestid populations or *T. castaneum*. Chlorfenapyr PI was significantly different in *T. castaneum* and *T. variabile* in the treated crevice arena.

Pyrethrin aerosol studies were conducted by holding the treatment at 0.5-hour, 1.0-hour, and 2.0-hour with either a fan or no fan moving the air inside the sheds. Arenas were placed on vertical shelves at three heights, uncovered, or five horizontal positions on the floor under boxes of 10-cm or 20-cm to imitate various warehouse scenarios. Eggs, larvae and pupae of *T. castaneum* and *T. inclusum* were placed on vertical shelves. There was no statistical difference in the time insects were held post-treatment. *T. castaneum* had higher larval and pupal survival rates than *T. inclusum* at 96-hours post-treatment. Insects on the lowest shelf had the lowest survival at both 96-hours post-treatment and three weeks post-treatment. No fan treatments had lower survival than treatments with fans. Of the five positions on the horizontal, only position 1 was in front of the boxes, uncovered. The other four positions were covered under boxes. In

general position 1 for both the fan and no fan treatments and both box heights had the lowest survival. At 96-hours post-treatment positions 2 through 5 had higher than 60% survival in the fan treatments. This differs from the no fan treatments where positions 2 and 3 typically had higher survival rates at 96-hours post-treatment than positions 4 and 5. In both the vertical and horizontal placements, the no fan treatments had lower survival.

Six bait and pheromone traps were tested against adult and larvae *T. variabile* and *T. inclusum*. *T. variabile* larvae were more attracted to the All Beetle food pellet bait station than the All Beetle pantry pest bait station whereas the *T. inclusum* larvae were mildly attracted to the All Beetle food pellet trap. Larvae of *T. inclusum* were negatively attracted to the four pheromone traps and larvae had nearly 0% attraction to any pheromone traps. Adult *T. variabile* were most attracted to the Trécé Dome with the Khapra beetle/warehouse beetle (KB/WB) lure whereas adult *T. inclusum* was most attracted to the X-Lure R.T.U. MST trap.

Fipronil gel bait is labeled for use against adult stored product pests. All six species tested had 4% or less survival after 24-hours exposure. *T. confusum* and *O. surinamensis* had the lowest adult survival after 24-hours, 20% and 5% respectively. By 96-hour post exposure, *T. castaneum*, *T. confusum*, and *O. surinamensis* adults all had 8% or less survival, with *T. confusum* having 0% survival.

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Acknowledgements

This work would not have been possible without the help and support of many, many people. To begin, my advisors, Dr. Jeff Whitworth and Dr. Frank Arthur. I appreciate all the support, extreme patience, prodding, guidance, and "get 'er done!" encouragement over the years of completing this work. Deanna Scheff, fellow PhD student turned lab supervisor, and Carlos Campabadal from the KSU Grain and Food Science Department for stepping in to round-out my 'PhD finishing' committee in the final months to complete my degree. Kun Yan Zhu, a very supportive committee member from the very beginning through the long finishing stages. Jim Stack who started on my committee and remained through my comp exams. Dr. James Campbell helped design the pheromone bait study and helped secure large growth chambers for studies. The following USDA Lab Technicians have helped with countless tasks from counting insects, experiment set-up, finding or building equipment, purchasing and so much more. I couldn't have done it without Brian Barnett, Rich Hammel, and Chris Hartzer. Kaitlyn Hilger, USDA undergraduate student, helped with a lot of concrete plate preparation, colony maintenance support, experimental set-up, and encouragement. Isaac Smith of the Kansas State University, Statical Consulting Laboratory, for his help with statistics, and code help on the bait station study. Jacqueline Maille and Mollie Toth, who provided writing support, statistical help, and 'brain breaks' for which I am extremely grateful. Cherie Copeland, for checking in and supporting from a distance. My colleagues at the Industrial Fumigant Company (IFC) and U.S. EPA Region 7, who allowed me to work on my degree while working full-time, always supporting my coursework and research needs. To the numerous friends and colleagues who encouraged me along the way, I can never say 'thank you' enough. In memoriam, Dr. Dennis Kopp and Gene White, fellow entomologists, who were very encouraging while they were here.

Dr. Kopp gave me the initial push into this adventure and encouraged me every step of the way through completing my comp exams. Most of all, to my husband, Jim, and children: Nelsa, Tyler and Bryce Beckman, who always allowed me the time and space required to get all this work completed, while tolerating my absence from school and family events.

Chapter 1 - An Introduction to Stored Product Pest Management

Stored product pest management in facilities

Stored product pests have long been an issue associated with food processing and storage facilities. Even archaeological site records document stored product pest fossils (Buckland, 1981). Within stored product insect populations, two types are of particular interest. Those insects which have economic importance because of the infestation of the product, and those which reside in residues in food facilities and equipment (Hagstrum and Flinn, 2014). Within facilities, many practices affect insect pest populations, including their distribution, and movements (Hagstrum and Flinn, 2014; Hagstrum and Athanassiou, 2019). Some of the factors affecting stored product pests include the amount of time products are stored, sanitation and management practices, temperature, and moisture (Kenkel et al., 1994) as well as the layout of the facility and other factors including building age, construction materials and type of construction, maintenance levels, facility relationship to outdoor areas and geographic location (Arthur et al., 2014, Williams et al., 2014). Seasonal changes can also affect pest pressures inside a facility (Arthur et al., 2014). Most of our current knowledge about stored product insect pest control for interior environments is based on studies conducted at feed or flour mills, or at food warehouses (Arthur, 2012). Through these studies in milling and processing facilities it was found that there are pest refuge areas where populations escape insecticide treatments and persist (Arthur, 2012; Campbell et al., 2010). These refuge areas consist of cracks, crevices, wall voids, and inside equipment or any other places food debris may accumulate that are hard to reach for cleaning purposes (Campbell et al., 2010).

Typically, pest concerns are directed at single components within a facility or area(s) where a pest problem occurs. Looking at a pest problem and the overall effect on other areas of a facility or processing system are not generally considered when discussing the single problem area (Kenkel et al., 1994). However, pest issues can enter the processing stream at any point from harvest and on-farm storage through processing and end-product storage to final export (Cuperus et al., 1990; Kenkel et al., 1994). Damage may include direct damage by insect feeding, increased humidity, excrement and larval feces (Vodjoudi et al., 2012). Insect damage can also lead to microbes on grain and in flour if left overtime.

Stored Product Pests

Agricultural pests take a toll on the world's food supply, from planted seeds to harvest and beyond (Williams, 1967). These insect pests come from the 0.1 percent of insects considered pest species, around 3,000 total which make up all pest categories including vectors of diseases for humans and animals (Williams, 1967).

Stored product pests have been an issue with commodities for centuries. Through the movement of commodities over time, the pests associated with them have moved as well on ships and cargo (Buckland 1981). Through this movement, most common stored product pests have spread worldwide. Tracing the source of a specific pest, especially in current international trade, is almost impossible to determine at which point along the travel route a commodity became infested.

Gräbner and Schmidt (1992) reported there are about fourteen stored product insects which occur in warehouse areas and more than thirty-three insect species of stored products associated with residences. Arthur et al. (2014) mentions four prominent species from pheromone traps at a United States Midwest warehouse facility which included *Tribolium*

castaneum (Herbst), red flour beetle, *Lasioderma serricorne* (Fabricius), cigarette beetle, *Oryzaephilus surinamensis* (Linnaeus), sawtoothed grain beetle, and *Trogoderma variabile* Ballion, warehouse beetle. Where the top six species were the same over a three-year period and several other species were found, but not specifically listed. This indicates that the same species are typically associated with stored product commodities within a given storage and processing area.

Dermestid Beetles

Dermestids are a family of beetles that are a significant economic pest of stored products worldwide. In some areas of the world, dermestids are a more serious pest due to the presence of the Khapra beetle (Hedges and Lacey, 1996). Prior to the mid-1950's, dermestids in stored grain were common, yet considered a minor issue not occurring or found in large numbers (White and McGregor, 1957). In 1954, *Trogoderma glabrum* (Herbst), the glabrous cabinet beetle, was found severely infesting wheat in Kansas (White and McGregor, 1957). This initiated the U.S. Department of Agriculture Stored-Product Insects Laboratory in Manhattan, KS to begin a more extensive study of the dermestid issue in grain (White and McGregor, 1957). Today, *Trogoderma granarium* (Everts), the khapra beetle, is the only dermestid of quarantine status in the United States (Arthur, 2012).

Beginning in the early 2010's dermestids came to the forefront as a stored product pest in grain storage and food processing facilities in the United States. Since then, it has remained one of the most requested identifications from many food processing facilities over the years since, especially the larval and cast skin stages (IFC Technical Services, personal experience). While there are many species of dermestids which can be pests in the food processing industry, four genera are most common including Anthrenus, Attagenus, Dermestes, and Trogoderma

(Hagstrum et al., 2012). A lot of focus in the food processing industry is on *Trogoderma variabile* Ballion, warehouse beetle, and *Trogoderma inclusum* Leconte, larger cabinet beetle, which are common in stored grain and food processing facilities. *T, variabile* and *T. inclusum* are classified as both primary and secondary pests since they feed on grain and are scavengers (Rees, 2004). Even though dermestids have a pest status on a wide variety of commodities, they are not overly well researched. A web of science search in May 2026 shows 1,052 publications on *Trogoderma*. If that is further broken down into *Trogoderma variabile* and *T. inclusum*, the number drops significantly to 167 and 85, respectively. Compared to other stored product pests such as *Tribolium*, these are very low numbers showing there is a need for further research on these common dermestid species.

Female *T. variabile* produce an average of 50 eggs per lifetime influenced by temperature and humidity (Davis, 1945; Smith and Whitman, 2007). However, a nutritive food source is not required for egg deposition. Loschiavo (1968) observed that *Trogoderma parabile* Beal (=*T. variabile*) oviposited the same number of eggs whether a nutritive food source was available or absent. Additionally, it was observed that males do not need to be continuously present for *T. parabile* to deposit a similar number of eggs compared to when males are continuously present. The trait was also observed for *T. inclusum* (=*T. versicolor*) (Norris, 1936). However, substrate with crevices is required for egg laying. Norris (1936) also found that neither food nor water are required for adult beetles to attain their maximum fecundity and longevity. When mating is delayed, *T. inclusum* typically deposits a larger number of eggs than *T. variabile,* resulting in larger numbers of individuals in the next generation for *T. inclusum* (Gerken and Campbell, 2017).

Early-stage *T. variabile* larvae initially begin feeding on grain dust, grain and food fragments, but later instars can attack and feed on whole grains (Rees, 2004). Larvae can molt from five to fifteen times, with an average of six molts, but molting more when conditions are not favorable (Rees, 2004; Mason 2018; Smith & Whitman, 2007). *Trogoderma variabile* larvae can grow up to 6.3 mm in length. Earlier instar larvae tend to be yellowish white in color and darken as they get older. Larvae and cast skins are two of the most common visible signs of an infestation. *Trogoderma variabile* larvae are known to enter diapause, which can make them hard to control and the parameters which control diapause are not known or understood (Wilches et al., 2016). There are several factors affecting diapause including temperature and number of larvae present in an area (Wright and Cartledge, 1994; Wilches et al., 2016). Wright and Cartledge (1994), looked at the effects of food volume and photoperiod on inducing diapause in *T. variabile*. When diapausing, larvae tend to hide in cracks and crevices which makes them harder to find, they can also live up to two years in a diapause state (Rees, 2004; Mason, 2018). Diapausing insects are more tolerant and harder to control due to their lower metabolic activity (Rees, 2004).

Trogoderma variabile larvae are covered in hairs, called setae, which are a known health concern (Hedges and Lacey, 1996). The barbed setae can cause stomach issues in sensitive populations such as infants and the elderly when ingested. For processing facilities that make products such as infant formula, there is an additional need for heightened awareness of pest pressure and pests present due to this risk. *Trogoderma variabile* were documented as the cause in a case of occupational respiratory disease in a pet food manufacturing facility worker which presented a negative reaction on a skin prick test in a published abstract (Berstein et al., 2009).

Pupation tends to occur nearer a food surface and lasts about four days (Mason, 2018). Prior to emergence from the pupal stage, adults tend to remain in the last skin for several days, mating soon after they exit. Adults are 3.2 mm in length, with an oval shape. Adult color is brown-black with variable yellow to yellowish-brown markings. Antennae are short with males having segments compactly joined in the club (Smith and Whitman, 2007).

Tribolium castaneum

Tribolium castaneum, is one of the most common stored product pests in the food processing industry. It is also one of the most researched with over 5,000 publications attributed to this species (7,909 publications on Web of Science search, May 2026). Campbell et al. (2022), described *T. castaneum* as a model insect which has affected many subject areas such as insect genetics, ecological, and behavioral studies, to list a few. Most pertinent to the studies in this dissertation are the contributions *T. castaneum* has made to pest management and monitoring procedures, insecticide efficacy and resistance mechanisms (Campbell et al., 2022). According to Smith and Whitman (1992), *T. castaneum* has origins in Indo-Australia. In the natural environment they can be found living under tree bark and in nests of animals feeding on decaying plant and animal refuse materials (Rees, 2004). When associated with stored products, *T. castaneum* are one of the most important stored product pests worldwide, considered a secondary pest, attacking a variety of processed cereal products and materials (Rees, 2004; Maier et al., 2006). Their general feeding damage is considered non-descript from other stored product species and their presence can cause off-odors in damaged products (Rees, 2004). The closely related *Tribolium confusum* Jacquelin du Val, confused flour beetle, is also associated with processed stored products and can occur in the same environment as *T. castaneum* competing for the same resources (Baldwin and Fasulo, 2003). Many times, when discussing the *Tribolium*

spp., both species are covered together since many attributes such as habitat, feeding behavior, food preferences, and life history attributes are similar (Rees, 2004; Baldwin and Fasulo, 2003; Mahroof and Hagstrum, 2012; Ryan et al., 1970).

Tribolium castaneum adults are long-lived compared to many other stored product pests. Female *T. castaneum* deposit eggs among food, singly, where they soon become coated with the food substrate due to their sticky surface (Rees, 2004). Ryan and Park (1970) showed that *Tribolium* spp. are carnivorous on eggs and pupae of both their own species as well as that of other *Tribolium* spp. Large larvae and adults may even be able to discriminate between species. Willis and Roth (1950) were able to demonstrate that *T. castaneum's* attraction to flour was, in part, due to the moisture content of the flour when beetles are starved and desiccated in arena studies. Beetles which were starved and kept in a dry environment moved toward whole wheat flour with the highest moisture contents. However, beetles which were not starved nor desiccated did not discriminate between flour types.

Tribolium castaneum adults are 3 to 4 mm in length, reddish-brown in color, and look very similar to *T. confusum*. The two species can be separated by morphological traits including the antennae, separation of the compound eyes on the underside of the thorax, and the ventral width of the compound eye (Smith & Whitman, 2007). *T. castaneum* have three enlarged antennal segments at the end of their antennae, whereas the antennae of *T. confusum* enlarge gradually from about half-way up the antennae. Female *T. castaneum* can produce as many as 300 to 500 eggs in or among food materials, by depositing up to 3 eggs per day over a span of two to three years. Eggs hatch in 5 to12 days depending on local conditions.

Larvae grow to a length of 4 to 5 mm. Older larvae have a hard body, which is wormlike in appearance. Larvae go through an average of 7 to 8 instars, but can have as many as 18. With

optimal conditions, a full life cycle from egg to egg can take approximately seven weeks. There can be as many as four to five generations in heated food processing or storage facilities (Smith & Whitman, 2007).

Tribolium castaneum populations are known to have insecticide resistance, in particular, malathion resistance (Vojoudi et al., 2012, Speirs et al., 1967). Speirs et al. (1967) published a paper on malathion resistance in *T. castaneum* showing the most resistant strain included in the study required 11.3 times the malathion rate at the LD50 level than a susceptible strain. Speirs (1967) also cited a study from as early as 1962 which showed a 52-fold level of resistance to malathion collected from stored peanuts in Nigeria. Speirs et al. (1967) showed that the level of resistance was directly correlated with the length of time malathion had been used in the facility from which the population was collected. This point is a strong argument for insecticide rotation and use of integrated pest management (IPM) in facilities. Halliday et al. (1988) tested 15 strains of red flour beetles for malathion resistance, of those, 13 strains showed malathion resistance, but they also found some resistance to dichlorvos and pirimiphos-methyl suggesting cross-resistance.

Similar to *T. variabile*, human health concerns exist with *Tribolium* species in that some, *T. confusum*, has shown to cause dermal allergic reactions, although is not known to spread disease (Alanko et al., 2000). Bell (2000) concluded that the *T. castaneum* has increased in pest importance throughout the 20th century even with the changes and advances in pest control practices.

IPM for Stored Product Pests

Integrated Pest Management (IPM) is the holistic, science based process that utilizes multiple tactics and tools for preventing, controlling, and managing stored product pests using

both preventative and curative methods (Karlsson Green et al., 2020). IPM utilizes physical, biological, cultural and chemical tools to minimize economic, health, and environmental risks to ensure the safe storage, processing, and distribution of stored products. Ehler (2006), says RJ Prokopy defines IPM as "…a decision-based process involving coordinated use of multiple tactics for optimizing the control of all classes of pests (insects, pathogens, weeds, vertebrates) in an ecologically and economically sound manner". Stored product IPM should provide pest management at the lowest possible cost while also protecting product quality at the highest levels in the food processing and milling industries (Hagstrum and Flinn, 2014).

An IPM program consists of a complex set of steps including inspections, monitoring, documentation, and review of an entire facility to focus on preventing pests, early detection and elimination, as needed, of pest problems (Hagstrum et al., 2012). Each step in an IPM program requires management, oversight and follow-through for a program to be effective. When defining IPM, references are sometimes made to an IPM pyramid (Karlsson Green et al., 2020) or IPM Wheel (Cornell University, 2015) comprising of steps to take beginning with pest identification and life cycle at the bottom of the pyramid and moving to the top where chemical control options are used as the smallest and last part of the pyramid or pest control steps. If using the pyramid schematic, each step of the pest control process would have its own set of tools to use to achieve the pest management within each step. Having a team to implement, and follow-through is the best approach.

IPM should also incorporate an economical analysis to aid in treatment decisions. First, an acceptable pest threshold must be established. Second, an economic threshold and economic injury level are determined, which would allow for some level of the pest to exist. Third, determine the point at which not doing a treatment would cost more compared to conducting a

treatment (Ehler, 2006, Campbell et al., 2012). However, there is no hard and fast rule to determine these components since every facility, commodity, location, etc. are unique and will not employ IPM programs in the same manner. In some instances, the tolerance level for a pest is 'zero', one example would be for flies in a commercial food processing facility which can carry and spread multiple human health diseases.

The food processing environment also requires a lower threshold of pests, compared to bulk storage, to avoid insects in final food products. If IPM is not managed properly, it can become integrated pesticide management managing the "discriminate" use of pesticides to quickly deal with pest issues (Ehler, 2006).

The general population is demanding a clean and safe food supply with fewer chemicals and pesticides used on food commodities. IPM programs are an opportunity to meet these consumer demands in the postharvest supply chain. This creates a balance between protecting food with having no chemicals or contaminants present in food (Athanassiou, 2012). There are several control and management tools available that consist of both non-chemical and chemical control options. However, determining the appropriate control method depends on the commodity, facility type, locations, and pest species to mitigate the infestation, prevent further infestations, and improve IPM effectiveness (Hagstrum and Athanassiou, 2019). Anukiruthika and Jayas (2025) describe IPM as "mandatory" for stored products to avoid grain losses.

In the food processing environment, there are multiple non-chemical and chemical control options available. Non-chemical options include sanitation, physical barriers, aeration, biological agents, trapping, and temperature control. Chemical control options include surface treatments, insect growth regulators, aerosols, insecticide incorporated packaging, and

fumigation. However, it is important to understand that no single method, chemical or non-chemical, will control and eliminate all pests and prevent their reinfestation (Kenkel et al., 1994).

Non-chemical control

There are several non-chemical control options for pest management of stored product pests. Of these methods, several are usually used in tandem with chemical control methods to enhance chemical performance (Williams et al., 2015). For organic food production, non-chemical controls in an integrated pest management system have been gaining in importance (Schöller, 2010).

Sanitation

One of the most basic and most important non-chemical controls is sanitation. Sanitation, keeping clutter and food debris out of areas, makes inspections easier and removes food particles and hiding places which supports harborages, feeding and egg laying for development of future pest generations. Toews et al. (2005) showed that *T. castaneum* trap catches at warehouses without food patches (where food was eliminated) more closely correlated with actual pest density, demonstrating that sanitation is an important component of pest monitoring. This was in contrast to warehouses with food patches where catches were lower, possibly due to the insects staying and aggregating in the food patches which may be more attractive than traps in that scenario. Sanitation needs to be done at large scale levels to clean up spills and larger messes. At the fine-detail levels of sanitation, deep cleaning of small particles in machines, cracks and crevices, and other hiding places need to be eliminated to prevent pests from being established in hard to find and reach places. Fine detail cleaning may involve breaking down machines to reach deep inside, power washing crevices in floors, or even taking wall panels down to get behind areas where food debris may be blown or washed up underneath

panels. The level of sanitation, while not the only factor influencing pest management, can affect the success of pest management (Williams et al., 2014, Toews et al., 2005, Said and Pradham, 2014). According to Said and Pradham (2014) sanitation can be 80 percent of a successful IPM program and can be the most cost-effective.

Exclusion

Next to sanitation exclusion is probably most important. Exclusion involves preventing pests from entering areas where commodities are stored or processed. In many cases this involves sealing all entryways into the structure such as doors and windows. Screen size to exclude pests is critical, and care must be taken to make sure air flow is not restricted when covering fans and louvers placing strain on mechanical components which could cause malfunction. Door seals may prevent entry into a facility or prevent movement between rooms. Cline and Highland (1981) tested twenty stored product adult pest beetles by allowing them to move through a series of sieves to determine the minimum sized holes that can be utilized for each of the species and sex ratio. Cline and Highland (1981) determined that the size variation in some species is larger than for other species with the range from 0.33mm to 2.25mm. Some beetles such as *Dermestes maculatus* De Geer, hide beetle, and *Tenebrio molitor* Linnaeus, the yellow mealworm, were too large to fit through the largest sieve size of 2.25mm. *Trogoderma inclusum*, *T. glabrum*, *Sitophilus oryzae,* Linnaeus, rice weevil, and *S. granarius* Linnaeus, granary weevil, were the most varied within a species. Whereas *T. castaneum* and *T. confusum* were some of the least varied species. With some beetles being able to breach the smallest size of 0.33 mm, exclusion needs to address very small, sometimes hard to find openings. Addressing exclusion also involves checking and sealing around any places where insects might enter a facility such as around windows, louvers, air intakes, screened areas, and others.

Exclusion also involves basic maintenance of structures to be maintained at the highest levels to prevent entry of all pests from insects to rodents. When sealing cracks and crevices, the proper sealing agent must be used for the type of construction materials, weather conditions, environmental elements, and situation it is being used for. Depending on the site and the environment, sealants may need to be food grade, or expandable to accommodate the site they are being used in without being compromised. Due to the environment, some sealants may last many years while others may need to be replaced or repaired more frequently to remain effective.

Monitoring

Monitoring and trapping devices, while indicating presence and numbers of pests, remove individuals from the local population, while also helping with monitoring and detecting species present and changes in species and/or numbers over time that are difficult to document with visual inspections (Toews and Nansen, 2012; Phillips et al.,2000). These devices should not be used as stand alone control devices, however, research continues to explore pheromones for control purposes (Phillips et al.,2000). Several different types of attractants are used depending on the device of interest. Insect trap performance can be affected by many factors including residual food, air movement, temperature, trap placement, and species of insects (Hagstrum and Flinn, 2012). Pest managers in a facility need to understand how each type of trap is used and how to operate traps within the confines of the physical parameters of a facility.

Pheromone traps can be hanging or floor models to detect either flying or crawling insects. In many food processing facilities pheromone traps are laid out in a near grid pattern. Per research, this grid trapping across entire facilities is recommended (Toews et al., 2005). These traps utilize chemicals, usually pheromones, which act as signals to other individuals of

the same species to either attract or repel individuals (Anukiruthika and Jayas, 2025). Pheromones include chemicals produced by insects that are classified as sex pheromones or aggregation pheromones to attract individuals are the most commonly used in their synthetic form to lure insects to trap devices (Phillips et al., 2000). The first pheromone discovered for a stored product pest was identified as early as 1966 for *Attagenus unicolor* (Brahm), black carpet beetle (Phillips et al., 2000). Food attractant lures and unbaited sticky traps may also be used as monitoring traps for stored product pests (Phillips et al., 2000). During the 1990's, use of pheromone traps increased greatly as an IPM tool in the food and baking industries (Phillips et al., 2000). This adoption and use of pheromone traps has continued to the present with pheromone trap research continuing to update and advance improvements in trap design. One example would be the Trécé Broad Spectrum DOME™ trap which originally had a one-piece basin bottom, and a later modified version Broad Spectrum QUICK CHANGE DOME™ with removable tray bottom preloaded with the pheromone and food oil for easier change-out and no cleaning required, both versions are still commercially available. Another improved trap would be the Insects Limited All Beetle Trap with Gel Attractant food source which originally came in a tube to squeeze into the bottom of the traps. Newer versions of the All Beetle Trap can be purchased with an RTU Gel Cartridge that is pre-loaded with the gel evenly spread on the bottom plate of the trap. Some improvements are for ease of application, while others are improvements in the pheromones themselves, the delivery systems, or the development of new synthetic pheromones so new species can be monitored by these trapping systems. For example, in 2023, the Industrial Fumigant Company (IFC) tested a new *L. serricorne* pheromone by Trécé in a warehouse field setting while Trécé worked on getting the product registered, which was a

complex task due to the number of compounds in synthetic lure to make it efficient. (Sharon Dobesh, personal communication with Trécé, 2023).

Toews et al. (2005) found that pheromone traps placed away from walls, but along objects such as shelving, may not capture as many insects unless a food source or food patch is present. Corners and hard-to-reach areas tend to capture more insects. Toews (2005) research suggests in stored product warehouses, concentrating monitors where insects most likely hang out such as corners, along walls, and along food storage areas, results in better trap data showing a more accurate picture of where beetle populations are occurring. This is consistent with the data found by Campbell and Hagstrum (2002) showing more beetles in corners than along walls.

Insect Light Traps

Insect light traps (ILTs) first appeared in 1913 and later improved in the 1920's beginning with the electrocutor type designs (Harris, 2006). ILTs were first used for insect trapping and monitoring around 1935 for agricultural use and have since been introduced into pest control in several industries for both indoor and outdoor use (Sliney et al., 2016). Later, in the 1950's, fluorescent lamps were used regularly as an insect attractant, and are still used today (Harris, 2006). There are many light trap designs which can be used in many scenarios. First, light traps usually use either electrocution or glue boards for trapping or killing insects. In addition to how they work they can be wall mounted, hung from ceilings, or portable and free-standing (Harris, 2006). Newer models today can have many of these designs for mounting in one unit for versatility. Light traps can be used in creative ways above drop ceilings and in other tight spaces and are used for trapping flying insects. Ultraviolet bulbs have been the standard for many years to attract insects with wavelengths of 315 to 400 nm (Toews and Nansen, 2012; Sliney et al., 2016). These bulbs should be changed every 6 to 12 months to maintain their

efficiency (Toews and Nansen, 2012). More recently, LED (light-emitting diode) bulb feasibility has been increasingly researched and used (Park and Lee, 2017; Miyatake et al., 2016). Energy efficiency and changing light strips less frequently are two aspects of LED light traps making them more popular more recently. While observing LED color based phototactic behaviors, Park and Lee (2012) observed that red and blue LEDs were more attractive to several stored product pest species including that red was more attractive to *T. castaneum*, but green was more attractive to *Plodia interpunctella* (Hübner), Indian meal moth. This research shows that more work needs to be done on the attractiveness of individual species to LED lights to make this emerging technology work optimally in field settings as use of LEDs becomes more popular.

In the last few years, LED units with cameras have been introduced into the pest control markets. While still in the early stages, the goal is to have artificial intelligence (AI) capabilities to not only report numbers of insects on glue boards, when they were caught, and types of insects, but to eventually be able to do insect identifications of major pests including large versus small flies and different groups of stored product pest beetles. These activities are still in the early stages of AI learning, and are continuing to progress, be more interactive, and more accurate over time. Currently more advanced camera ILT models have an interface with an online portal where users can watch glue boards in real time and see numbers on a dashboard for daily or weekly activities. Cameras can be programmed for timing photo captures of glue boards, which can be from hourly to daily.

Environmental Controls

Environmental controls involve altering the environment where pests occur to make it less desirable for them, or unable to reproduce, develop, or even kill pests. These tactics involve altering the temperature or using different lighting types or wavelengths that are not attractive to

pests. Some developed countries have also applied additional storage techniques as part of their IPM programs to protect grains. Other countries, including India, are beginning to use these techniques (Said and Pradham, 2014). The techniques include refrigerated storage, aeration, modified atmospheres, and hermetic storage. While not used as regularly as traditional methods such as exclusion, pheromone monitoring, and chemical controls, these methods offer additional opportunities when facilities have the equipment and space for these control methods.

Biological Control

Biological control agents for stored product pests occur naturally and are available but not typically used in stored products or processing facilities in the United States. One reason for this is that having dead insects or insect parts found in food has restrictions and can be considered contamination or adulteration of food products. The United States Food and Drug Administration publishes the Food Defect Levels Handbook (2018) in which pests, including insects, pest parts or pest evidence may be defined as contamination, or any foreign matter found in food due to the presence of pests. However, since pests, including insects, are present at all stages of food production from field to finished product, it is nearly impossible to have a 'zero' amount of insect parts or 'debris' in food requiring set limits. For example, the allowable rate of 'insect filth' in wheat flour is currently "Average of 75 or more insect fragments per 50 grams" and must not exceed these levels. However, Schöller (2010) points out that biological control has been developing in Europe for stored product pests with traditional pest management companies using both parasites and predators to control insects. Schöller (2010) points to several publications on biological control for several stored product pests including *P. interpunctella* and *Tribolium* spp. In instances where biological control has been used for over 10 years continuously, pheromone traps were still used to help document populations over time.

Following a five-year study in Europe on biological control, Hansen (2007) identified three situations where biological control is most likely to succeed. These include empty room treatment, preventative treatment in bulk commodities, and preventative applications of egg parasitoids to protect packaged products from moth infestations. Empty room treatments are not always practical in food processing facilities where shifts run 24/7 in some cases. Schöller (2010), also points out that development of effective guidelines for releasing natural enemies has not been fully explored in terms of looking at numbers of biological control agents to be released and timing for optimal effectiveness. These areas need more study on many biological control systems.

Heat Treatment

Heat treatment is a control method which has been around for over 100 years (Dean, 1911; Mahroof et al., 2005; Campolo et al., 2013). With the loss of methyl bromide as a fumigant, heat treatments were one of the more popular alternative considerations (Phillips and Throne, 2010; Hulasare et al., 2010). This method involves raising the temperature of a structure or warehouse to a minimum of 50° C to 60°C and maintaining that temperature range for 24 to 36 hours for an entire facility to kill stored product insects (Mahroof et al., 2005; Hulasare et al., 2010). The length of time a heat treatment lasts depends on the size and type of facility being treated, as well as the species of insect being treated. The time-mortality responses have been explored for *T. castaneum* and *T. confusum*, in general, there is a linear response with the longer and hotter a heat treatment is, the more control is obtained (Boina and Subramanyam, 2004; Mahroof et al., 2005).

Treatments can be conducted in an entire building or smaller areas which can be properly sealed off to retain heat. It can also be a good tool in places where insecticide resistance may be

an issue. However, different species and life stages have differing susceptibilities ranging from about 54% to 100% mortality (Campolo et al., 2013). Boina and Subramanyam (2004) showed that eggs and young larvae were the most susceptible stages to heat treatment in *T. confusum*, compared to older larvae and adults. Due to these results, Boina and Subramanyam (2004) suggest using older larvae to measure the effectiveness of a heat treatment. Mahroof et al. (2005) also showed that *T. castaneum* exposed to heat at 50°C as pupae and young adults caused decreased fecundity and progeny production in individuals exposed to a sublethal dose. The effectiveness of a heat treatment can differ based on location in a facility and can be enhanced by use of other methods such as sanitation and exclusion (Boina and Subramanyam, 2004; Hulasare, et al., 2010; Campolo et al., 2013; Semeao et al., 2013). While insect movement can be a concern that they will move away from a heated area to survive, Semeao et al. (2013) showed by a beetle mark and recapture program that while there is movement of insects, there is not an increased rate of movement of beetles to other floors of a flour mill to escape a heat treatment. Another concern with heat treatments is damage to heat sensitive equipment and age of buildings, in that older structures could be damaged or affected by the higher temperature exposure (Phillips and Throne, 2010).

Ionizing Radiation

Ionizing radiation is a method used for several reasons including sterile insect technique, phytosanitary irradiation for shipping and export purposes, to facilitate augmentative biological control rearing of predators and parasites, and to suppress pest populations in stored products (Hallman, 2013). Currently, the largest use of this technology is in the spice industry for the purpose of controlling microorganisms which cause disease. However, insects which are present during treatment can be killed as well. While several countries approve the use of ionizing

radiation, fourteen countries allow use of this technology for stored products (Hallman, 2013). In the United States, irradiation is cleared for use on wheat and wheat flour. One downside of irradiation is the possibility of adversely affecting quality from either high doses or excessive doses applied to stored products (Hallman, 2013). Another issue applying irradiation is when applying it to streams of grain. Higher energies are required for thicker grain streams, yet grain closer to the source of the irradiation receives higher doses than grains further from the source resulting in uneven treatment. Lower doses are required to prevent adult insect reproduction of stored product pests with ranges from 0.05 kGy to 0.45 kGy across the 37 species in 4 orders listed in Hallman (2013).

There are other types of detection. Neethirajan et al. (2007) reviews many detection techniques. Some techniques such as near-infrared reflectance (NIR) spectroscopy, acoustical methods, electrical conductance, machine vision, and X-ray imaging require specialized equipment. Each of these techniques has sensitivities or hurdles to making them easy to implement. An example would be that the machine vision technique is not able to detect infestations of internal feeders. NIR spectroscopy instruments require regular maintenance and calibration which can drive the cost of the detection methods higher. Continued research may help new technologies develop or be created.

Chemical control

In the world of IPM, chemical control is viewed as the last tool which should be utilized when other strategies have not been sufficient to control the pests of concern. The exception is when fumigants are used in quarantine situations to ensure no foreign pests are entering an area where they do not already exist. In those cases, items or grain products are fumigated regardless of whether pests are found or not. These are cases of zero tolerance.

Chemical controls vary in toxicity and consist of both natural and synthetic products. In the cases of insect growth regulators, the products are synthetic, and mimic natural insect processes. Williams (1967) discusses the various pros and cons of the three generations of pesticides, focusing on third-generation pesticides, insect growth regulators, but showing how the previous two generations had faults of being too broad affecting all insects, pests or otherwise, and the environment in unintended ways including pests developing insecticide resistance.

Insecticides differ in efficacy against different insect species. Formulation type is one important factor in affecting insecticide residual efficacies. Studies by Arthur (1994) showed wettable powder (WP) formulations had more residual efficacy than emulsifiable concentrates (ECs). A second factor affecting efficacy depends on the surface-type it has been applied to (Vojoudi et al., 2012). Surfaces that result in the highest efficacies are glass, then ceramic tile. More porous surfaces followed in efficacy levels with a paper disc surface being the least efficacious of the surface-types tested. In many food processing facilities, ceramic tile, concrete, and stainless steel would be the most common surfaces treated (Vojoidi et al., 2012; Arthur, 2012). Efficacy could be increased by sealing concrete to reduce insecticide absorption into the pores (Arthur, 1994). Unfortunately, newer research on insecticide absorption on surfaces by newer classes of insecticides such as neonicitinoids or pyrolles has not been conducted (Arthur, 2012).

A third factor affecting contact insecticide efficacy is the presence or absence of food materials (Arthur, 2013). Although there is an increased rate of survival when a food substrate is present in lab studies by Arthur (2013), it remains unclear whether survival is due to increased health and nutrition caused by the presence of food or if the food source being present following

pesticide exposed insects being added to the Petri dishes pulls pesticide residues off the insects now allowing them to affect the insects.

The concept of IPM in food processing facilities tries to keep chemical control as a last resort in practice. When fumigation is unavailable, a combination of residual treatments and aerosols can be important to include in pest management plans. This is especially important in states where whole facility fumigations may be limited to one or two per year (New Jersey, Department of Environmental Protection, 2023). A few states have more recently started using air quality rules to restrict the number of fumigations per year in a facility, limiting pest control options if pest populations explode unexpectedly. Planning for these events becomes more critical and all options need to be considered.

Insect Growth Regulators

Insect growth regulators (IGRs), sometimes referred to as third generation pesticides (Williams, 1967; Oberlander and Silhacek, 2000), are synthetic mimics of the insect's natural hormones found in the molting process. The most common are juvenile hormone analogues, chitin inhibitors, or ecdysone agonists. Juvenile hormone analogues affect larval development preventing advancement to the adult stage, maintaining insects in the larval stage, this however can lead to increased larval feeding and damage. The overall effect is the prevention of future generations of insects and increasing populations (Oberlander and Silhacek, 2000; Mondal and Parween, 2000). Chitin synthesis inhibitors affect insect's ability to create chitin which is a primary component of the insect exoskeleton which is produced in the epidermis (Oberlander and Silhacek, 2000; Mondal and Parween, 2000). Ecdysone agonists act differently from the other two groups of IGRs in that they disrupt the molting process causing death (Oberlander and Silhacek, 2000; Mondal and Parween, 2000). Ecdysone agonists can also cause disruption in

feeding, which, when applied with a juvenile hormone such as methoprene, can cause an early death to larvae which cannot mature to the next stage (Oberlander and Silhacek, 2000).

Insect growth regulators do not cause immediate death or quick knock-down of insect populations and can be used in combination with conventional pesticides (Mondal and Parween, 2000). In practice, this is usually an adulticide to control adult populations, while also keeping the next generation from maturing to reproductive adults. Arthur et al. (2014; 2019) found that using an IGR in combination with synergized pyrethrins contributes to residual aspect controlling immature stages of stored product insects after the pyrethrins are no longer active. Specificity to insects also makes IGRs a good choice for low toxicity insecticides that will not affect non-target species (Oberlander and Silhacek, 2000).

Four IGRs are commonly used today for control of stored product insects in the commercial setting. According to labels of products currently used, these are methoprene, pyriproxyfen, hydroprene and novaluron. Newer formulations also include multiple IGRs in one easy to mix or ready-to-use (RTU) product. In general, IGRs, due to their targeted selectivity, are considered to be safer as grain protectants and for the environment (Mondal and Parween, 2000). Methoprene was one of the first introduced into pest control in 1973 and is the most closely related to natural juvenile hormone (Oberlander and Silhacek, 2000). Hydroprene is the most volatile of the IGRs resulting in less residual control than its relatives, methoprene and pyriproxyfen (Arthur, 2012).

Mondal and Parween (2000) pointed out the many advantages and disadvantages of IGRs, where the advantages greatly outweighed the disadvantages citing the many studies showing these trends. A few noteworthy advantages mentioned include that IGRs do not affect parasites and predators, slow down population build-up in enclosed environments, generally do

not cause insecticide resistance and may help control insecticide resistant pests, do not affect seed germination, are affective in small amounts, and can be easily formulated. Disadvantages included prolonging the larval stage. Life stage timing is crucial, and that a few insecticide resistant insects may show cross-resistance to IGRs (mainly houseflies and mosquitoes with questions about *T. castaneum*) Mondal and Parween (2000).

Essential Oils

A lesser used control is essential oils, a natural plant chemical derived control method. While studies exist to look at their use, either due to lack of commercially available products or underdeveloped usable technology, these are not widely used in industry but are continually evaluated. Some essential oils have shown properties against *T. castaneum* including contact toxicity, repellency, having killing effects against eggs, larvae, and pupae, and being an antifeedant (Upadhyay e. al., 2018). One issue with essential oils is their complex chemical composition which can be different depending on the geographic area it is sourced from. Harvesting, using proper plant part, and extraction methods can also alter the chemical composition (Upadhyay et al., 2018). To be used successfully in commercial formulations, standardization and consistency are required to ensure the stabilization of toxicity. If not for the difficulty in overcoming the commercial obstacles, essential oils are of interest for use in pest control as they are generally considered to be readily available, and non-toxic to humans (Campolo et al., 2018). Interest in this area continues to be examined.

Aerosols (Fogging)

Aerosol treatments, also known as fogging, are one type of treatment used for whole-facility pest management. Insecticides are poured into a mechanical device to be atomized and dispersed as fog or mist. Insecticides can also come in cylinders to be dispensed directly without

mixing. Particle sizes for this type of application usually range from 5 to 50 µm (Peckman and Arthur, 2005). These devices can be hand-held mobile units, stationary stand units, or permanent wall mounted units (Toews et al., 2010). Asuncion et al. (2020) tested the different application devices and found that hand-held sprayers generate larger droplet sizes and a wider droplet distribution compared to compressed gas or cylinderized product devices which generate significantly smaller droplet sizes with better coverage.

Aerosol applications can be very confusing to many people for several reasons. First, because it is one of a couple types of space treatments which would also include fumigations (Peckman and Arthur, 2006). Next reason is due to the language used to define aerosol application which include terms such as aerosols, foggings, ultra low dose/ultra low volume (ULD/ULV), mists and more (Peckman and Arthur, 2006). In the food processing industry, most call these applications aerosols or foggings even though they are still sometimes confused with fumigations (Peckman and Arthur, 2006). Aerosol/fogging materials are almost always the same chemicals that can be used in general contact applications. The most popular chemicals in use today for aerosols are dichlorvos, IGRs, pyrethrins and pyrethroid materials. Some pyrethrins are used in combination with piperonyl butoxide (PBO) to enhance efficacy (Sutton et al., 2011).

Many of the terms used to discuss aerosol applications are different due to the equipment used to make the applications. Different equipment delivers a different range of particle sizes which is important for dispersing the chemical throughout the space to be treated (Peckman and Arthur, 2006). Many studies have looked at the importance of particle size and dispersal in food plants and food mills to try to determine best delivery factors efficacy of chemicals (Subramanyam et al., 2014; Sutton et al., 2011; Arthur et al., 2019). Equipment used to mechanically deliver chemical in particle sizes in the 5-50 µm size are defined as an aerosol or

fog. However, particles in the 5-15 μm range, a median particle size, has performed best for treating food processing areas, which allows for longer 'hang time' and allows for more opportunity of exposed insects to come into contact with the active ingredient (Bennett et al., 1997; Peckman and Arthur. 2006; Toews et al., 2010).

Fumigants

Fumigation is a pesticide treatment which consists of filling a building, structure, or other sealed off area (such as a tarped off grain pile) with the application of a toxic gas, to control pests (El-Aziz, 2011; Derrick et al., 1990). Some structures may also be filled with stored products. Sealed structures can take many forms including warehouses, silos, railcars, barges and ships, portable enclosures or even piles of grain under gas-proof plastic sheets (Bell, 2000; Phillips et al., 2012). Fumigation has a long history with stored product pests which includes early attempts of using plant materials with fumigant action along a wood ash and dry soil mixture to cause dehydration (El-Aziz, 2011). One attribute of using a fumigant is that they do not leave residues (Derrick et al., 1990).

There are three main gases used in the United States (Phillips et al., 2012). Most commonly used are phosphine and sulfluryl fluoride. The third, methyl bromide, was phased out of common use in developed countries by 2005 since it was listed as an ozone depleter in 1992, according to the Monreal Protocol, yet still maintains some uses for quarantine work (Bell, 2000; Fields and White, 2002; Arthur, 2012). Applicators must have a written fumigation management plan in place prior to conducting a fumigation. This plan is a detailed document required by the United States Environmental Protection Agency (U.S. EPA) to help applicators comply with the fumigant label and provide a safe and effective fumigation job. Detailed information including emergency numbers, facility contacts, and specific information about the site and fumigation job

to be performed must be included. This document needs to be kept on file with the fumigation company for reference and future use (Phillips et al., 2012). Facility personnel, such as supervisors, should help create and review or update the fumigation management plan plus the pest management provider for accuracy before each fumigation.

Fumigation can be effective at killing all insect life stages. Fumigant effectiveness against each species and life stage of each species differs. Some insect life stages, such as the egg and pupal stages, have lower metabolic levels or may be more protected, thus require longer exposure times (Derrick et al., 1990). To achieve efficacy, the Law of Habor needs to be observed, which is the level of toxicity of the fumigant, exposed to the pest at the appropriate gas level (concentration) and is proportionate to the length of time exposed in order to ensure control of the target pest population (Cotton et al., 1944; Derrick et al., 1990). Humidity levels and temperature can also affect the efficacy of fumigants (Derrick et al., 1990).

Prior to 2000, phosphine misuse was occurring through short exposures at higher temperatures and poorly sealed structures which led to resistance development in many insects (Bell, 2000; Rajendran and Sriranjini, 2007; Said and Pradham, 2014). Phosphine is available in several forms such as pellets, tablets, ropes, and gas cylinders to accommodate various situations. Because of its flexibility phosphine was used for years in on-farm storage against insect pests.

Sulfuryl fluoride (SF) is an alternate fumigant. It fills a gap created by other fumigants in that it works well when used against phosphine resistant insects. Initially SF was used as a fumigant for structures, initially developed for control of drywood termites in the 1950s (Derrick et al., 1990).

One complicating factor when fumigating stored products is sorption. Sorption occurs when the product being fumigated absorbs some of the gas meant to kill insects making it unavailable to the pests, also leading to longer term off-gassing following the fumigation due to the slower release of the gas from the products or packaging, which may not be able to be accurately measured and accounted for. Cotton et al. (1944) showed the effects that sorption can have on *T. confusum* when individuals removed immediately from flour bags after a short 30-minute fumigation with hydrocyanic acid were still alive, yet other individuals left in bags of fumigated flour for 48-hours were dead at an 83 percent rate. Cotton et al. (1944) showed that in all cases the 48-hour kill rates were much higher than those immediately following the conclusion of the fumigation.

Detection equipment is required for all fumigations. There are many types of detection equipment and the equipment used should be approved for the fumigant it is used to detect. Detection equipment can be used to detect gas leaks from the sealed fumigation chamber or structure. Detection equipment that has the capability to measure low concentrations of fumigant are required for clearing a fumigation before turning a facility or commodities back for reoccupation (Phillips et al., 2012). Personal low concentration detectors known as a 'badge' can also be worn by applicators and workers in a fumigation to let them know what levels they have been exposed to during a fumigation (Phillips et al., 2012).

Introductions for Current Studies

Of all the research on stored product insects, one area which continues to be a major issue for pest management is managing crack-and-crevice areas which harbor pests and contribute to pest control failures. In particular, *T. variabile*, have become a more prominent pest from the later 2000's to the present. Most of the pesticide tolerance (bioassay) research literature was

conducted prior to the 1990's on older chemistries, many of which are no longer used in commercial food processing facilities. Reasons these products are no longer used are that stored product insects may now show insecticide resistance, or uses against stored product pests were discontinued/cancelled as in the case of many organophosphates, such as malathion and chlorpyrifos, and discontinued uses on and around food products and food work areas.

Experiment 1: Bioassay on Crevice Arenas

Arthur (2013) showed that chlorfenapyr is highly effective against *T. castaneum*, with a less than label rate, residual dose being effective. No comparative studies have been conducted on *T. variabile* or *T. inclusum* with chlorfenapyr. Most studies on these dermestids have focused on life history, biology, distribution, and dispersal (Campbell and Mullen, 2004). Arthur (2012) indicates that there is a need for this type of residual evaluation on *Trogoderma* species, especially with their status as an emerging pest and few studies comparing dermestids to flour beetles (Arthur, 2012). Campbell and Mullen (2004) showed that dermestids are regularly captured in trapping studies. Since stored product pests also reside in the same environments as many urban pests, data on susceptibility may also be used for future label reviews (Arthur, 2008).

Crevices are a major sanitation issue in the food processing and warehousing industry (FDA.gov, 2004). Crevices can be utilized by insect pests as harborages and frequently have debris which naturally falls into them or accumulates when regular sanitation washes debris into them (FDA.gov, 2004). Crevices can consist of several types of surfaces or surfaces coming together. The depth and width of crevices can vary widely. Deep cleaning crevices is sometimes overlooked and usually requires extra time and attention to details. Newer sanitation products, such as biocleaners, which come in many formulations, have been a welcomed sanitation tool.

Trematerra and Fleuara-Lessard (2015) state that filling of crevices should be systematically conducted including crevices and cracks in walls and floors, edges and column-floor junctions. No previous studies were found exploring the affects on insects in a crevice scenario with only concrete surfaces (Arthur, 2008).

Our goal for this study was to evaluate four insect populations against four treatment arrangements using two formulations of chlorfenapyr, testing for residual effectiveness on a concrete surface with crevice. The concrete surface was chosen since it is one of the main floor types used in processing facilities (field observations). Arthur (2008) noted that not many studies exist using newer insecticides, such as chlorfenapyr, for stored product insects. Arthur's (2008) study was the first to establish a chlorfenapyr baseline, which is less than label rate, in the SC formulation for *T. castaneum* and *T. confusum*, since they are worldwide common pests. Chlorfenapyr needs to be further evaluated so that pest management and facility operators can make informed decisions regarding newer insecticides and their efficacy on a variety of stored product pests. Our study, using *T. castaneum* also compares *T. variabile* and *T. inclusum* to provide baseline studies for those species to chlorfenapyr in both the SC and the PI formulations, noting that the SC formulation is labeled for use in food production facilities, although the PI formulation is only labeled for non-food areas in food processing facilities.

Experiment 2: Barrier to Food

Arthur and Fontenot (2012) explored food source provisioning with barrier in arenas to test if food availability would affect the efficacy to *T. castaneum*. No information exists presenting data relative to this concept with *T. variabile* and *T. inclusum*. The goal of this study was to test if having a barrier to food would affect the efficacy of chlorfenapyr to *Trogoderma* species.

Experiment 3: Label versus Effective Rate for Chlorfenapyr

Arthur (2008) discovered that a less than label rate of chlorfenapyr is effective for *T. castaneum*. The goal of this study was to test if there was a difference between the effective and maximum label rate in having a higher mortality rate or speed of efficacy in dermestid larvae since the larval dermestid stage had a higher survivability rate than dermestid adults in our first experiment testing efficacy of chlorfenapyr in crevice arenas. There are no studies comparing an effective rate versus label rate of chlorfenapyr or other insecticides in the literature. Arthur (2008) regularly used the less than label rate in subsequent studies. In Kansas, a less than label rate application is allowed by commercial applicators if the applicator supplies in writing to the customer that a less than label rate was applied, unless prohibited by the label (Shawn Hackett, EPA Region 7 Pesticide Program, personal communication, May 2026). In some states a less than label rate application is not allowed. Commercial applicators need to check state laws to see if a less than label rate application is allowed.

Experiment 4: Pyrethrin Aerosol Bioassay

Tribolium spp. and *Trogoderma* spp. are commonly found in food processing facilities such as flour mills, processing plants, and food product warehouses (Arthur, 2008(b)). Since methyl bromide was given an end-use date beginning in 2005 (Fields and White, 2002), several alternative control methods have been explored as replacements for whole facility treatments (Arthur, 2012). Space treatments, such as aerosols, are used to control many types of insect pests such as stored-product beetles, crawling insects, and occasional invaders entering facilities from outdoors and may be represented by many different insect orders (Arthur and Campbell, 2008; Bernhard and Bennett, 1981). Monitoring for pests and management plans typically focus on the interior of facilities (Semeao et al., 2013(a)).

While focusing on treating indoor spaces of facilities, exterior outdoor populations are important since they may enter facilities unnoticed through daily facility activities. Several facilities may operate on a 24/7 basis which may allow invaders such as night fliers and product pests to enter a facility when personnel open exterior doors, when loading trucks with roll-up doors, or when opening doors, louvers, and windows for air flow when the building is hot. These actions can inadvertently allow exterior pests into a facility. While most occasional invaders are not a direct concern for food products, some, such as *P. interpunctella*, *Trogoderma* spp., and *Tribolium* spp., can enter during these opening events and become established inside facilities if not trapped or killed by space treatment.

Several lab and operational facility studies have been conducted looking at the effects of aerosolized pyrethrin products on *T. confusum* and *T. castaneum*, either with or without an insect growth regulator (Bernhard and Bennett, 1981; Arthur and Campbell, 2008; Toews et al., 2010; Campbell et al, 2014; Kharel et al., 2014a; Kharel et al., 2014b; Arthur et al., 2019; Scheff et al., 2019). Most studies focused on *T. confusum*, and none of these studies included dermestid beetles, even though they are a common pest of processing facilities. In Scheff et al. (2019) adult *T. confusum* exposed to a cylinderized pyrethrin with pyriproxyfen IGR resulted in adults closest to the point of application had greater than 60% affected individuals with the IGR component offering continued residual control after 6-weeks post exposure. Arthur and Campbell (2008) and Kharel et al. (2014b) looked at the effects of food resources being available to individuals finding that survival and recovery from knockdown increased when flour was present. Kharel et al. (2014a) evaluated different life stages of *T. confusum* and *T. castaneum* by treating individuals and allowing them to develop following exposure to either treated or untreated flour.

Kharel et al. (2014a) found that larvae were more tolerant compared to eggs or pupae which are immobile stages.

Many of these studies were conducted with insects placed in Petri dishes on the floor of sheds. Bernhard and Bennett (1981) evaluated insects with partial harborage and on shelves in a cabinet at two different levels. While they had significant differences for shelf height and time of exposure for rice weevil, Sitophilus oryzae (L.), their results for *T. confusum* were inconclusive due to low mortality. However, exposure of *T. confusum* to the treatment for 120-minutes without harborage resulted in mortality greater than 40%. Campbell et al. (2014) found that aerosol applications in flour mills, due to the complexity of their interiors with various machines, can influence the deposition of aerosol droplets. Their findings indicate that walls and corners were areas where lower efficacy occurred, especially those behind the point of application. These are areas which may need to be targeted for better control since many insects may also hide or avoid insecticide contact in these areas.

The purpose of this study was to determine the effects of aerosol pyrethrin treatments on different life stages of *T. inclusum* and *T. castaneum*, in simulated warehouse situations. Two tests were included, 3 heights of vertical shelving (low, medium and high) to emulate stacked goods in warehouse storage, and horizontal treatment under 2 heights of covered boxes at 5 different positions under each height which emulates depth of stacked goods. The overall goal was to determine the number of viable individuals (larvae, pupae or adults) which survive following treatment at each of the different positions mimicking warehouse storage. Knowing the effectiveness of aerosols to these species, especially immature Tribolium stages, would be impactful in a complete pest management program (Arthur, 2008b).

Experiment 5: Bait Station Bioassay

Insect pheromone traps are one of the most used tools for integrated pest management for stored product pests (Campbell & Toews, 2010). These devices are used for trapping and monitoring local pest populations. Traps can help detect populations or determine changes in populations which are hard to document with regular visual inspections of a facility (Toews and Nansen, 2012; Phillips et al., 2000).

Several studies have been conducted looking at the use of pheromone traps and capture rates for *T. castaneum* (Buckman and Campbell, 2013; Doud and Phillips, 2020). This study explores the capabilities of capture for dermestids using several available commercial bait and pheromone traps. The purpose of this study was to determine which of six commercially available baited traps and/or food lures were most effective after short-term exposure.

Experiment 6: Fipronil Gel Bait Bioassay

MaxForce® FC Magnum is labeled as a fipronil based cockroach gel bait. It also carries label language for use against adult stages of 'pantry pests' including some of the most common stored product pests such as *T. castaneum*, *T. confusum*, *Trogoderma* spp., *T. variabile*, *L. serricorne*, *O. surinamensis*, and others. For sensitive areas such as food and feed handling areas, MaxForce® is a spot or crack and crevice option which is labeled for multiple pest species which may harbor in the same spaces. Fipronil is an active ingredient with common uses in field crop, urban and veterinary medicine applications (Kavallieratos et al., 2010). Fipronil gel baits have been used in the United States against cockroaches since 1998 (Gondhalekar and Scharf, 2012).

The goal of this study was to evaluate the efficacy of fipronil gel bait formulation on six species of stored product insect pest adults and larvae. While the product is labeled for use on

adult beetles, there are no studies on the efficacy to species listed on the label. This is especially critical for species which are not required to feed as adults such as *T. variabile* and *T. inclusum*.

Conclusion

The purpose of these studies was to look at applied IPM tools currently used in the field and assess the efficacy of products which may be used in grain mills, food processing facilities, warehouses, and related industries. The products explored in this dissertation include contact insecticides, pyrethrin aerosol insecticide application, pheromone bait stations, and a gel bait labeled for use against stored product pest adults. The aim is to determine the effectiveness of these products on *T. variabile* and *T. inclusum* adults and larvae. This research is necessary and helpful to the pest control industry to know which products are most effective against larvae and adults. Arthur (2012) discussed that species such as *T. variabile* and other dermestids have not had recent trials conducted to compare their insecticide susceptibility to flour beetles. This is a major reason for conducting studies involving *T. variabile*, *T. inclusum, T. castaneum* and other stored product pests.

Chapter 2 - Materials and Methods

Insect Populations

Dermestids

Three dermestid populations were used in these studies, one lab colony of *Trogoderma variabile* Ballion, warehouse beetle, and two field collected populations of *Trogoderma inclusum* Le Conte, larger cabinet beetle. The lab population of *T. variabile* has been reared at the USDA Agriculture Research Service, Center for Grain and Animal Health Research in Manhattan, KS for over 30 years. The two field populations of *T. inclusum*, one from Arkansas and one from Kansas, were both field collected in August 2012, and had been exposed to normal field conditions, including pesticide applications to that time.

T. variabile and *T. inclusum* colonies were reared in colony at 30°C at 64% RH, and a 16-hour light/8-hour dark light cycle on one of three diets. Either (1) a diet of Appeal Meal Replacement Drink Mix™ (NSE Products, Inc., Provo, Utah, 84601) covered with quick oats (Kroger Co., Cincinnati, OH, 45202) for harborage, (2) one-part vanilla Appeal Meal Replacement Drink Mix™ and two-parts ground Purina One Lamb and Rice Formula™ dog food mix (Purina, Neenah, WI, 54957) covered with quick oats for harborage, or (3) only ground Purina One Lamb and Rice Formula™ dog food covered with quick oats in one-quart canning jars. A slightly damp one-half sheet of paper towel was put over the top of the quick oats, squeezed out with just enough moisture for the insects to become established. Canning jar lids were replaced with screen mesh for ventilation.

The diet for dermestids was modified over time after the vanilla Appeal Meal Replacement Drink Mix™ was no longer available. Adult *Trogoderma* spp. used for all experiments were < 48-hours old of mixed sexes. A tight control of adult ages was required

since the adult lifespan is < 14 days, and to ensure that adults would survive the length experiments to avoid adult natural mortality.

Tenebrionids

Two lab strains of tenebrionid beetles, *T. castaneum* and *T. confusum,* had been reared in colony at the USDA Agriculture Research Service, Center for Grain and Animal Health Research in Manhattan, KS for over 30 years. A second *T. castaneum* population was used for the bait station study and the fipronil cockroach gel bait study which was field collected from the Kansas City area in 2005. All three *Tribolium* spp. populations were reared at 27.5°C, 60% RH, and a 16-hour light/8-hour dark light cycle on a diet of whole wheat flour (Hudson Cream, Hudson, KS 67545).

Tenebrionid adults were aged 0 to 144 hours (0-6 days) for use in experiments. Tenebrionid adults could be slightly older than dermestids due to their longer adult stage which would more easily survive one-week studies.

Other Species

Two other species were used in the fipronil cockroach gel bait study, *Lasioderma serricorne* (Fabricius), cigarette beetle, and *Oryzaephilus surinamensis* (Linnaeus), sawtoothed grain beetle. Both species were reared in colony at the USDA Agriculture Research Service, Center for Grain and Animal Health Research in Manhattan, KS for over 30 years. *Lasioderma serricorne* was reared on whole wheat flour (Hudson Cream, Hudson, KS 67545) with quick oats (Kroger Co., Cincinnati, OH, 45202) layered over the top. *Oryzaephilus surnamensis* were reared on quick oats (Kroger Co., Cincinnati, OH, 45202). Both populations were reared in a growth chamber at 28°C, 64% RH. For studies, adults < 48-hours old of mixed sexes were used in all studies.

Oviposition Jars for Larval Testing

Each oviposition jar was set up by placing 20 adults in All Purpose Kroger flour (Kroger Co., Cincinnati, OH, 45202) sifted through a #60 sieve with catch pan (U.S. Standard Sieve Series; Dual Manufacturing Co., Chicago, Illinois) and allowing them to oviposit for 48±2 hours. Adults were removed from the flour by sifting out adults using a #30 sieve with catch pan (U.S. Standard Sieve Series; Dual Manufacturing Co., Chicago, Illinois), eggs and flour returned to oviposit jars to develop. Eggs were allowed to hatch naturally. Larvae were left to grow to medium size for each species at 30°C, 64% RH. Oviposition jars were set up and timed to have all species being used in any given experiment to be at medium size at the same time for each species being tested. Age consideration was made by starting oviposition jars at the appropriate time for each species to grow and arrive at medium size synchronized for the start time of the experiment to avoid larvae pupating for the duration of the experiment.

Bioassay Arena Preparation

Bioassay arenas were prepared using concrete patch (Rockkite™, Heartline Products Co Inc., Cleveland, OH), which was purchased from a local hardware store and prepared according to package directions, by mixing approximately 2000g Rockkite™ with 1.0 L tap water, stirred to make a slurry, and then poured into Petri dishes to a depth of 5-mm and allowed to dry at ambient conditions for at least 48-hours prior to use in experiments.

Once the concrete bioassay arenas had dried, polytetrafluoroethylene (Fluon®, Sigma Aldrich, St. Louis, Missouri0) was applied to the sidewall of the bioassay arenas with a cotton swab and allowed to dry for at least 12-hours. This was done to prevent insects escaping the bioassay arenas.

Crevice Bioassay Arenas

Crevice plates were created similar to above with the addition of using two drinking straws (150 mm × 25 mm), cut to the length of a 150 mm Petri dish and taped together with clear scotch tape, covering the indentation between the stacked straws. The straws were placed in the center of the Petri dish (Figure 2.1) and the concrete slurry was poured into the Petri dish up to the height of the bottom straw. After 2 hours, the straw stack was removed, creating a crevice from the void left behind. Bioassay arenas were allowed to dry at ambient conditions for at least 48-hours prior to use in experiments (Figure 2.2).

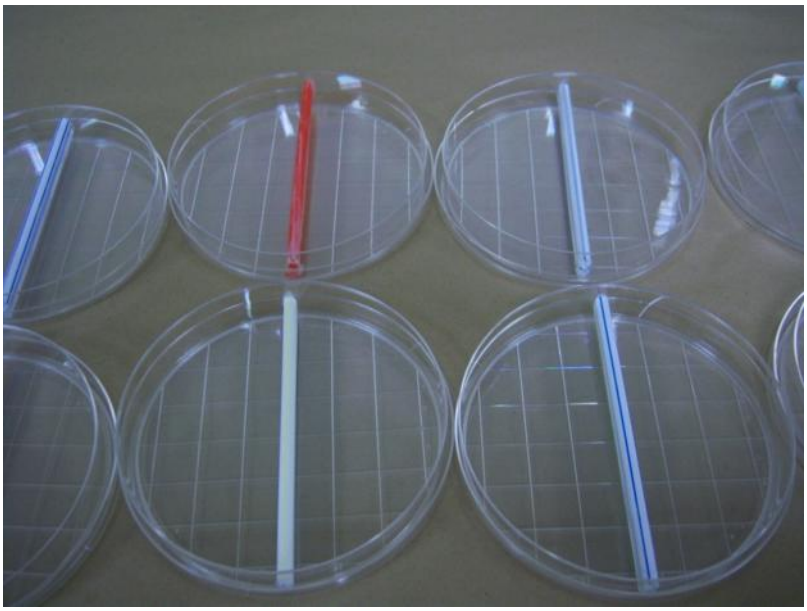


Figure 2.1. Petri dish stacked straw arrangement prior to pouring Rockkite®.

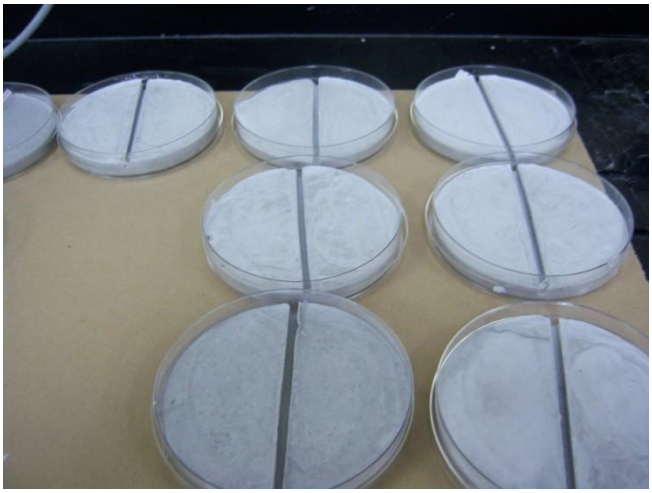


Figure 2.2. Prepared crevice bioassay arenas.

Pesticide Formulations:

Two formulations of chlorfenapyr were used in our studies. The first was Phantom® Termiticide-Insecticide concentrate with the active ingredient (AI) 21.45% chlorfenapyr and 78.55% other ingredients (EPA registration number 241-392, BASF, Research Triangle, North Carolina). The chlorfenapyr SC formulation, a suspension concentrate (SC), was diluted in water to prepare a 55 mg active ingredient (AI)/m^2 formulation. The formulations was applied using a Badger 100 artist's airbrush (Franklin Park, Illinois, (Figure 2.3) to spray the required volume onto each of the treatment arenas.

The second formulation was Phantom® Pressurized Insecticide (PI) which contains 0.5% chlorfenapyr and 99.5% other ingredients (EPA registration number 7969-285, BASF, Research Triangle, North Carolina). The chlorfenapyr PI formulation is a ready-to-use (RTU) formulation which comes in a aerosol spray can. The PI formulation was applied at label rate of 1 sec spray at approximately 30.5 cm above the 154 cm^2 bioassay arena (Figure 2.4).

The control was to spray 1.2 ml tap water on the arenas treatment areas to mimic normal wash down in a food processing facility.

There is a difference in the appearance of the chlorfenapyr SC versus the chlorfenapyr PI formulations in that after the SC formulation dried, there is no visually detectable residue on treated surfaces. The chlorfenapyr PI formulation, however, leaves a visually detectable crystalline residue on the surface.

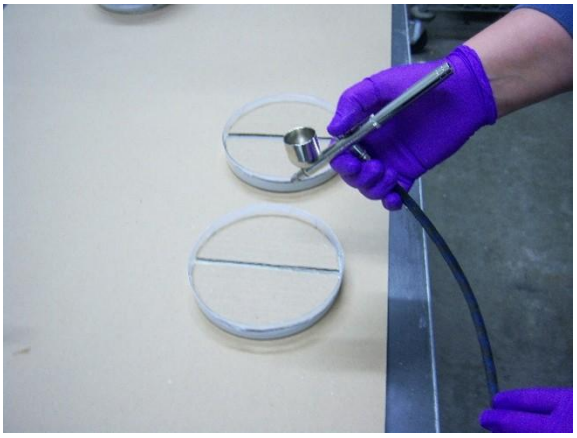


Figure 2.3. Badger 100 Artist's Airbrush

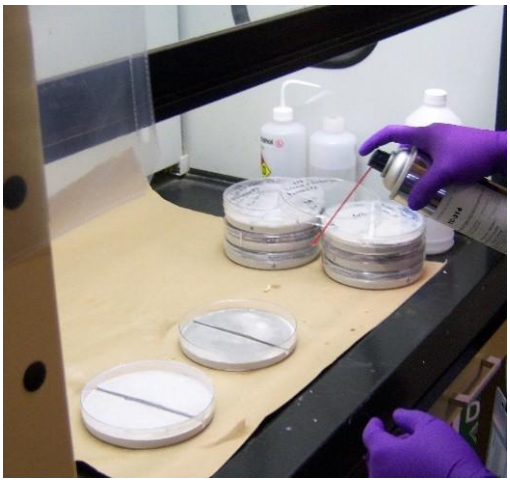


Figure 2.4. Chlorfenapyr PI application straw

For the shed aerosol study, Entech Fog-10 Space Spray with 1.0% pyrethrins (AI), 5.0% Piperonyl Butoxide and 94% other ingredients (EPA registration number 40391-14, Entech

Systems Corporation, Lenner, Louisiana) at the label rate of 1oz. per 1000 ft^3. A Fogmaster Jr., Model 5330 (Fogmaster Corporation, Deerfield Beach, Florida) handheld fogging machine (Figure 2.5) which delivers a particle size of 15-40 microns. Control sheds were no treatment.



Figure 2.5. Fogmaster Jr. Handheld fogging machine.

A gel bait bioassay was conducted using MaxForce® FC Magnum which contains 0.05% fipronil (AI), and 99.95% other ingredients (EPA registration number 432-1460, Bayer Environmental Science, Research Triangle Park, North Carolina). The formulation of this gel bait is a proprietary combination of fipronil in a gel matrix and food attractant, mainly marketed and used for cockroach control by consumption. "Pantry Pests" are listed as a target pest on the label, specifically listing the adult stage of the following beetles: *Trogoderma* spp., *T. confusum*, *T. castaneum*, *L. serricorne*, and *O. surinamensis*, among others. The gel bait was applied using the ready-to-use syringe applicator inserted into a Maxforce® Bait Injector for the best control of bait quantities (Figure 2.6). Control arenas were left blank with no gel bait added.



Figure 2.6. Maxforce® Bait Injector

Beetle Assessments

Tribolium castaneum adults were classified as life, knocked down or dead when observed in all studies. Live adults were in an upright position either moving or motionless unless gently prodded with a straight probe. Individuals determined as 'knocked down' were upside down with legs waving in the air or upright moving slowly and/or stumbling (included shaking, unstable movements, difficulty walking). Dead adults were those that were upside down on their backs, legs curled in, and did not respond to gentle prodding with a straight probe (BioQuip, Rancho Dominguez, California).

All three adult dermestid populations, *T. variabile* and *T. inclusum* populations, were classified as live, knocked down or dead when observed in all studies. A straight probe was used to gently stroke or brush insects to check for movement. Normal live movement was either moving or motionless in an upright position. Knocked down movements included being upside down with legs waving in the air or upright moving slowly and/or stumbling (included shaking, unstable movements, difficulty walking). If there was no movement of any kind, generally with their legs pulled in tightly, and no response after stroking with a straight probe they were

considered dead. Some hard to assess Trogoderma beetles were set aside for about 20 minutes while evaluating others in the Petri dish to wait for signs of movement since they tended to appear dormant but would eventually move within a few minutes of being left alone.

Larvae of all species were assessed in a similar manner. Live larvae were running, walking or sitting still; knocked down larvae had upright movement with difficulty walking or with shaky, unstable movements; dead larvae were motionless, and if dead for a few days, may have begun to darken in color.

Experiment 1: Bioassay on crevice arenas

Bioassay Arenas

Arenas were prepared as above for the crevice bioassay arenas. Arenas for each of four treatments plus control was prepared for each of four populations and two formulations. Five replications were conducted.

Insect Populations

Four stored product pest beetle and larval populations were used in this experiment, which included the laboratory colonies of *T. castaneum* and *T. variable*, and two field collected populations of *Trogoderma inclusum* (larger cabinet beetle), one from Arkansas and one from Kansas, were also tested.

Pesticides

Two formulations of chlorfenapyr were used, Phantom® SC and Phantom® PI by BASF. Arenas were prepared as described above using the less than label rate of the chlorfenapyr SC formulation and label rate of chlorfenapyr PI formulation.

Treatments

There were four treatments and an untreated control. The four treatments were crevice only (C), surface only (S), crevice and surface (All), and food-in-crevice (FC). Cardboard half-circles were cut to custom fit each Petri dish in the shape of the surface to be protected from treatment (untreated) for crevice only and food-in-crevice treatments. The double straw configuration, described under 'crevice bioassay arenas', was placed back into the crevice by pinching and pressing which plugged the crevice to conduct the surface only treatment without contaminating the crevice. For the food-in-crevice treatments, 2.5 grams of vanilla supplement was spread evenly throughout the crevice using a #10 paint brush, and then the pesticide application was made over the top of the food in crevice.

Experimental Setup

Adults and larvae were aged as described above for each of the four species used. Individual insects were selected at random, not sexed, using mixed-sex populations in each bioassay.

Ten adults 0 to 48-hours for dermestids and 0 to 144-hours (0 to 6 days) old for tenebrionid species were introduced to each peri dish in the crevice. Petri dishes were set at room temperature of 27°C. Evaluations were conducted every 24-hours ± 2 hours for four days. Beetles were categorized as alive/running (r), knocked down (kd), or dead (nr = no response). *T. variabile* and *T. inclusum* were sometimes gently 'stroked' or 'probed' to validate a response since they tended to appear unresponsive during data collection.

Experimental Assessment:

Adults and larvae of *T. castaneum*, *T. variabile* and *T. inclusum* were evaluated as described previously.

Experiment 2: Barrier to Food

Bioassay Arena Preparation

A barrier and food were placed between the treatment on the opposite side of the bioassay arena. Bioassay arenas were prepared by taping a 7.6 cm section of plastic angled corner barrier to the bottom of a 100 × 20 mm Petri dish prior to the addition of concrete. Concrete patch (Rockkite™) was prepared as described above. The slurry was poured into bioassay arena in a thin layer approximately 4 mm to 5 mm in depth to represent a concrete floor. Plates were allowed to dry thoroughly for a minimum of 48-hours before treatment.

After curing, 2 grams of all-purpose flour for food patch was placed behind the clear plastic barrier for red *T. castaneum*. 2 grams of vanilla protein supplement was placed behind the clear plastic barrier for *T. variabile*. The opposite side of the bioassay arena was treated with chlorfenapyr. A piece of cardboard was used to separate the treated from untreated area of the arena to prevent drift onto the untreated portion of the Petri dish. The treated area was about 20.6 cm2 or about 14.4% of the total area (Arthur & Fontenot, 2012). See Figure 2.7.

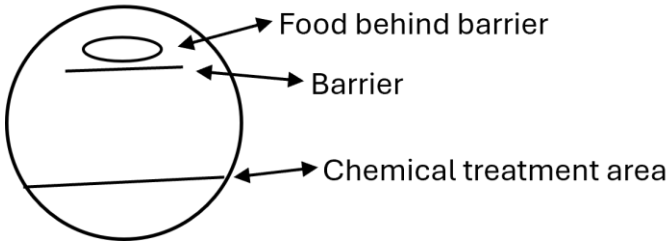


Figure 2.7. Barrier Petri Dish Configuration

Insect Populations

Four beetle populations consisting of three species were used in this experiment. Colonies included the lab reared *Tribolium castaneum* and *Trogoderma variabile*, and two field collected populations of *Trogoderma inclusum*. All three dermestid colonies consisting of *T. variabile* and both *T. inclusum* colonies were reared on a 1/3 vanilla Appeal Meal Replacement Drink Mix™ (NSE Products, Inc., Provo, Utah, 84601) and 2/3 ground Purina One Lamb and Rice Formula mix covered with quick oats for harborage at 30°C.

Pesticides

Bioassay arenas were partially treated with either Phantom SC or Phantom PI. Arenas were treated with either the effective rate or label rate of chlorfenapyr SC and label rate of chlorfenapyr PI formulation. Control plates were untreated. Four arenas for each treatment were prepared for each of four replications.

Experimental Setup

Ten mixed sex adults, <48-hours for *T. variabile* or <144 hours for *T. castaneum* or ten mid-sized larvae of either *T. variabile* or *T. castaneum* were introduced onto each plate in the space between the treatment and the barriered refuge. Plates were set at room temperature, approximately 24°C. Evaluations were made every 24 ± 2 hours for four days. Following the four-day daily readings, surviving larvae were allowed to continue to adult emergence on the same arenas.

Experimental Assessment

Adult and larvae, dermestids and tenebrionids, were evaluated as described above.

Experiment 3: Label versus Effective Chlorfenapyr Rate for Chlorfenapyr

Bioassay Arenas

Bioassay arenas were set up as described above for crevice bioassay arenas.

Insect Populations

Larvae of lab reared populations of lab colonies *T. castaneum* and *T. variable*, and two *T. inclusum* populations were tested in this experiment. Larvae were raised using the oviposition jar method described previously.

Pesticides

Two formulations of chlorfenapyr were used, Phantom® SC and Phantom® PI by BASF. Arenas were prepared as described previously using the effective rate of the chlorfenapyr SC formulation of 55 mg (AI)/m^2, maximum label rate of chlorfenapyr SC of 1.1g active ingredient (AI)/m^2 (Arthur, 2013), and label rate of 1 sec spray at approximately 30.5 cm above the 154 cm^2 bioassay arena chlorfenapyr PI formulation.

Treatments

There were four treatments and an untreated control: crevice only (C), surface only (S), crevice and surface (All), and food in crevice (FC). Cardboard half-circles were cut to custom fit each Petri dish in the shape of the surface to cover non-treated areas of crevice only and food in crevice treatments. Four treatments plus control arenas were prepared for each species and pesticide. Four replications were conducted.

Experimental Setup

Ten mid-sized larvae were introduced to each peri dish crevice. Petri dishes were set at room temperature of 27°C. Evaluations were conducted every 24-hours for four days. Larvae were categorized as alive/running (r), knocked down (kd), or dead (nr = no response).

Trogoderma variabile and *T. inclusum* larvae were 'stroked' or 'probed' to validate a response since they tended to appear unresponsive during data collection.

Experimental Assessment

Tribolium castaneum larvae and all three dermestid populations, *T. variabile* and both the Arkansas and Kansas *T. inclusum* populations were classified as live, knocked down or dead status. A straight probe was used to gently stroke or brush larvae to check for movement. If they moved normally, they were considered alive, if they moved as if affected, sluggish, swaying or staggered walking, they were considered knocked down, if there was no movement of any kind, they were considered dead.

Experiment 4: Pyrethrin Aerosol Bioassay

Aerosol Sheds

Three sheds were used measuring 45.24 m^3 (6 m × 2.9 m × 2.6 m) in Manhattan, KS (Kharel et al., 2014(a)) with the interior completely covered with 6 mil-thickness Husky™ clear plastic sheeting to avoid contamination of the shed interiors. HOBO units were used to log the ambient temperatures for the duration of the treatment and experiments which, in hourly checks, ranged from 21°C to 25°C and 58% to 62% RH (Onset Computer Corporation, Bourne, MA) over all three repetitions which were conducted one per week for three weeks in September.

Insect Populations

Two species of stored product insects were used for this experiment, *T. inclusum* and *T. castaneum.*

T. castaneum, a lab reared colony which was field collected in 2005 from the Kansas City area and maintained on a diet of wheat flour at 27.5°C, 60% RH, and a 16-hour light/8-hour dark

light cycle, without exposure to insecticides since acquisition at the USDA Agriculture Research Service, Center for Grain and Animal Health Research in Manhattan, KS since 2005.

Trogoderma inclusum, a field population collected from central Kansas in August 2012. *Trogoderma inclusum* was maintained at 30°C on a diet of two parts ground dog food (Purina lamb and rice) and one part vanilla protein supplement with quick oats over the top for harborage.

Three life stages (egg, larvae, and pupae) of *T. castaneum*, and *T. inclusum*, were exposed to Entech Fog-10 Space Spray aerosol in sheds using a Fogmaster Jr. handheld fogging machine. The three sheds used were 45.24 m^3 (6 m × 2.9 m × 2.6 m) in Manhattan, KS (Kharel et al., 2014) with the interior completely covered with 6 mil-thickness Husky™ clear plastic sheeting to avoid contamination of the shed interiors. HOBO units were used to log the ambient temperatures which ranged from 21°C to 25°C and 58% to 62% RH (Onset Computer Corporation, Bourne, MA).

Pesticide

Entech Fog-10 Space Spray was applied at the label rate of 0.03 L per 28 M^3. There were two treatments: no fan for still air during treatment or fans for air movement. Fogging was conducted with the fogger held one meter above the floor, pivoting the aerosol device slowly from side to side, for 2 minutes and 15 seconds for each treatment to achieve label rate.

Experimental Setup

Petri dishes containing eggs, larvae and pupae were placed on vertical shelves and Petri dishes containing only larvae were placed horizontally on the shed floor under a box cover which was either 10-cm or 20-cm in height. These placements were made to replicate what insects in a stacked warehouse would be exposed to. The vertical shelves were at 0.30-m, 0.91-m, and 1.83-

m heights from the floor and described as placed at low, medium and high levels. Six open Petri dishes were placed randomly on shelves at each of the three levels (low, medium and high), consisting of two Petri dishes of each life stage, egg, larva, and pupa.

Vertical Petri Dish Setup

Petri dishes used on the vertical shelves at the back of the sheds were standard 100-mm × 15-mm plates that had 10 eggs each, late instar larvae or pupae, placed into the center of each plate. Egg arenas differed in preparation from larval and pupal arenas in that double-sided scotch tape was placed on the center bottom of the plate. Ten eggs were gently placed in two rows of five onto the tape using a 2-hair paint brush. Flour or vanilla protein supplement was then added to coat the eggs and sticky tape to coat any remaining sticky areas so when the larvae hatched, they could crawl off the tape and out into the plate. Ten larvae or pupae were only placed onto the plates for treatment. All Petri dish sidewalls were coated with Fluon® to prevent insects from climbing out of the dishes. Each Petri dish had 400 mg of food added to larval and pupal plates for food; vanilla protein supplement for *T. inclusum* and wheat flour for *T. castaneum*. Egg plates had 200 mg of either vanilla protein supplement or wheat flour added as above.

Horizontal Box and Petri Dish Setup

Petri dishes used on the horizontal set up (placed on the floor) of the shed were 50 mm × 15 mm Petri dish arenas which had 5 late-instar larvae placed into the center of each. Each arena had sides coated with fluon to prevent larvae from escaping. Each arena had 200-mg food added. Wooden boxes covering the plates were one of two heights, 10-cm high × 20-cm wide × 99-cm long or 20-cm high × 20-cm wide × 99-cm long, placed over the plates with two plates at each of five positions for a total of ten plates. Both ends of the boxes were open for air flow. Position #1 was placed 7.5 cm in front of the boxes, position #2 at 7.5 cm inside the box,

position #3 at 35.5 cm inside the box, position #4 at 63.5 cm and position #5 at 91.5 cm inside from the front end of the box.

Aerosol Treatment

Following treatment, the sheds were sealed to allow the aerosol to settle. Treatments were held for 0.5, 1.0 or 2.0 hour(s) post-application before the Petri dishes were removed from the treatment area. Fan treatments were created using two HDX 9-inch 3-speed table fans (model number TF-810S) on the lowest setting of '1'. Control arenas were placed in sheds with no application, then collected at the appropriate time for 0.5, 1.0 or 2.0 hour(s).

Experimental Assessment

Following treatment, Petri arenas were collected and held in a 27.6°C growth chamber to monitor survival and growth progression through life stages. Results were recorded weekly, with the first reading being at 96-hours ± 2 hours post-treatment, then at full-week intervals in subsequent weeks. Classifications for eggs were (unhatched/dead or larvae), larvae were (dead, knock down or alive), pupae (dead or alive). If required, larvae were probed to check for viability as described above. If the life cycles advanced to adult emergence, adults were either dead or alive. Final results are based on total live individuals at three weeks, regardless of life stage due to the differing rates of development between the two species. Three repetitions were conducted.

Experiment 5: Bait Station Bioassay

Insect Populations

Populations of two beetle species were used in this experiment. *Trogoderma variabile*, lab strain, and *T. inclusum*, collected in Central Kansas, had been exposed to normal field and storage conditions on rice, including pesticide applications until their time of collection in

August, 2012. *Trogoderma variabile* and *T. inclusum* colonies were reared on a one-part vanilla Appeal Meal Replacement Drink Mix™ (NSE Products, Inc., Provo, Utah, 84601) and two-parts ground Purina One Lamb and Rice Formula™ mix covered with quick oats for harborage in one-quart canning jars. A slightly damp one-half sheet of paper towel was put over the top of the oatmeal, squeezed out with just enough moisture for the insects to become established. Canning lids were replaced with screen mesh for ventilation. Colonies were held in a growth chamber at 30°C.

Experimental Setup

Adults for release into test arenas were prepared by placing an 8.9 cm × 8.9 cm corrugated cardboard square into a square 9.2 cm × 9.2 cm Petri dish. Forty adults, < 72-hours old, were placed into each Petri dish to hide in the cardboard for later transfer to the test arenas. 48-hours were allowed for beetles to enter cardboard. This allowed the adult beetles to enter a refuge and have minimal effect on release.

Twelve 90 quart/85L Sterilite™ tubs measuring 80 cm length × 31.8 cm width × 30.5 cm height were used as the test arena. Arena set-up contained a bait station, a mineral oil bait station, and a 8.9 cm by 8.9 cm cardboard containing the adults to release (Figure 2.8). After placing the cardboard containing adults into the tub, any beetles which were not in the cardboard were poured from the Petri dish on top of the cardboard into the tub so all 40 adults were placed into the tub arenas.

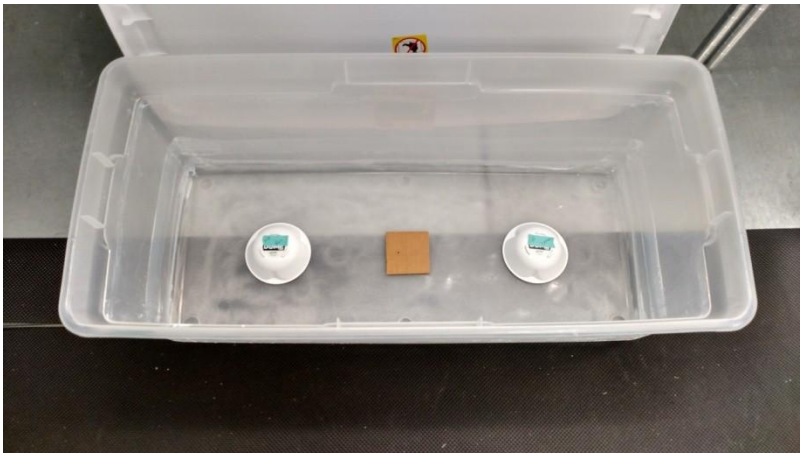


Figure 2.8. Sterilite™ tub arena setup.

The six bait stations used were: Trécé Storgard® Dome™ trap KB/WB pheromone lure and food bait oil; Trécé Storgard® Quick-Change™ Dome™ trap with KB/WB lure; Trécé Storgard® Quick-Change™ Dome™ trap with multi-combi lure; Insects Limited All Beetle trap with Pantry Patrol Insect Pheromone Attractant; Insects Limited All Beetle trap with food pellet bait and Tanglefoot to trap insects; J.F. Oakes LLC X-Lure R.T.U. MST (multi-species trap) beetle floor trap containing lure. Prior to use, all three Trécé pheromones and the X-Lure pheromone trays were opened under a ventilated air hood for 24 ± 1 hours before arenas were set up.

Mineral oil stations were prepared with just enough mineral oil to coat the bottom of the trap. For the Trece Quick-Change traps and the L-Lure trap, bottoms for mineral oil were created using a Petri dish with filter paper lining the bottom to mimic the commercial trap which have a paper liner in their trays. The All Beetle traps only had mineral oil on the bottom since only the gel bait or food pellet was in the bottom tray.

Prepared Sterilite tubs had each replicate randomly arranged by generating six random patterns using www.randomizer.org in a 30°C growth chamber.

Experimental Assessment

Number of adults in and on bait stations were recorded at 24-, 48-, and 72-hours. Six reps were conducted.

Experiment 6: Fipronil Gel Bait Bioassay

Bioassay Arenas

All Petri dishes were first prepared with concrete bases using concrete patch (Rockkite™), purchased locally. Rockkite™ was prepared according to directions by mixing approximately 2,000 g Rockkite™ with 1.0 L tap water, stirred into a slurry. The slurry is then poured into Petri dish plates in a thin layer covering the bottom of the Petri dish, approximately 2 to 3-mm in depth. Petri dishes were allowed to dry for at least 48-hours. Once the concrete bottoms were dried, fluon was applied to the sidewall of the Petri dishes and allowed to dry to prevent insects from crawling out.

Insect Populations

The following six insect species were tested: *T. variabile*, lab colony; *T. inclusum*, collected from Central Kansas; *T. castaneum*, collected in 2005 from the Kansas City area; *T. confusum*, lab colony; *L. serricorne*, lab colony; and *O. surinamensis*, lab colony. Adult insects were aged to be 24-hours to 48-hours old at the start time of the experiment. All six species tested are specifically listed on the MaxForce label in addition to ten other species. The MaxForce FC Magnum label specifies only adult beetle control.

Adult Rearing

Adult insect species were reared following protocols described above. All species were reared to time the emergence of adults to be aged properly for each species at the time of the experiment.

Larval Rearing

Larval stages were aged using the oviposition jars described above. Adults of each species were allowed to oviposit in flour for 48 ± 2 hours. Adults were sifted off the flour and eggs allowed to hatch and grow to mid-sized larvae for use in experiments. Mid-sized larvae would still actively feed but not pupate during the duration of the experiment.

Pesticide

MaxForce FC Magnum Roach Killer Bait Gel was applied to Petri dishes in a strip of 6.35 mm (0.25 inch) using the plastic applicator tip included with the product reservoir.

Experimental Setup

Five repetitions were conducted using four treatment plates and four control arenas with no gel bait per repetition for each insect species. MaxForce® FC Magnum, active ingredient fipronil, was applied to each 100 mm × 20 mm arena in the crevice between the concrete floor and sidewall of the Petri dish. To measure the amount of MaxForce® gel bait in grams, ten sentinel plates per repetition, 60 mm × 15 mm were used. Bait was applied in 6.35 mm (0.25 inch) amount to the crevice between concrete bottom and the sidewall of the Petri dish. Weights were taken of pre-treated Petri dishes, Petri dishes immediately after treatment of gel bait was applied, and a second weight taken at 24-hours after treatment, to allow for drying shrink, at room temperature of 27° C on an analytical balance (Mettler AE163). The 24-hour dried gel bait average weight was 0.018 g gel bait per arena.

MaxForce® gel bait was allowed to dry for 24-hours before introducing adult or larval insects onto the plate. Ten insects, adults or larvae, were introduced onto the center of each Petri dish.

Experimental Assessment

Data readings were taken every 24 ± 1 hour for four days after introducing insects. Data was recorded as running (live), knocked down, or dead based on criteria outlined above for assessment.

Statistical Analysis

For all experiments except experiment 5, data were analyzed using the Statistical Analysis System (SAS) version 9.4 using the General Linear Models (GLM) Procedures and the Means Standard Error Procedures. The raw data was converted into percent survival. The General Linear Models Procedure was used to separate differences in pesticide formulation. The Tukey's Studentized Range (HSD) Test (0.05%) was used for separation differences in pesticide formulations and species.

For Experiment 5, the pheromone/bait station study, a difference between the total bait station and total mineral oil captures was used. The raw data was converted into percentages of percent survival.

Chapter 3 - Results

Experiment 1: Bioassay on Crevice Arenas

Table 3.1 lists the comparisons of means with standard errors for each of the four populations tested and both formulations of chlorfenapyr. All chlorfenapyr PI formulations were significant at $P<0.0001$. In the chlorfenapyr SC formulation, the two lab populations, *T. castaneum* and *T. variabile*, were significant at $P<0.0001$, both *T. inclusum* populations were significant at $P=0.0002$. Overall percent survival was lower in the PI treatments for all adult populations compared to the SC formulations. When evaluating each species individually, there are survival differences in treatments where the crevice-only and the crevice-plus-surface treatments were the most effective. Surface only treatments and food-in-crevice treatments are less effective with the surface only treatment the least effective against adults, in most cases.

Within both the PI and the SC formulations, both adult lab populations had lower survival than the two field populations. In all populations and chlorfenapyr formulations, the surface only treatment had the highest survivorship, with the exception of the Arkansas *T. inclusum* populations when exposed to the PI formulations where the food-in-crevice had the highest survivorship.

Table 3.1. Mean percent adult survival of four species of stored product beetles with standard errors for four treatments against two formulations of chlorfenapyr after 96-hours. Mean separations are indicated by letter within formulation for each treatment and species. (PI: $F(2,12) = 1.22$, $P < 0.3441$; SC: $F(3,12) = 3.64$, $P < 0.0970$). (PI) Pressurized Insecticide Formulation, (SC) Suspension Concentrate Formulation.

Treatment	*T. castaneum*	*T. variabile*	Arkansas *T. inclusum*	Kansas *T. inclusum*
	PI Formulation			
Untreated Control	100.0 ± 0.0 a	97.5 ± 2.5 a	96.0 ± 2.5 a	90.0 ± 3.2 a
Crevice	0.0 ± 0.0 b	0.0 ± 0.0 b	4.0 ± 2.4 d	16.0 ± 6.0 c
Surface	0.0 ± 0.0 b	10.0 ± 4.1 b	34.0 ± 9.3 bc	54.0 ± 9.3 b
All	0.0 ± 0.0 b	0.0 ± 0.0 b	8.0 ± 4.9 cd	10.0 ± 5.5 c
Food in Crevice	0.0 ± 0.0 b	2.5 ± 2.5 b	42.0 ± 9.7 b	36.0 ± 7.5 bc
	SC Formulation			
Untreated Control	100.0 ± 0.0 a	97.2 ± 2.8 a	98.0 ± 2.0 a	98.0 ± 2.0 a
Crevice	0.0 ± 0.0 b	10.0 ± 7.1 bc	38.0 ± 9.7 c	38.0 ± 4.9 bc
Surface	11.1 ± 7.2 b	28.6 ± 5.8 b	78.0 ± 6.6 ab	70.0 ± 5.5 ab
All	0.0 ± 0.0 b	5.0 ± 2.9 c	30.0 ± 10.5 c	32.0 ± 14.0 c
Food in Crevice	5.0 ± 2.9 b	7.5 ± 2.5 c	47.5 ± 13.3 ab	50.0 ± 11.0 bc

Mean percent adult survival of four species of stored product beetles with standard errors for four treatments against two formulations of chlorfenapyr after 96-hours. Mean separations are indicated by letter within formulation for each treatment and species**.** PI - Pressurized Insecticide Formulation, SC - Suspension Concentrate Formulation.

Table 3.2 lists the P-values at $P \leq 0.05$ for each treatment with significant differences in the formulation for each of the four populations. When the formulation is the factor, only four treatment/population combinations show a significant difference between formulation performance at 96-hours (day 4). They are the surface only treatment for the *T. variabile* population, the crevice only treatment and the surface only treatment for the Arkansas *T. inclusum* population, and the crevice only treatment for the Kansas *T. inclusum* population.

Table 3.2. Significan Differences of chlorfenapyr formulation (PI versus SC) to adults for each treatment/insect populations at 96-h post-treatement. (*) indicates significance at $P < 0.05$.

Treatment	*T. castaneum*	*T. variabile*	Arkansas *T. inclusum*	Kansas *T. inclusum*
Untreated Control	---	0.943	0.537	0.168
Crevice	---	0.207	0.024*	0.100
Surface	0.248	0.039*	0.012*	0.320
All	---	0.134	0.059	0.337
Food in Crevice	0.134	0.207	0.294	0.484

Table 3.3 outlines the mean percent survival of larvae exposed to chlorfenapyr in crevice arenas at 96-hours. *T. castaneum* had the lowest survival rate for both the PI and the SC formulations of all four populations. Based on this data and the lack of mortality to chlorfenpyr exposure, larval discriminations were made after an additional week. Further evaluations were made at two weeks post-treatment.

Table 3.3. Mean percent survival of four species of larvae with standard errors for four treatments against two formulations of chlorfenapyr at four days (96-hours). Mean separations are indicated by letter within formulation for each treatment and species. (PI: $F(3,12) = 53.47$, $P < .0001$; SC: $F(3,12) = 1.39$, $P < 0.2922$). **(**PI**)** Pressurized Insecticide Formulation, **(**SC**)** Suspension Concentrate Formulation.

Treatment	*T. castaneum*	*T. variabile*	Arkansas *T. inclusum*	Kansas *T. inclusum*
	PI Formulation			
Untreated Control	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	95.0 ± 5.0 ab
Crevice	32.5 ± 10.3 c	100.0 ± 0.0 a	92.5 ± 4.8 a	100.0 ± 0.0 a
Surface	45.0 ± 13.2 bc	100.0 ± 0.0 a	92.5 ± 7.5 a	100.0 ± 0.0 a
All	30.0 ± 9.1 c	100.0 ± 0.0 a	97.5 ± 2.5 a	100.0 ± 0.0 a
Food in Crevice	80.0 ± 9.1 ab	100.0 ± 0.0 a	95.0 ± 2.9 a	85.0 ± 2.9 b
	SC Formulation			
Untreated Control	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Crevice	72.5 ± 10.3 a	97.5 ± 2.5 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Surface	90.0 ± 7.1 a	97.5 ± 2.5 a	100.0 ± 0.0 a	100.0 ± 0.0 a
All	85.0 ± 11.9 a	100.0 ± 0.0 a	100.0 ± 0.0 a	97.5 ± 2.5 a
Food in Crevice	97.2 ± 2.8 a	100.0 ± 0.0 a	92.5 ± 4.8 a	89.4 ± 6.1 a

Table 3.4 shows treatment efficacy for larvae at two weeks post-treatment. Larval survival was still higher than expected overall. *T. castaneum* survival of less than 15% for the PI formulation and survival less than 22% for all treatments except the food-in-crevice are the lowest survival rates of all 4 populations. The food in crevice treatment survival for all larval species is generally lower than for other treatments except for *T. castaneum* in the SC formulation. For all species and both chlorfenapyr formulations, the surface only treatment has a higher survival rate than when the crevice is treated. Both field collected populations of *T. inclusum* larvae also had higher survival rates for the PI formulation in the crevice only and all treatments than *T. variabile*. However, the SC formulation still had no mortality effect on *T.*

variabile in the crevice only treatment after two weeks of exposure. Allowing the larvae the additional week of continued exposure to chlorfenapyr caused further mortality. Larvae had lower survival overall to the PI formulation than the SC formulation.

Table 3.4. Mean percent survival of four populations of stored product larvae with standard errors for four treatments against two formulations of chlorfenapyr after two weeks. Mean separations are indicated by letter within formulation for each treatment and species. (PI: $F(3,12) = 4.78$, $P < .0.0204$; SC: $F(3,12) = 19.68$, $P < .0001$). (PI) Pressurized Insecticide Formulation, (SC) Suspension Concentrate Formulation.

Treatment	*T. castaneum*	*T. variabile*	Arkansas *T. inclusum*	Kansas *T. inclusum*
	PI Formulation			
Untreated Control	94.4 ± 5.6 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Crevice	0.0 ± 0.0 b	67.5 ± 13.1 a	72.5 ± 16.0 a	72.5 ± 18.0 a
Surface	15.0 ± 15.0 b	64.4 ± 21.7 a	92.0 ± 4.7 a	80.0 ± 12.3 a
All	0.0 ± 0.0 b	45.0 ± 20.6 a	63.8 ± 3.6 a	57.5 ± 16.0 a
Food in Crevice	5.0 ± 5.0 b	63.9 ± 8.0 a	65.0 ± 13.2 a	56.1 ± 12.9 a
	SC Formulation			
Untreated Control	87.5 ± 12.5 a	100.0 ± 0.0 a	100.00 ± 0.0 a	100.0 ± 0.0 a
Crevice	13.2 ± 10.2 b	100.0 ± 0.0 a	92.5 ± 7.5 ab	95.0 ± 2.9 a
Surface	13.8 ± 5.5 b	97.5 ± 2.5 b a	100.0 ± 0.0 a	97.5 ± 2.5 a
All	22.0 ± 16.5 b	94.7 ± 3.1 a	100.0 ± 0.0 a	97.5 ± 2.5 a
Food in Crevice	41.4 ± 14.0 ab	28.3 ± 17.4 b	73.3 ± 8.3 b	66.1 ± 9.5 b

Experiment 2: Barrier to Food

96-hours after exposure to a partial chlorfenapyr treatment with food behind a barrier Figure 3.1 shows that the chlorfenapyr PI formulation affected adults more than larvae. *T. castaneum* adults showed the largest effect to the partial treatment, followed by the Kansas *T. inclusum* adults. Larvae were affected less, with the Arkansas *T. inclusum* and the Kansas *T. inclusum* populations showing the most affect from treatment after 96-hours of exposure.

Following these results, insects were held for one month post-treatment to observe survival with no further exposure to the insecticide.

Fewer larvae were affected by the partial chlorfenapy treatment. Many of the larvae were found in the food patch and may have avoided the treated segment of the Petri dish, which could explain the low mortality rate of the larvae. The Arkansas *T. inclusum* population had the lowest larval survival rate of all four populations at 96-hours post-treatment.

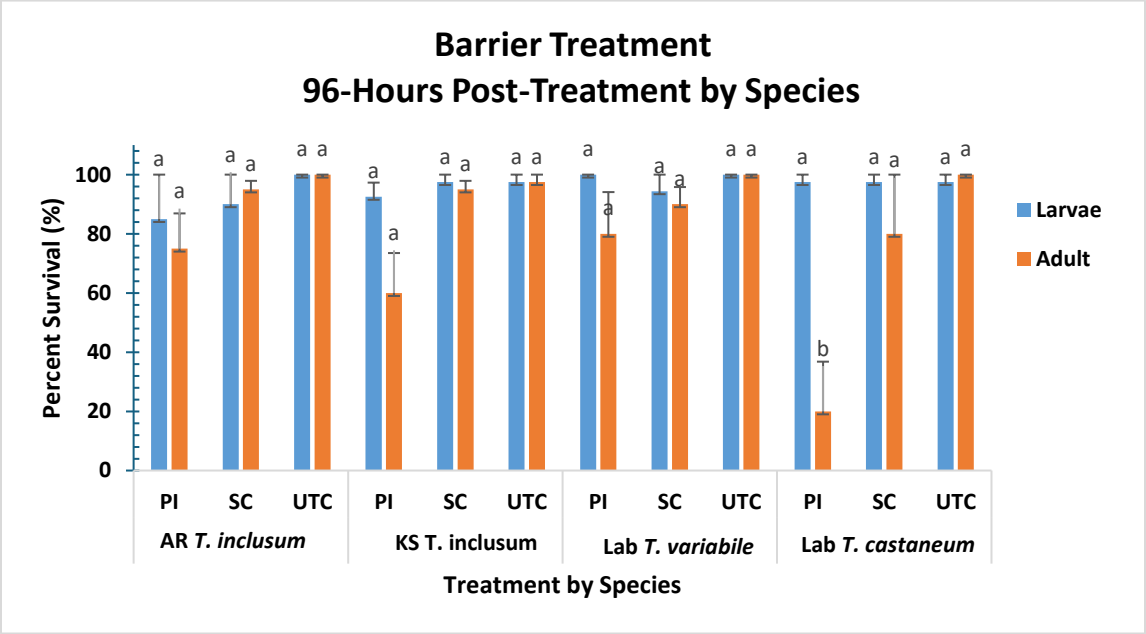


Figure 3.1. Mean percent survival of four populations of stored product pest species exposed to chlorfenapyr after 96-hours with barrier to food. (AR *T. inclusum*: $F(1,6) = 0.27$, $P < 0.6202$; KS *T. inclusum*:$F(1,6) = 5.12$, $P < 0.0643$; Lab *T. variabile*: $F(1,6) = 2.0$, $P < 0.2070$; *T. castaneum*: $F(1,6) = 20.74$, $P < 0.0039$). (PI) Pressurized Insecticide Formulation, (SC) Suspension Concentrate Formulation, (UTC) Untreated Control.

After one month, Figure 3.2, the untreated control survival shows the survival of untreated populations for both adults and larvae. The adult populations of *Trogoderma* species untreated controls show the normal decline due to their shorter adult life cycle which is around one month total. *Trogoderma variabile* untreated controls demonstrated a higher rate of survival at one month than both *T. inclusum* populations. *Tribolium castaneum* had the highest one-

month survival which is expected due to the longer life cycle of *Tribolium* species which can be over one year. Only 15% of the *Trogoderma* species adults living after the 96-hour post-treatment data readings were still alive at one-month post-treatment. However, approximately 95% of the 96-hour *T. castaneum* surviving adults exposed to chlorfenapyr PI and chlorfenapyr SC were still alive one month following treatment. In contrast, at one month post-treatment, over 87% of the 96-hour post-treatment surviving larvae were still alive for all four populations. These higher rates of larval survival allows all four populations to continue development towards the adult stage, becoming reproductive individuals.

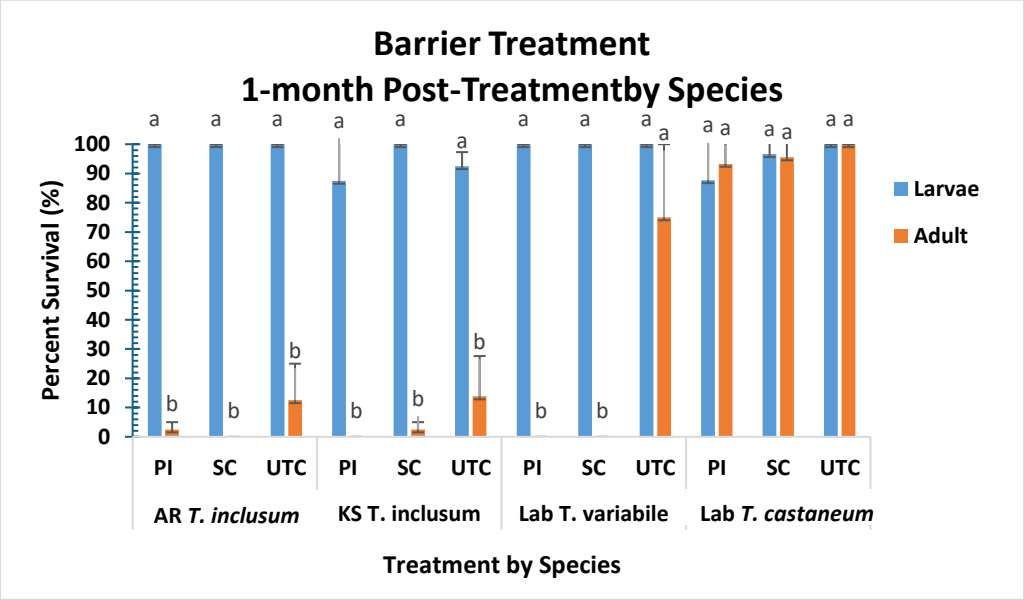


Figure 3.2. Mean percent survival of four populations of stored product pest species exposed to chlorfenapyr one month post-treatment with barrier to food. (AR *T. inclusum*: $F(1,6) = 0.62$, $P < 0.4626$; KS *T. inclusum*:$F(1,6) = 1.00$, $P < 0.3559$; Lab *T. variabile*: $F(1,6) = 1.0$, $P < 0.3559$; *T. castaneum*: $F(1,6) = 17.16$, $P < 0.0061$). (PI) Pressurized Insecticide Formulation, (SC) Suspension Concentrate Formulation, (UTC) Untreated Control.

Experiment 3: Label versus Effective Rate for Chlorfenapyr

As a follow up to the first study's results with the higher larval survival rates at the effective rate of chlorfenapyr based on Arthur (2013), we decided to test the effective rate

against the label rate of the chlorfenapyr SC formulation. The chlorfenapyr PI formulation had lower survival rates in the first experiment, but the PI formulation was included as a reference in this study to compare against the chlorfenapyr effective rate and the chlorfenapyr label rate.

Results are presented in Figures 3.3 through 3.7, each Figure represents a different treatment arena type as in experiment 1. *Tribolium castaneum* had the greatest mortality to the clorfenapyr label rate in all treatment strategies except for the Food in Crevice arenas. There were no significant differences between the clorfenapyr SC effective rate and label rate except in the Food in Crevice arenas for the Kansas *T. inclusum* and *T. castaneum* and *T. castaneum* in the Surface arena.

In the All treatment arena, *T. castaneum* had the lowest survival to chlorfenapyr SC and PI formulations. The crevice treatment with chlorfenapyr PI had the greatest efficacy on *T. variabile* and *T. castanuem*.

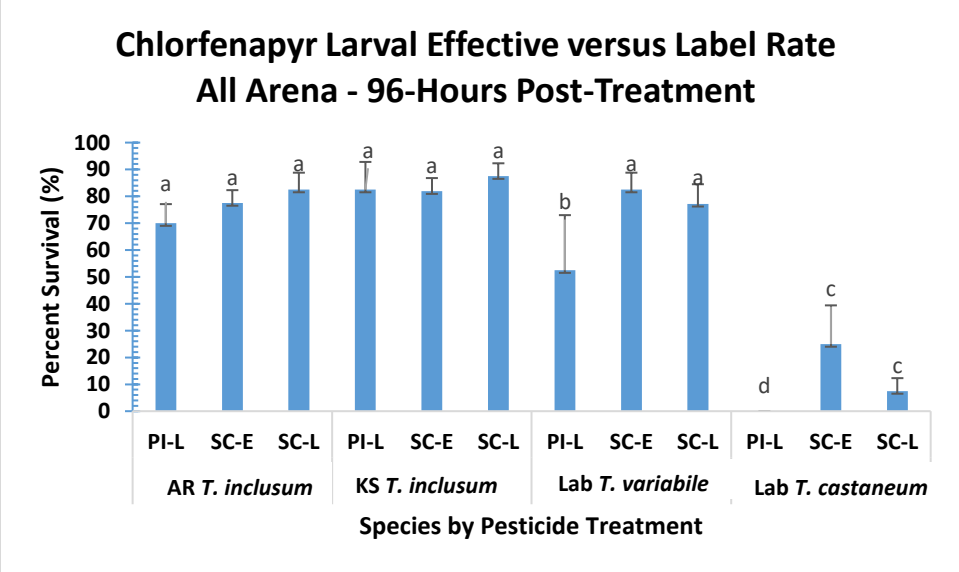


Figure 3.3. Mean percent survival of four populations of stored product larvae with standard errors for three pesticide treatments of two formulations of chlorfenapyr after 96-hours in the All treatment arena. (AR *T. inclusum*: $F(1,6) = 0.40$, $P < 0.5504$; KS *T. inclusum*:$F(1,6) = 0.66$, $P < 0.4490$; Lab *T. variabile*: $F(1,6) = 0.30$, $P < 0.6051$; *T. castaneum*: $F(1,6) = 1.32$, $P < 0.2936$). (PI-L) Pressurized Insecticide Label Rate Formulation, (SC-E) Suspension Concentrate Effective Rate Formulation, (SC-L) Suspension Concentrate Label Rate Formulation.

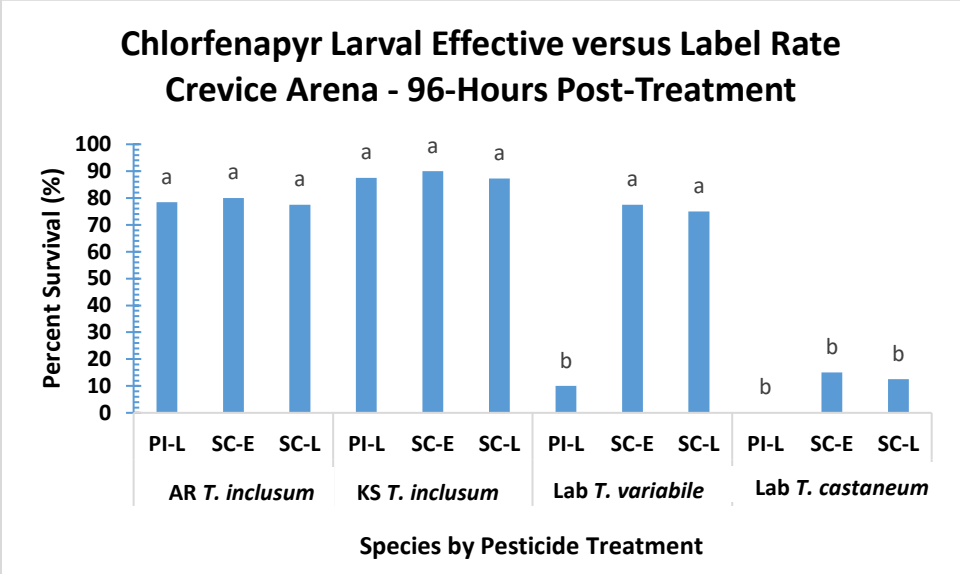


Figure 3.4 Mean percent survival of four populations of stored product larvae with standard errors for three pesticide treatments of two formulations of chlorfenapyr after 96-hours in the Crevice only treatment arena. (AR *T. inclusum*: $F(1,6) = 0.09$, $P < 0.7796$; KS *T. inclusum*:$F(1,6) = 0.14$, $P < 0.7229$; Lab *T. variabile*: $F(1,6) = 0.08$, $P < 0.7908$; *T. castaneum*: $F(1,6) = 8.34$, $P < 0.0278$). (PI-L) Pressurized Insecticide Label Rate Formulation, (SC-E) Suspension Concentrate Effective Rate Formulation, (SC-L) Suspension Concentrate Label Rate Formulation.

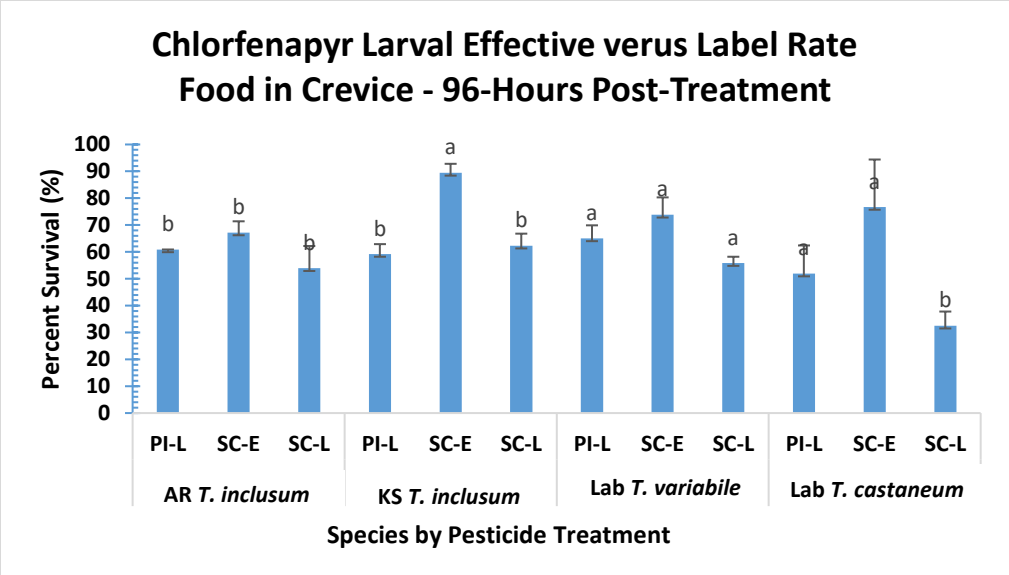


Figure 3.5. Mean percent survival of four populations of stored product larvae with standard errors for three pesticide treatments of two formulations of chlorfenapyr after 96-hours in the Food in Crevice treatment arena. (AR *T. inclusum*: F(1,6) = 2.17, P < 0.1912; KS *T. inclusum*:F(1,6) = 16.54, P < 0.0066; Lab *T. variabile*: F(1,6) = 2.78, P < 0.1468 ; *T. castaneum*: F(1,6) = 23.20, P <0.0029). (PI-L) Pressurized Insecticide Label Rate Formulation, (SC-E) Suspension Concentrate Effective Rate Formulation, (SC-L) Suspension Concentrate Label Rate Formulation.

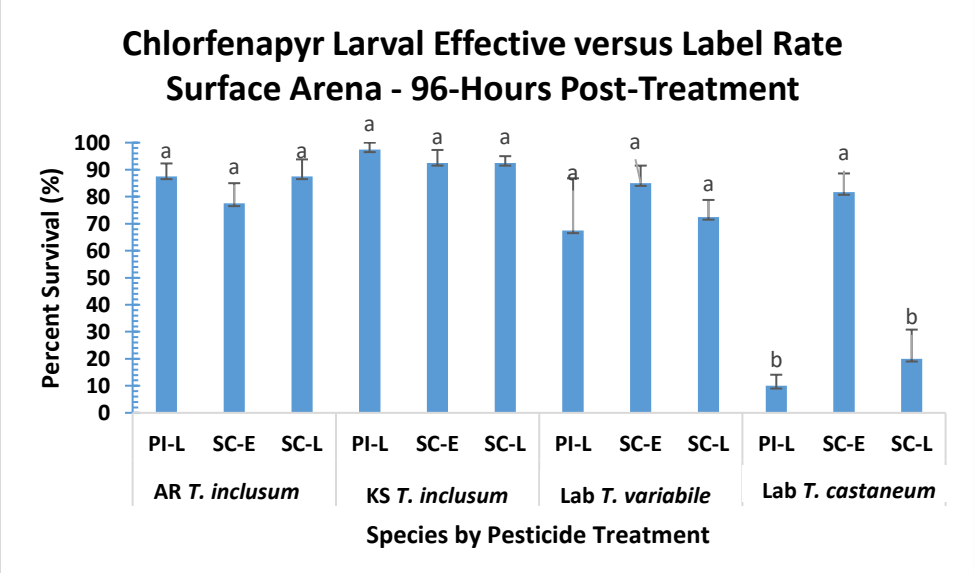


Figure 3.6. Mean percent survival of four populations of stored product larvae with standard errors for three pesticide treatments of two formulations of chlorfenapyr after 96-hours in the Surface only treatment arena. (AR *T. inclusum*: F(1,6) = 1.0.4, P < 0.3464; KS *T. inclusum*:F(1,6) = 0.00, P < 1.0000; Lab *T. variabile*: F(1,6) = 2.78, P < 0.1468; *T. castaneum*: F(1,6) = 23.20, P <0.0029). (PI-L) Pressurized Insecticide Label Rate Formulation, (SC-E)

Suspension Concentrate Effective Rate Formulation, (SC-L) Suspension Concentrate Label Rate Formulation.

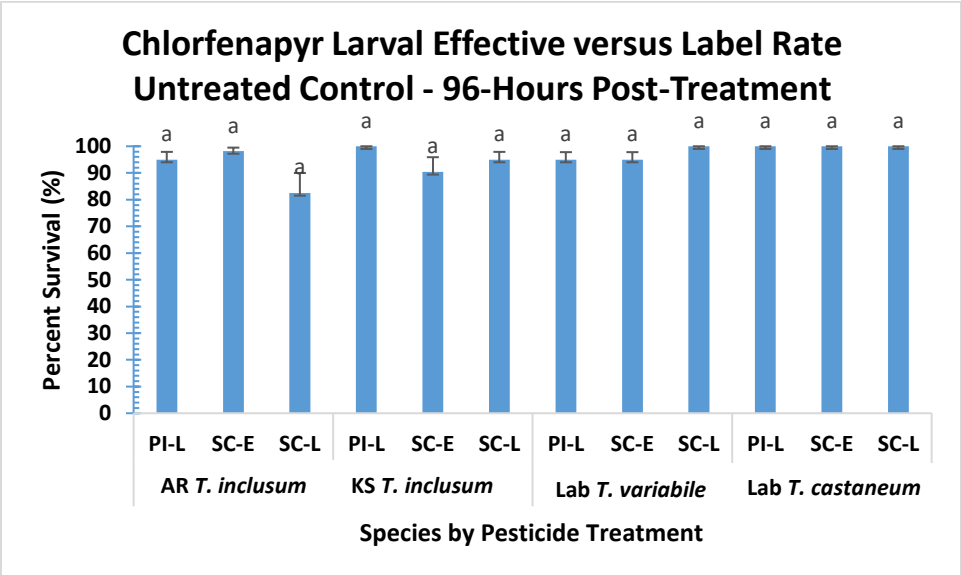


Figure 3.7. Mean percent survival of four populations of stored product larvae with standard errors for three pesticide treatments of two formulations of chlorfenapyr after 96-hours in the Untreated Control arena. (AR *T. inclusum*: $F(1,6) = 1.33$, $P < 0.2932$; KS *T. inclusum*: $F(1,6) = 0.53$, $P < 0.4933$; Lab *T. variabile*: $F(1,6) = 0.09$, $P < 0.7796$; *T. castaneum*: $F(1,6) = 3.00$, $P < 0.1340$). (PI-L) Pressurized Insecticide Label Rate Formulation, (SC-E) Suspension Concentrate Effective Rate Formulation, (SC-L) Suspension Concentrate Label Rate Formulation.

Experiment 4: Pyrethrin Aerosol Bioassay

Vertical results:

Pyrethrin results were recorded post-treatment from treated sheds after 0.5 hours, 1.0 hours, and 2.0 hours exposure. Results reported are for weeks one and three. Results are presented for the two treatments, 'fan' and 'no fan'. All untreated controls for 0.5 hour, 1.0 hour, and 2.0 hour treatments were at 96.3 to 100.0 percent survival (Figure 3.8) for both the 96-hour and three week evaluations.

Fan treatments:

In the fan treatment *T. castaneum* had the highest survival across all three time holding periods. There was no significant difference between species survival at either 96-hours or at 3 weeks post-treatment.

Figure 3.9 shows that, at 96-hours after the 0.5-hour treatment, more *T. inclusum* eggs remained viable than *T. castaneum* eggs. In contrast, more *T. castaneum* larvae and pupae remained alive than *T. inclusum* larvae and pupae. At 3 weeks post-treatment, more *T. castaneum* larvae and pupae were still alive, but no *T. inclusum* eggs survived (Figure 3.10). *T. castaneum* larvae survival was significantly higher, statistically, than all other life stages at all three height levels for *T. castaneum* and *T. inclusum* at the 0.5-hour treatment time. Scheff et al., 2026 (unpublished data) has shown that holding a treatment for 0.5-hours post-treatment is effective at controlling pest populations. For the vertical 0.5-hour no-fan treatment, Figure 3.15, eggs of *T. inclusum*, larvae and pupae of both *T. inclusum* and *T. castaneum* were still alive at 96-hours post-treatment. When surviving 0.5-hour no-fan treatment individuals were held to three weeks, Figure 3.16, the only surviving individuals were *T. castaneum* pupae at 13.3% survival.

At the 1.0-hour fan treatment 96-hour data shows a lower survivorship than at the 0.5-hour treatment 96-hour post-treatment where the higher survivorship were the *T. castaneum* larvae (Figure 3.11). At the medium and high vertical levels, having 40.0% and 43.3% surviving, respectively. The larvae in general have the highest survivorship in both species. By three weeks post 1.0-hour fan treatment, that survivorship drops to 6.7% at the medium vertical height and 9.7% at the high vertical height for *T. castaneum*. *T. inclusum* has the highest surviving individuals at three weeks post-treatment with 13.3% larval survivorship (Figure 3.12).

Figure 3.13 shows the results of 2.0-hour pyrethrin vertical treatment at 96-hours post-treatment. Compared to 0.5- and 1.0-hour results, these are the lowest number of survivors. The results differ in that the *T. inclusum* larvae survive in larger numbers than *T. castaneum* at the low, medium and high levels. At three weeks post-treatment (Figure 3.14), the only remaining *T. inclusum* are larvae at the vertical high level at 16.7%. *T. castaneum* larvae survive at 6.7% at the low vertical level, and 10.0% at the medium and high vertical levels.

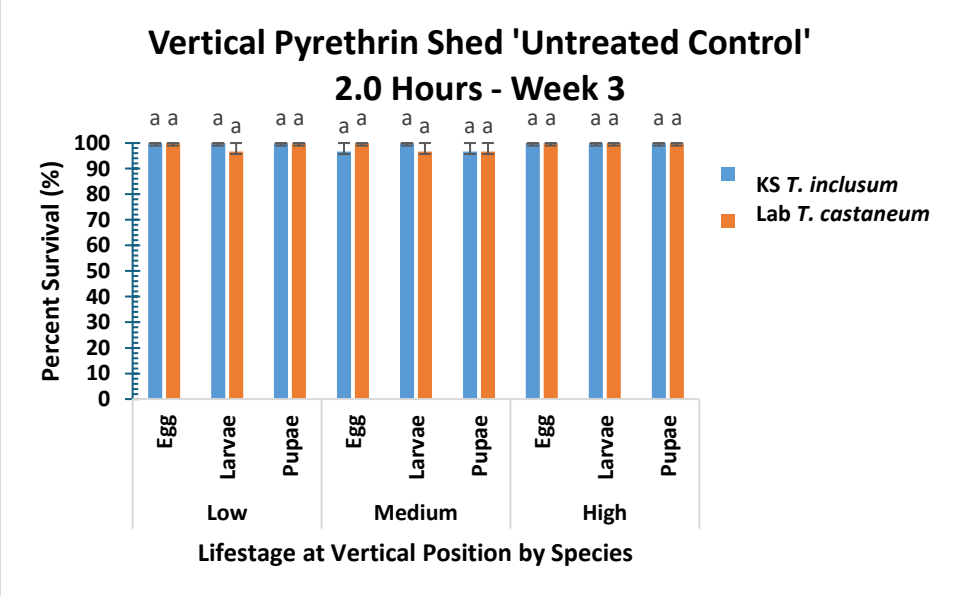


Figure 3.8. Mean percent survival of two populations of three stored product species life stages with standard errors for pyrethrin control shed at three vertical heights for 2.0 hours, at three weeks post-treatment is representative of all untreated controls. (KS *T. inclusum*: $F(2,6) = 1.00$, $P < 0.4219$; *T. castaneum*: $F(2,6) = 4.00$, $P < 0.0787$).

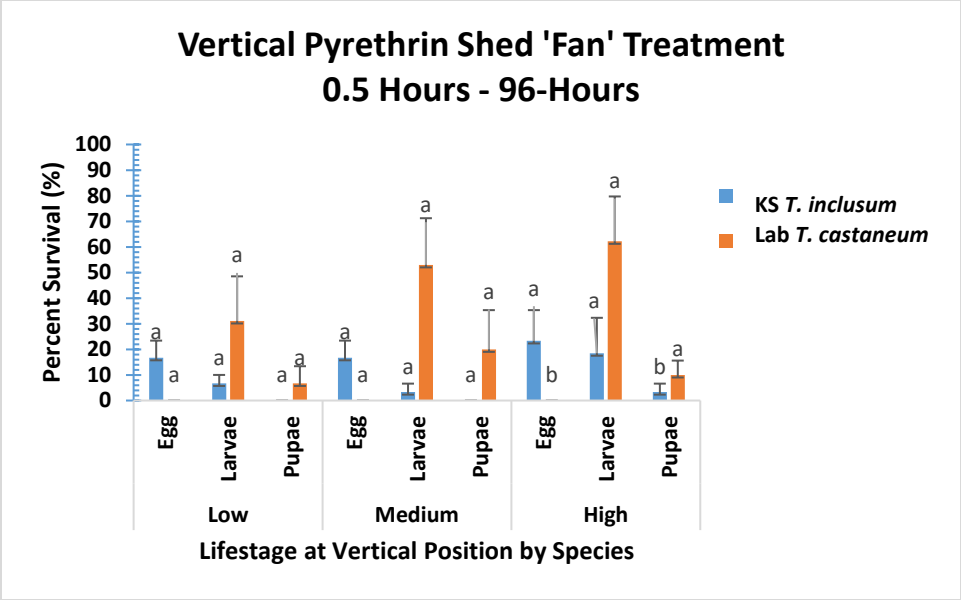


Figure 3.9. Mean percent survival of two populations of three stored product species life stages with standard errors for pyrethrin fan treatment at three vertical heights for 0.5-hour, 96-hours post-treatment. (KS *T. inclusum*: $F(2,6) = 0.90$, $P < 0.4568$; *T. castaneum*: $F(2,6) = 0.82$, $P < 0.8056$).

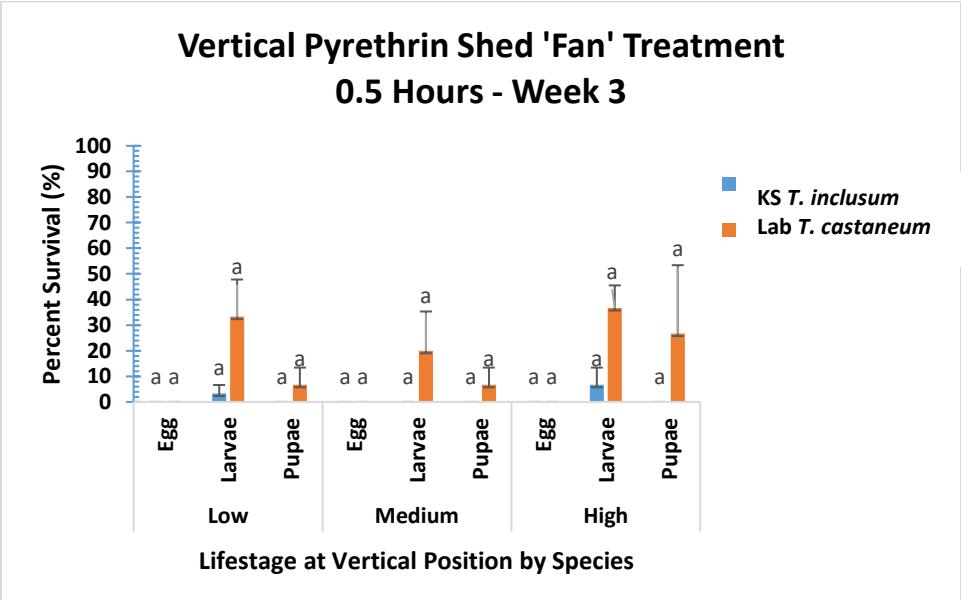


Figure 3.10. Mean percent survival of two populations of three stored product species life stages with standard errors for pyrethrin fan treatment at three vertical heights for 0.5-hour, three weeks post-treatment. (KS *T. inclusum*: $F(2,6) = 0.60$, $P < 0.5787$; *T. castaneum*: $F(2,6) = 0.45$, $P < 0.6593$).

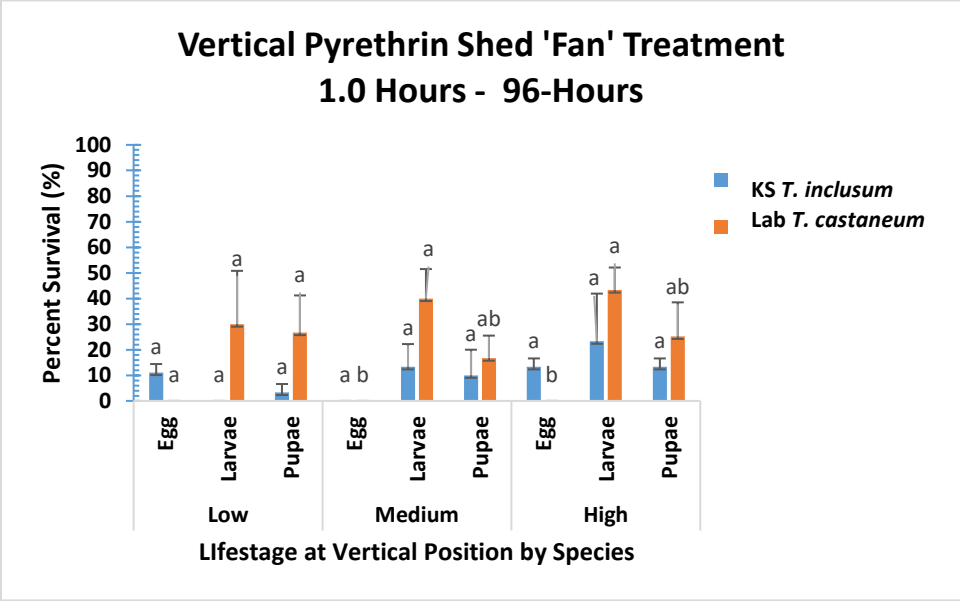


Figure 3.11. Mean percent survival of two populations of three stored product species life stages with standard errors for pyrethrin fan treatment at three vertical heights for 1.0-hour, 96-hours post-treatment. (KS *T. inclusum*: $F(2,6) = 0.97$, $P < 0.4303$; *T. castaneum*: $F(2,6) = 0.22$, $P < 0.8056$).

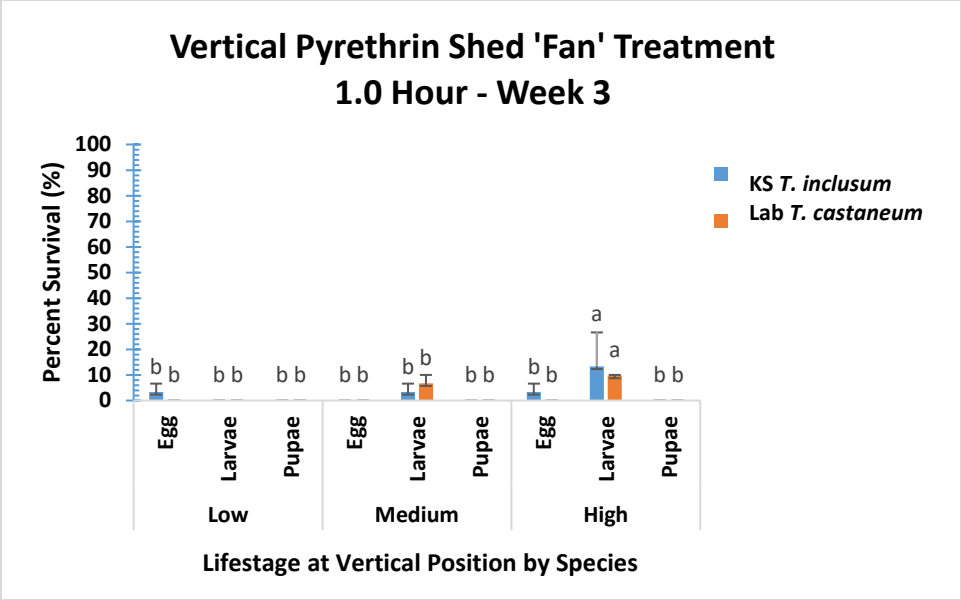


Figure 3.12. Mean percent survival of two populations of three stored product species life stages with standard errors for pyrethrin fan treatment at three vertical heights for 1.0-hour, three weeks post-treatment. (KS *T. inclusum*: F(2,6) = 0.50, P < 0.6297; *T. castaneum*: F(2,6) = 6.59, P <0.0306).

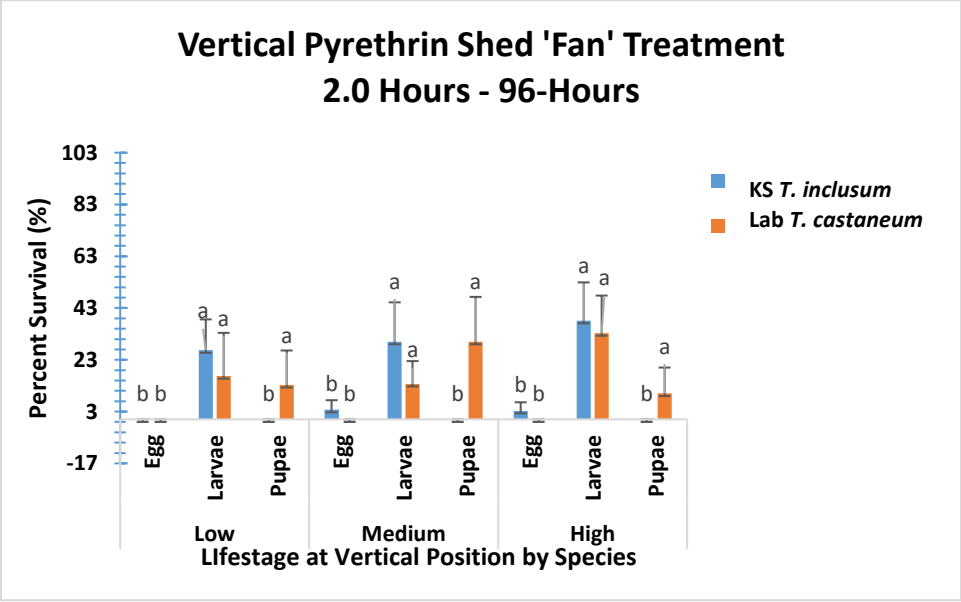


Figure 3.13. Mean percent survival of two populations of three stored product species life stages with standard errors for pyrethrin fan treatment at three vertical heights for 2.0-hours, 96-hours post-treatment. (KS *T. inclusum*: F(2,6) = 0.08, P < 0.9252; *T. castaneum*: F(2,6) = 0.59, P < 0.5816).

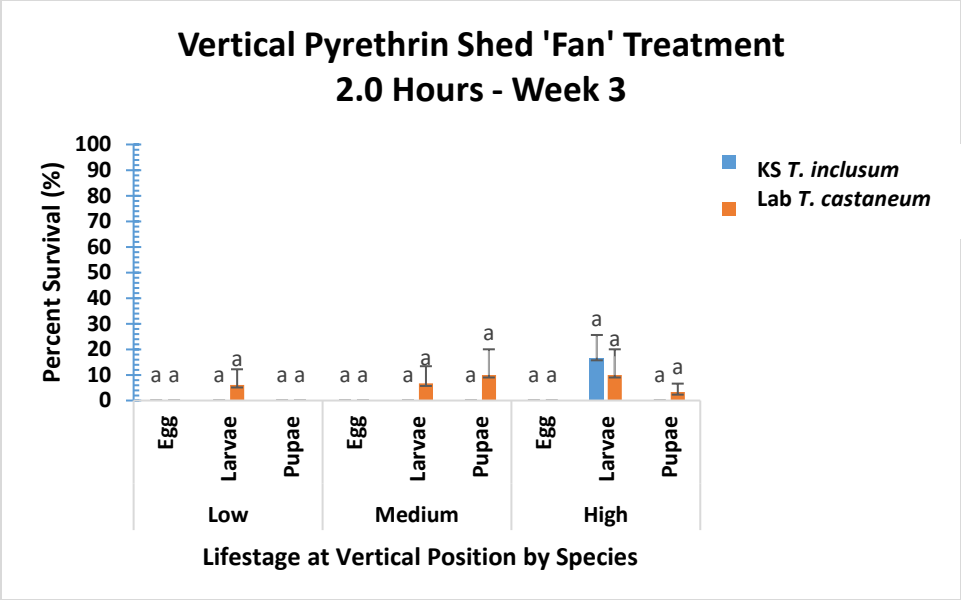


Figure 3.14. Mean percent survival of two populations of three stored product species life stages with standard errors for pyrethrin fan treatment at three vertical heights for 2.0-hours, three weeks post-treatment. (KS *T. inclusum*: $F(2,6) = 3.57$, $P < 0.0951$; *T. castaneum*: $F(2,6) = 0.07$, $P < 0.9290$).

No Fan treatments:

In the no-fan treatment, *T. castaneum* had the highest percentage of survival across all three time holding periods. There was no significant difference between life stages at all vertical levels of low, medium or high within 96-hours post-treatment or at week three post-treatment. The no-fan treatments at week three post-treatment have fewer survivors than the fan treatments at week three. However, the treatments are not significantly different by time (week) post-treatment.

The 96-hour post-treatment results for the 0.5-hour treatment resulted in no egg survival for *T. castaneum* at all three vertical levels (Figure 3.15). *Trogoderma inclusum* had very low egg survival rates for at all three vertical levels of 6.7% at the low and medium levels, and 3.3% at the high vertical level. Pupae had the highest survival rates at all three levels for *T. inclusum* and for the low and medium levels for *T. castaneum*, with the larvae having the most

survivability at the high vertical level (Figure 15). By week three post-treatment for 0.5-hour treatment, the only survivors were *T. castaneum* pupae at 13.3% (Figure 3.16).

The 1.0-hour no-fan post-treatment results show that *T. inclusum* pupae have higher survival rates at the low (31.8%) and medium (16.7%) vertical levels and that *T. castaneum* larvae had the higher survival rate (23.3%) at the high vertical level (Figure 3.17). Similar to the 0.5-hour pyrethrin treatment the 1.0-hour treatment survival rates at three weeks post-treatment drops to 3.3% and under. *Tribolium cataneum* larvae survive at 3.3% each for low level and high vertical levels. *Trogoderma inclusum* eggs survived at 3.0% at the medium vertical level (Figure 3.18).

Pyrethrin treatments for 2.0 hours only had larval and pupal survivors 96-hours post-treatment (Figure 3.19). *Tribolium castaneum* pupae had the highest survival at all three height levels, 30.0% at the low and medium vertical levels and 26.7% at the high vertical level. Larvae only survived at the medium and high vertical levels. *Trogoderma inclusum* pupae only survived at the low (3.3%) and medium (10.0%) heights. All three *T. inclusum* life stages survived at the medium vertical height level. At three weeks post-treatment (Figure 3.20) only 6.7% of *T. inclusum* larvae survived.

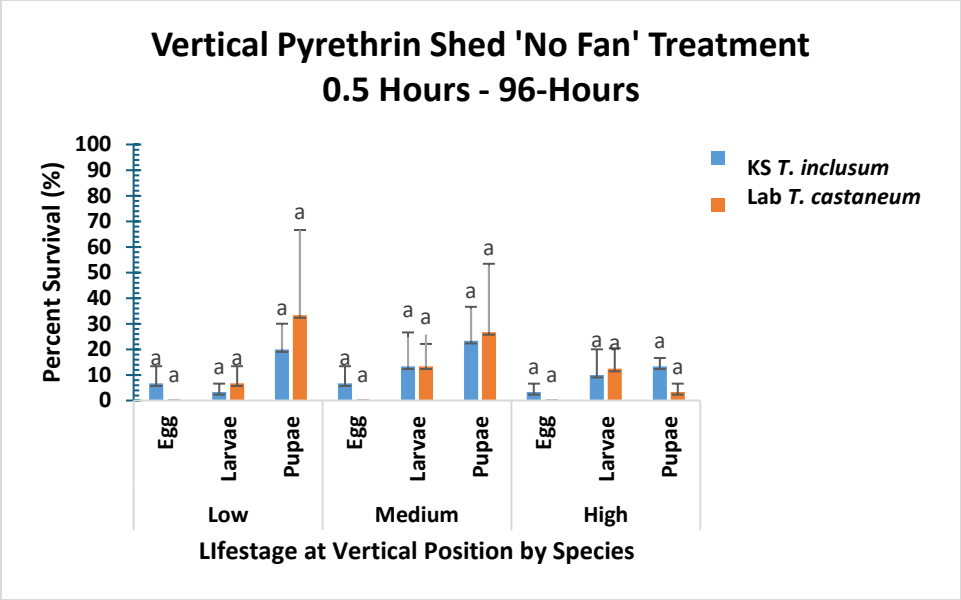


Figure 3.15. Mean percent survival of two populations of three stored product species life stages with standard errors for pyrethrin fan treatment at three heights for 0.5-hour, 96-hours post-treatment. (KS *T. inclusum*: F(2,6) = 0.60, P < 0.5787; *T. castaneum*: F(2,6) = 0.45, P <0.6593).

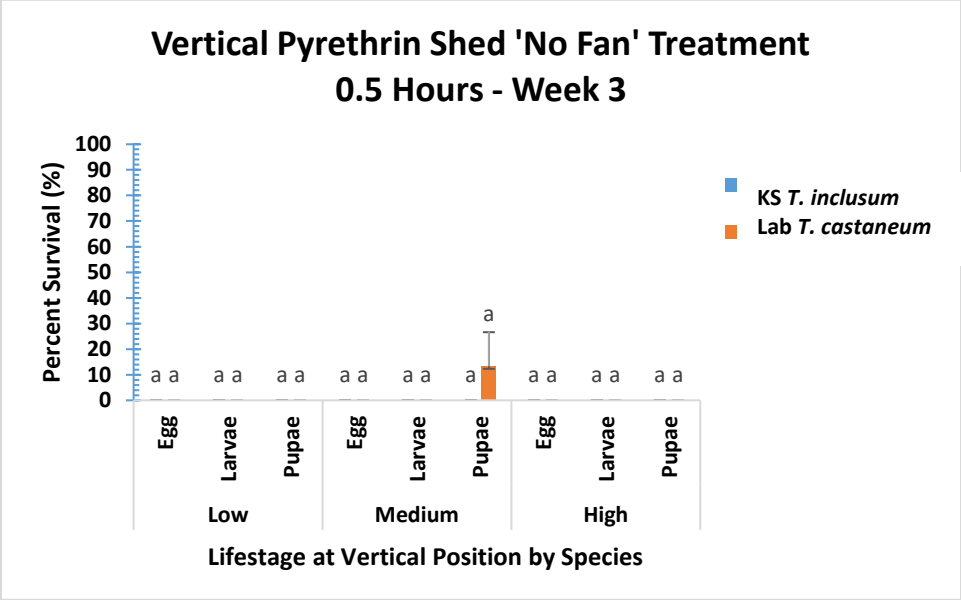


Figure 3.16. Mean percent survival of two populations of three stored product species life stages with standard errors for pyrethrin fan treatment at three heights for 0.5-hour, three weeks post-treatment. (KS *T. inclusum*: F(2,6) = 0.00, P < 0.00; *T. castaneum*: F(2,6) = 1.00, P <0.4219).

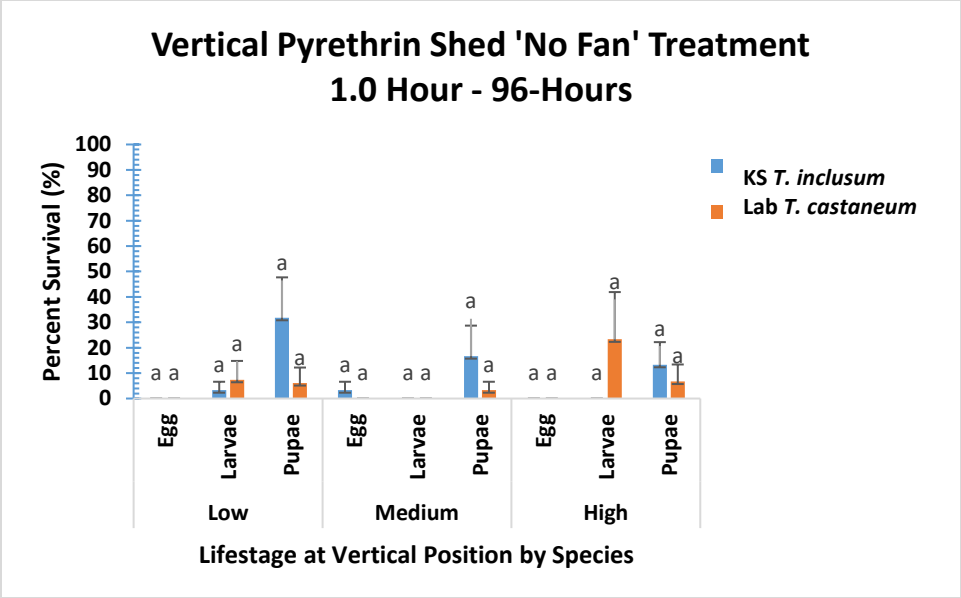


Figure 3.17. Mean percent survival of two populations of three stored product species life stages with standard errors for pyrethrin fan treatment at three heights for 1.0-hour, 96-hours post-treatment. (KS *T. inclusum*: F(2,6) = 1.00, P < 0.4219; *T. castaneum*: F(2,6) = 1.07, P <0.4011).

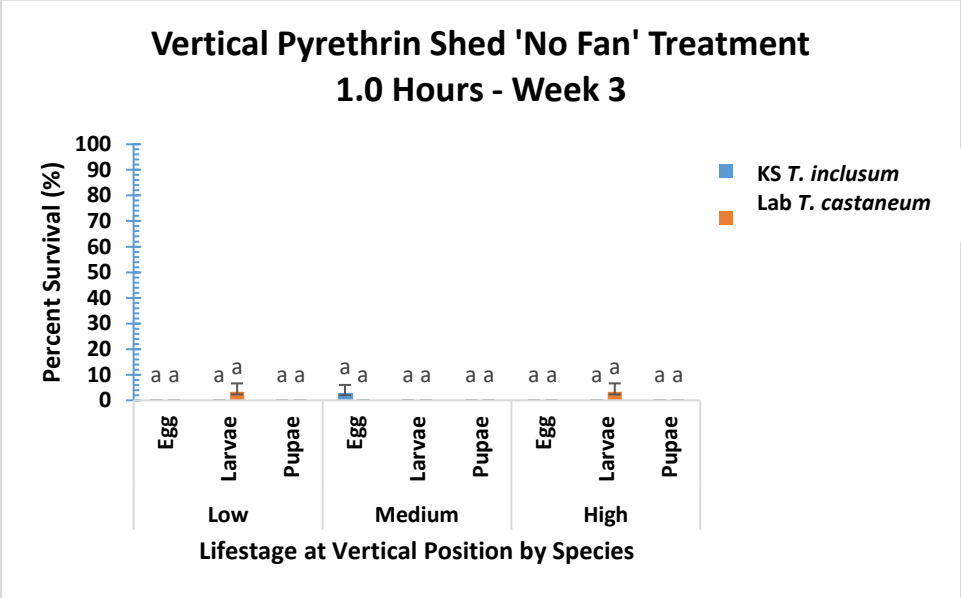


Figure 3.18. Mean percent survival of two populations of three stored product species life stages with standard errors for pyrethrin fan treatment at three heights for 1.0-hour, three weeks post-treatment. (KS *T. inclusum*: F(2,6) = 1.00, P < 0.4219; *T. castaneum*: F(2,6) = 0.50, P <0.6297).

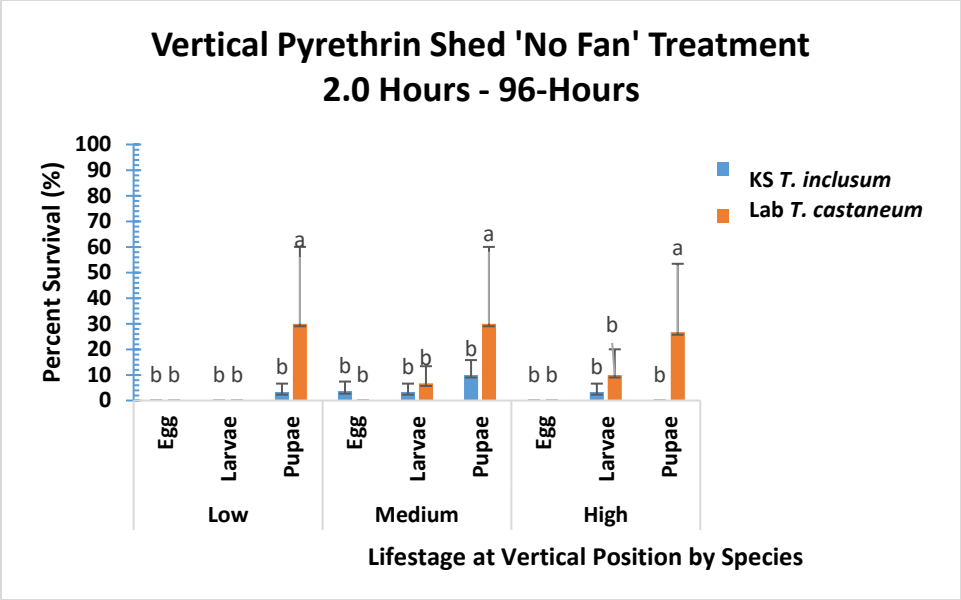


Figure 3.19. Mean percent survival of two populations of three stored product species life stages with standard errors for pyrethrin fan treatment at three heights for 2.0-hours, 96-hours post-treatment. (KS *T. inclusum*: F(2,6) = 0.08, P < 0.9252; *T. castaneum*: F(2,6) = 0.59, P <0.5816).

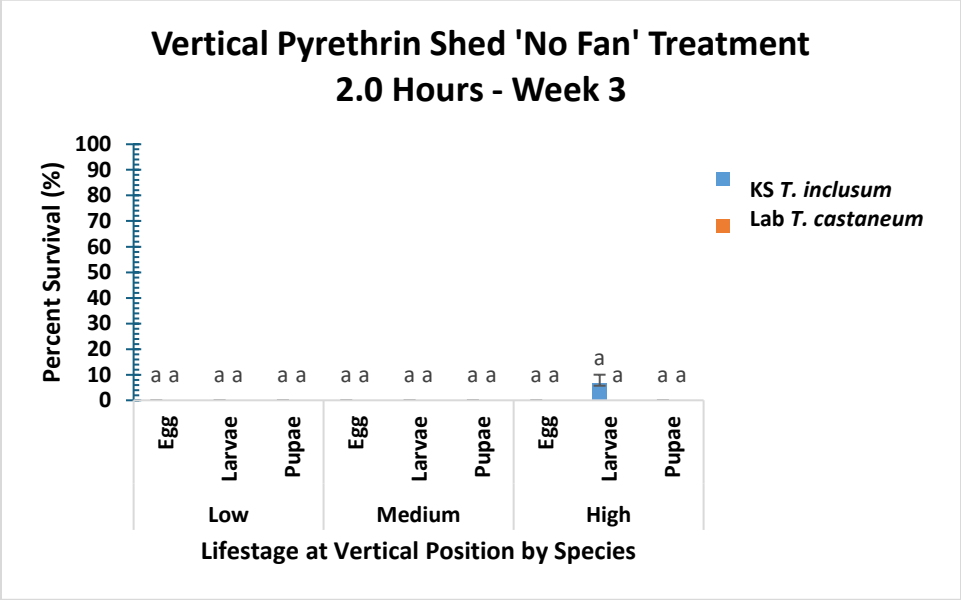


Figure 3.20. Mean percent survival of two populations of three stored product species life stages with standard errors for pyrethrin fan treatment at three heights for 2.0-hours, three weeks post-treatment. (KS *T. inclusum*: F(2,6) = 3.57, P < 0.0951; *T. castaneum*: F(2,6) = 0.07, P <0.9290).

The vertical results show how pyrethrin aerosol treatments may work in a stacked warehouse where there is ample room between shelves for exposed life stages of *T. inclusum* and *T. castaneum* to be exposed. The differences in the fan versus no-fan treatments, while not statistically significant, are consistent with other research conducted by Frank Arthur (personal communication, 2017). This data shows that pyrethrin aerosol treatments are an effective tool for controlling *T. inclusum* and *T. castaneum* in vertical settings. Even though the fogging machine was held below the medium and high vertical height levels, the fogging action from the machine was pointed upwards and filled the space where insects at higher levels were exposed to an effective dose of pyrethrin. Longer treatment times resulted in slightly, but not significantly, higher levels of mortality at 96-hours post-treatment. However, by three weeks post-treatment, survival had dropped to zero in many treatments, most under 13.3%. There were a few survivors over 13.3% to 36.7%. Of those with higher survivability, most were the larval life stage, followed by the pupal stage and in the fan treatment at the 0.5-hour treatment time.

Horizontal results

The horizontal results for *T. inclusum* and *T. castaneum* larvae differ from the vertical height data in that four of the horizontal positions (2-5) are under a solid box cover, similar to insects being in stacked box areas or insects hiding in facility sites that are not open to direct aerosol treatment. Horizontal position 1, placed 7.5-cm in front of the horizontal box, is the only Petri dish without a cover. All untreated control treated arenas for the horizontal results under the 10-cm and 20-cm boxes and for both species, *T. inclusum* and *T. castaneum*, ranged from 93.3% survival to 100.0% survival at both 96-hours post-treatment and three weeks post-treatment. Figure 3.21 is representative of the overall control survival at the longest treatment time of 2.0-hours and three weeks post-treatment.

Fan treatment:

At 0.5-hour treatment, 96-hours post-treatment, *T. inclusum* larvae had the lowest survival rate at position 1 for both the 10-cm box height (40.0%) and the 20-cm box height (73.3%) (Figure 3.22). At the 10-cm box height, position 1 had less than half the survival of positions 2 through 5. *Tribolium castaneum* had a lower survival rate at position 1 for the 20-cm box height than the 10-cm box height. Position 2 had the lowest survival rate for the *T. castaneum* 10 cm box height. *Trogoderma inclusum* had 100.0% survival under the 10-cm box height for positions 3, 4, and 5 indicating that there was no exposure to the treatment. There was no significant difference between the 10-cm or the 20-cm box height for the *T. inclusum* or *T. castaneum* survival. No survival rate was below 40% at 96-hours post-treatment. Survival rates under the boxes for positions 2 through 5 were 68.1% and higher for both species tested. When held for three weeks post-treatment (Figure 3.23) *T. inclusum* survival for position 1 dropped to 13.3% and 65% at the 10-cm and 20-cm box heights, respectively. *Tribolium castaneum* had 26.7% and 56.7% survival for the 10-cm and 20-cm box heights, respectively.

When the treatment was held for 1.0-hour, data for the 96-hour post-treatment (Figure 3.24), where *T. inclusum* had 20.0% survival at the 10-cm box height and 30.0% at the 20-cm box height, was significantly lower survival rate than *T. castaneum*. *Tribolium castaneum* survival results were similar to the 0.5-hour treatment where the lower survival rate for position 1 was inverted that the lower survival rate was at the 20-cm box height. *Tribolium castaneum* had a survival rate of 86.7% at 10-cm and 73.3% at 20 cm box height. At 96-hours post-treatment all positions under the box height, positions 2 through 5, had a survival rate of 63.3% and higher in the 1.0-hour fan treatment. After holding samples for three weeks post-treatment, while survival dropped, the general data pattern resembles more of a curve under the box (Figure

3.25) than it did at 96-hours post-treatment (Figure 3.24). Also, at three weeks post-treatment, *T. inclusum* had a similar survival rate in positions 1 through 3 versus positions 4 and 5 for the 10-cm box height. Whereas *T. castaneum* had similar survival rates for positions 1 and 2 compared to positions 3, 4, and 5 for the 10-cm box height.

A similar pattern occurs in the 2.0-hour treatment at 96-hours post-treatment where the lowest survival rate is at position 1 for both *T. inclusum* and *T. castaneum* within species (Figure 3.26) for the 10-cm box height. At three weeks post 2.0-hour treatment, *T. castaneum* had the lowest survival rate at the 20-cm box height for positions 1 (13.3%) and 5 (20.0%). *Trogoderma inclusum* had the lowest survival rate at positions 1 and 3 under the 10 cm box height where both were 40.0% (Figure 3.27).

Looking across timed treatments, positions 3, 4, and 5 had the most 100.0% survival rates at 96-hours post-treatment, all were *T. inclusum* (Figures 3.22, 3.24, and 3.26). At the 0.5-hour treatment, all 100.0% survival rates were under the 10 cm box height at positions 3, 4, and 5. For the 1.0 hour, the only 100.0% survival rate site was position 5 under the 10-cm box height. And for the 2.0-hour treatment, there were three 100.0% survival rate sites and those were position 3 under the 10-cm and 20-cm box heights and position 4 under the 10-cm box height. This is an indication that either the pyrethrin aerosol did not reach those areas, or at least did not reach those areas with enough active ingredient to have an effect on the *T. inclusum,* 96-hours post-treatment.

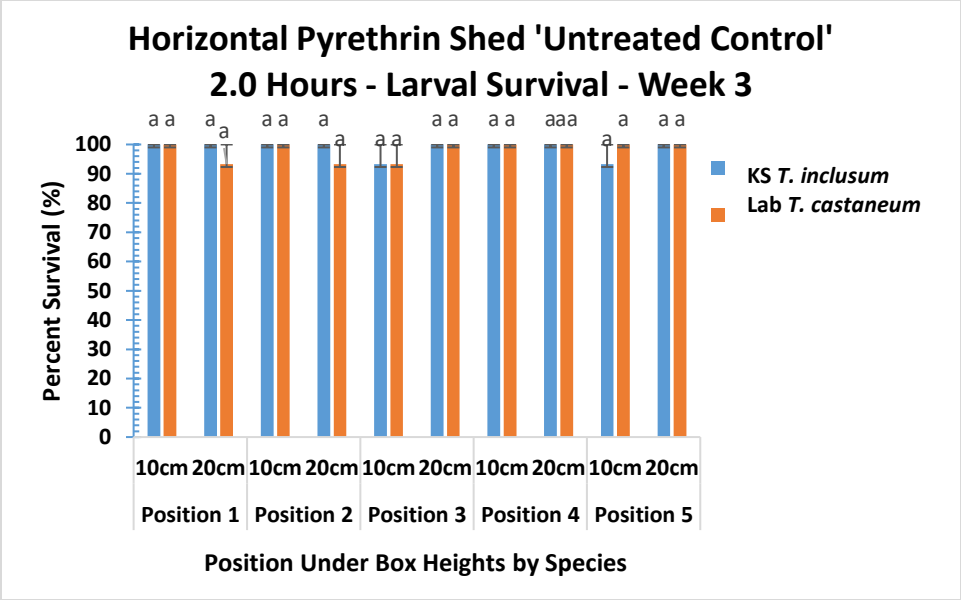


Figure 3.21. Mean percent survival of two larval populations with standard errors for pyrethrin untreated control shed at five horizontal positions under a 10-cm height box and 20-cm height box for 2.0-hours, three weeks post-treatment. (Position 3: KS *T. inclusum*: F(1,4) = 1.00, P < 0.3739; *T. castaneum*: F(2,6) = 1.00, P <0.3739).

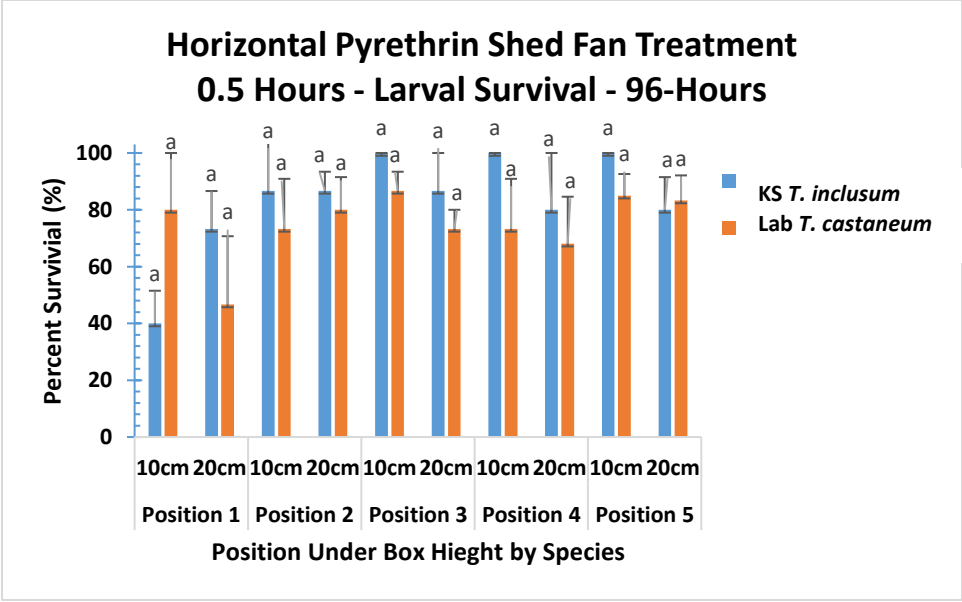


Figure 3.22. Mean percent survival of two larval populations with standard errors for pyrethrin fan treatment at five horizontal positions under a 10-cm height box and 20-cm height box for 0.5-hour, 96-hours post-treatment. (Position 3: KS *T. inclusum*: F(1,4) = 1.00, P < 0.3739; *T. castaneum*: F(2,6) = 2.00, P <0.2302).

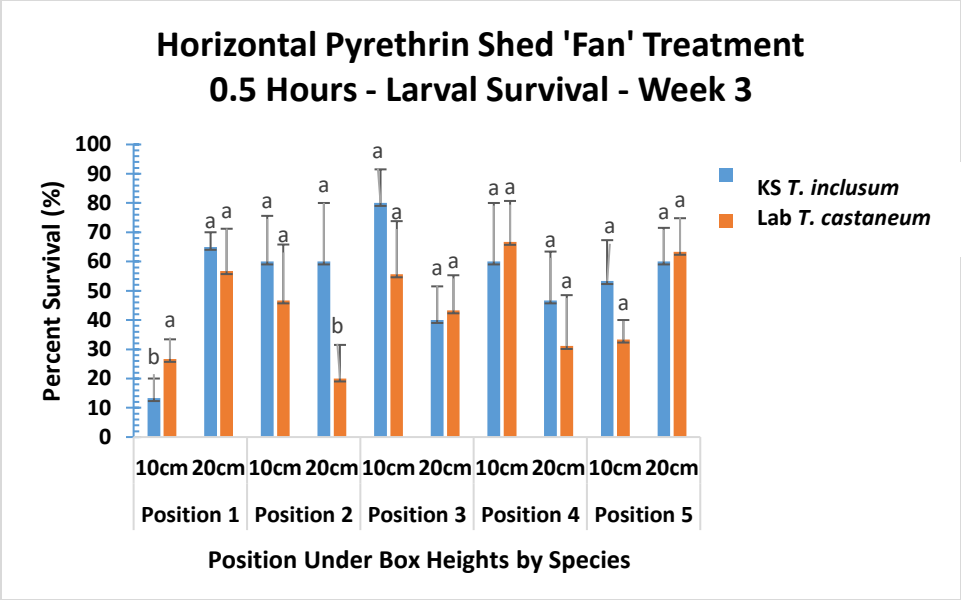


Figure 3.23. Mean percent survival of two larval populations with standard errors for pyrethrin fan treatment at five horizontal positions under a 10-cm height box and 20-cm height box for 0.5-hour, three weeks post-treatment. (Position 3: KS *T. inclusum*: F(1,4) = 6.00, P < 0.0705; *T. castaneum*: F(2,6) = 0.31, P <0.6050).

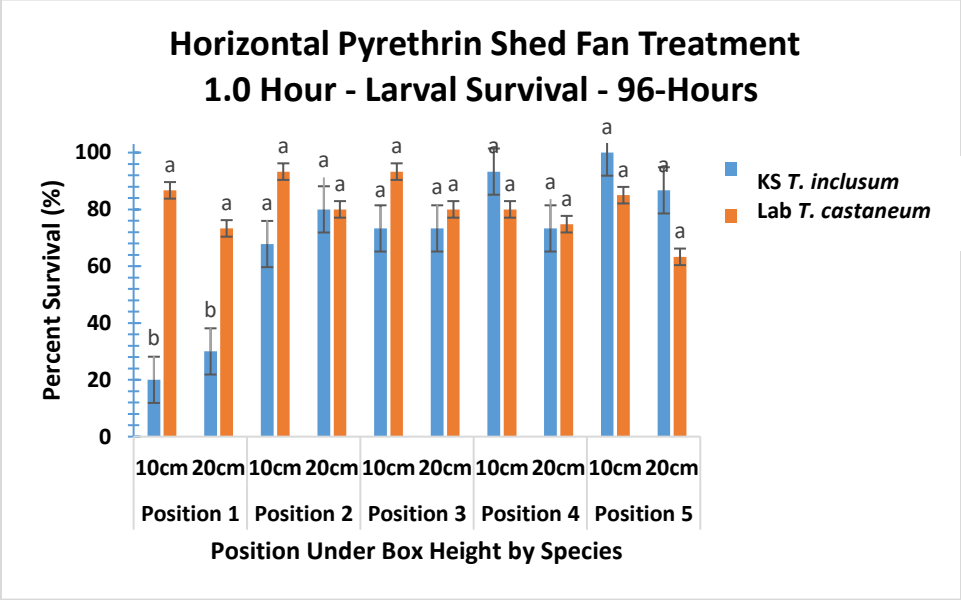


Figure 3.24. Mean percent survival of two larval populations with standard errors for pyrethrin fan treatment at five horizontal positions under a 10-cm height box and 20-cm height box for 1.0-hour, 96-hours post-treatment. (Position 3: KS *T. inclusum*: F(1,4) = 0.00, P < 1.0000; *T. castaneum*: F(2,6) = 1.00, P <0.3739).

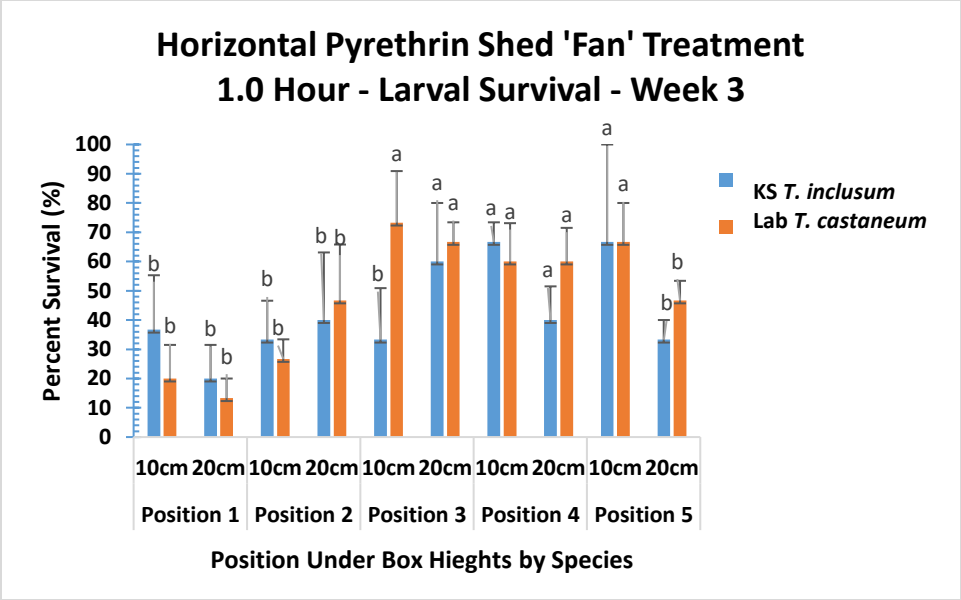


Figure 3.25. Mean percent survival of two larval populations with standard errors for pyrethrin fan treatment at five horizontal positions under a 10-cm height box and 20-cm height box for 1.0-hour, three weeks post-treatment. (Position 3: KS *T. inclusum*: F(1,4) = 1.00, P < 0.3739; *T. castaneum*: F(2,6) = 0.12, P <0.7415).

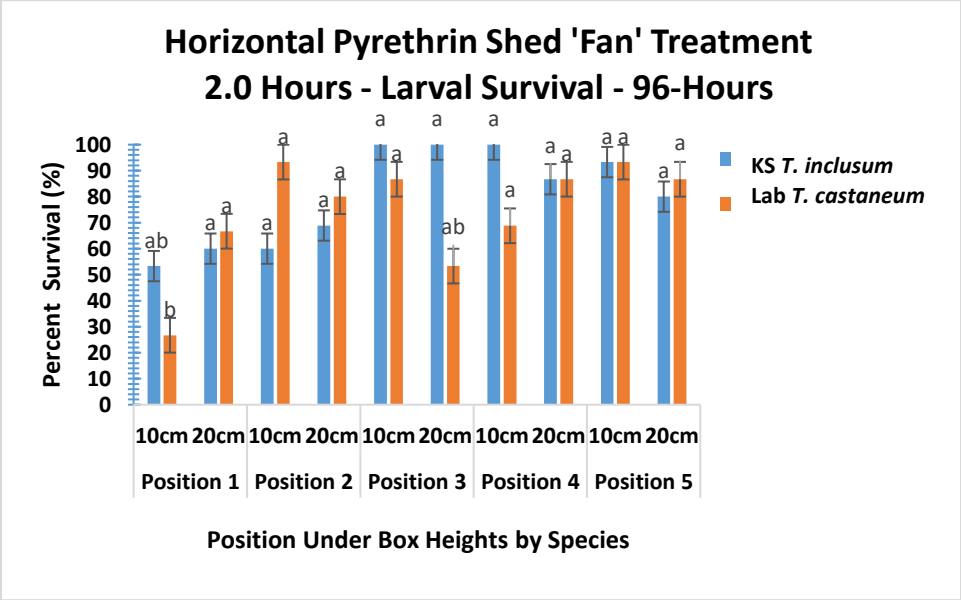


Figure 3.26. Mean percent survival of two larval populations with standard errors for pyrethrin fan treatment at five horizontal positions under a 10-cm height box and 20-cm height box for 2.0-hours, 96-hours post-treatment. (Position 3: KS *T. inclusum*: F(1,4) = 0.00, P < 0.0000; *T. castaneum*: F(2,6) = 3.13, P <0.1518).

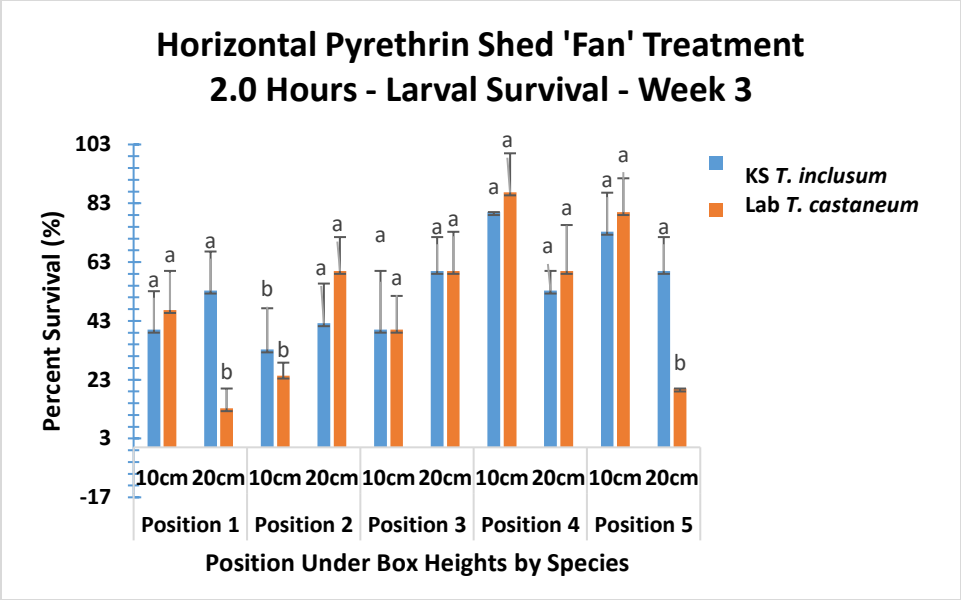


Figure 3.27.

Mean percent survival of two larval populations with standard errors for pyrethrin fan treatment at five horizontal positions under a 10-cm height box and 20-cm height box for 2.0-hours, three weeks post-treatment. (Position 3: KS *T. inclusum*: $F(1,4) = 0.75$, $P < 0.4353$; *T. castaneum*: $F(2,6) = 0.38$, $P < 0.5734$).

No-Fan Treatment:

The 0.5-hour larval survival with no fan after 96-hours post-treatment, *T. inclusum* had a higher survival rate at the 10-cm box height versus the 20-cm box height except at position 5 where the survival rate is the same at both heights (Figure 3.28). *Tribolium castaneum* also shows the same trend except at position 1 where the 20-cm box height had a higher survival than the 10-cm box height (Figure 3.28). Figure 3.29 shows week three post-treatment at the 0.5-hour post-treatment time *T. inclusum* had 0.0% survival at the 20-cm box height, position 1 and at the 20-cm box height, position 5. *Tribolium castaneum* had 20.0% survival and higher three weeks post-treatment.

At the 1.0-hour treatment, 96-hours post-treatment, the 10-cm box height, position 3 site (Figure 3.30), *T. inclusum* had 100.0% survival, by week three post-treatment (Figure 3.31) that

same site had 40% survival. *Tribolium castaneum* also had the highest survival rate at the 10-cm box height, position 3 site. For *T. castaneum*, positions 2, 3, and 4, under box height of 10-cm had higher survival rates than larvae under the 20-cm box height (Figure 3.30). At week three post-treatment, all survival rates were under 45.0% except for the *T. inclusum* 10-cm box height at position 2 (60.0%) and *T. castaneum* 20-cm box height at position 3 (60.0%) (Figure 3.31).

2.0-hours treatment, 96-hours post-treatment (Figure 3.32) resulted in the highest overall average survival rate compared to the 0.5-hour and the 1.0-hour treatment holding times. *Tribolium castaneum* survival is higher than *T. inclusum* at all recorded points ecxept the 10-cm box height at positions 3 and 4. Figure 3.33 shows the survival rates of larvae at 2.0-hours holding time treatment at three weeks post-treatment. In general, all survival rates were lowest after three weeks post-treatment than 0.5-hour and 1.0-hour post-treatment for both species.

Except for the 2 positions at 0.5 hour, 3 weeks post-treatment, none of the sites resulted in 0.0% survival rate using the pyrethrin aerosol application. Looking at the graphed data, there is a general trend of a survival curve which is highest for both box heights towards the center of each of the 10-cm and 20-cm box heights, typically positions 2 through 5 which are all under the box cover.

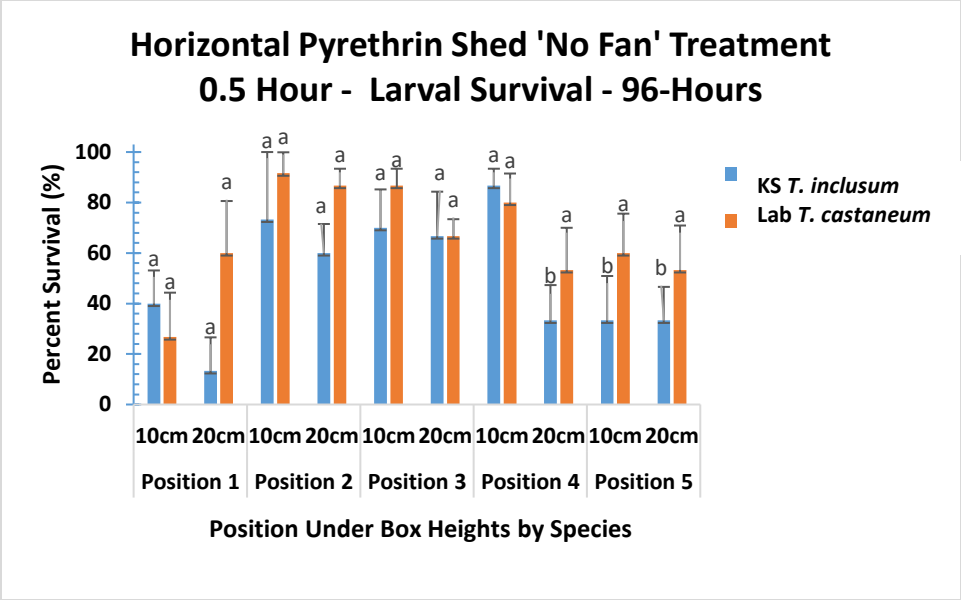


Figure 3.28. Mean percent survival of two larval populations with standard errors for pyrethrin no-fan treatment at five horizontal positions under a 10-cm height box and 20-cm height box for 0.5-hour, 96-hours post-treatment. (Position 3: KS *T. inclusum*: $F(1,4) = 0.02$, $P < 0.8933$; *T. castaneum*: $F(2,6) = 4.50$, $P < 0.1012$).

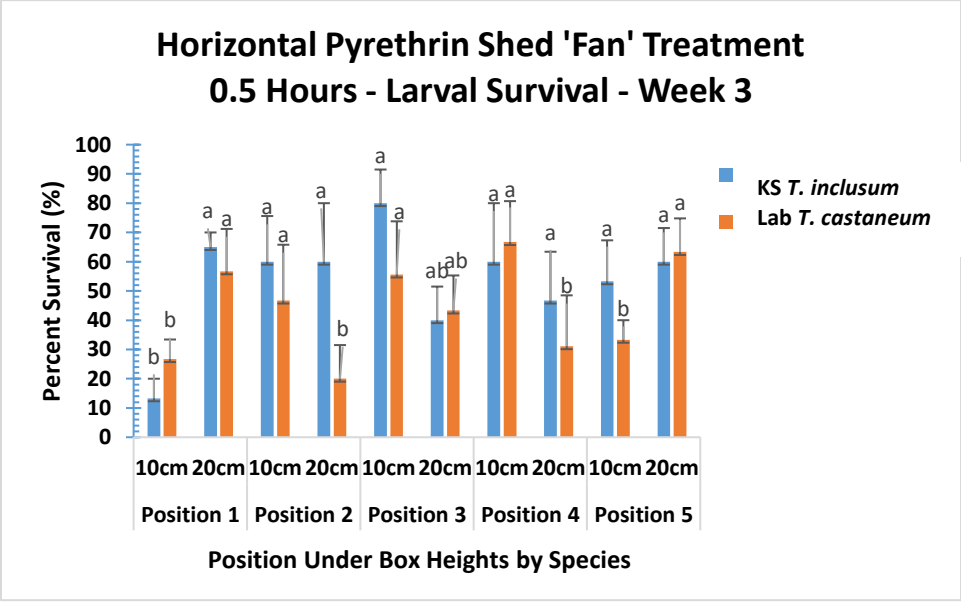


Figure 3.29. Mean percent survival of two larval populations with standard errors for pyrethrin no-fan treatment at five horizontal positions under a 10-cm height box and 20-cm height box for 0.5-hour, three weeks post-treatment. (Position 3: KS *T. inclusum*: $F(1,4) = 0.13$, $P < 0.7376$; *T. castaneum*: $F(2,6) = 0.38$, $P < 0.5734$).

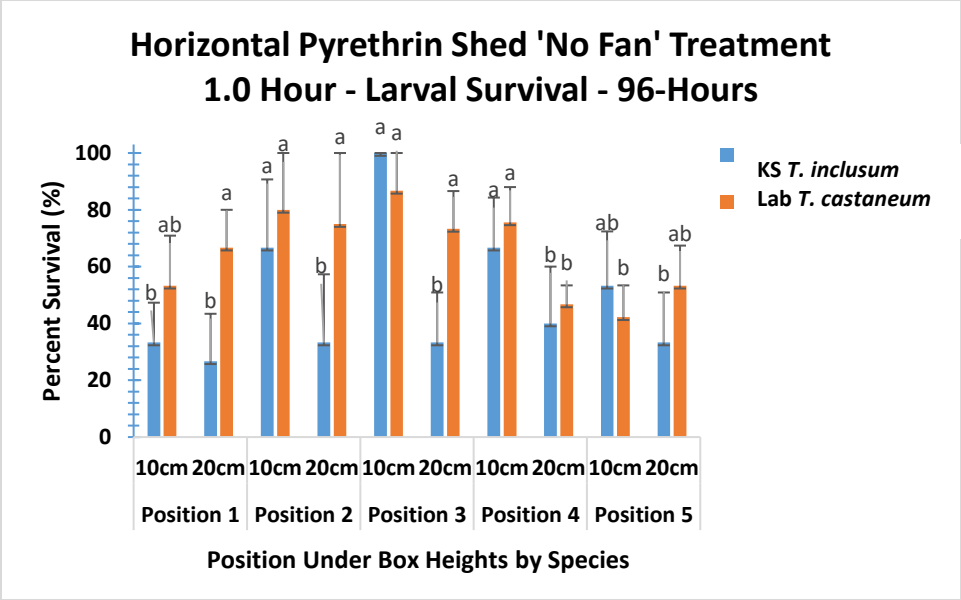


Figure 3.30. Mean percent survival of two larval populations with standard errors for pyrethrin no-fan treatment at five horizontal positions under a 10-cm height box and 20-cm height box for 1.0-hour, 96-hours post-treatment. (Position 3: KS *T. inclusum*: $F(1,4) = 14.29$, $P < 0.0194$; *T. castaneum*: $F(2,6) = 0.50$, $P < 0.5185$).

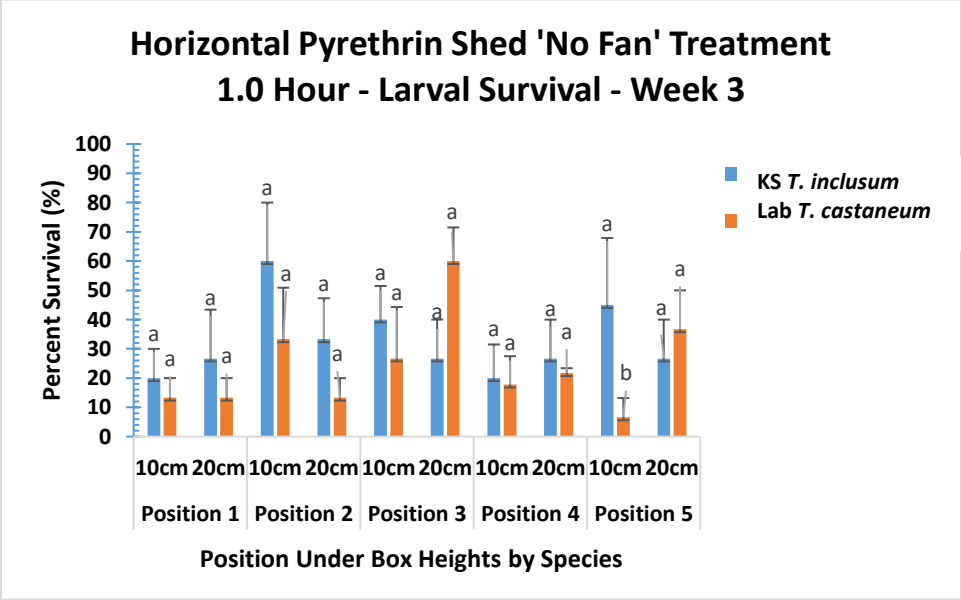


Figure 3.31. Mean percent survival of two larval populations with standard errors for pyrethrin no-fan treatment at five horizontal positions under a 10-cm height box and 20-cm height box for 1.0-hour, three weeks post-treatment. (Position 3: KS *T. inclusum*: $F(1,4) = 0.57$, $P < 0.4918$; *T. castaneum*: $F(2,6) = 0.05$, $P < 0.8340$).

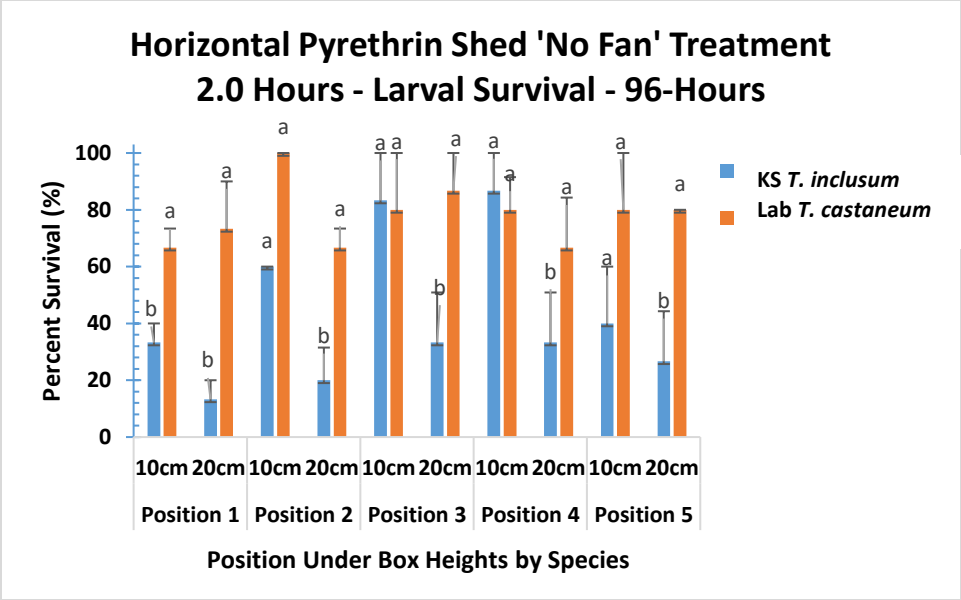


Figure 3.32. Mean percent survival of two larval populations with standard errors for pyrethrin no-fan treatment at five horizontal positions under a 10-cm height box and 20-cm height box for 2.0-hours, 96-hours post-treatment. (Position 3: KS *T. inclusum*: $F(1,4) = 4.25$, $P < 0.1084$; *T. castaneum*: $F(2,6) = 0.08$, $P < 0.7953$).

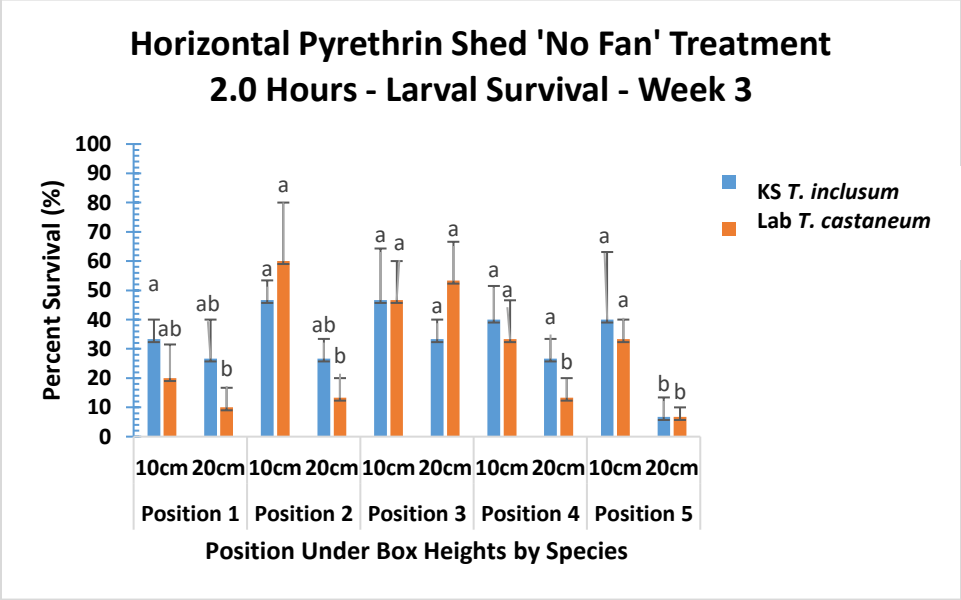


Figure 3.33. Mean percent survival of two larval populations with standard errors for pyrethrin no-fan treatment at five horizontal positions under a 10-cm height box and 20-cm height box for 2.0-hours, three weeks post-treatment. (Position 3: KS *T. inclusum*: $F(1,4) = 0.50$, $P < 0.5185$; *T. castaneum*: $F(2,6) = 0.12$, $P < 0.7415$).

Experiment 5: Bait Station Bioassay

This experiment was analyzed by the difference in trap captures between the commercial bait traps and mineral oil traps. Evaluations were made of trap captures every 24 ± 2 hours up to 72 ± 2 hours. Table 3.5 shows mean differences with standard errors. Negative differences in data indicate more captures in mineral oil traps than in baited traps. Actual data will appear in Appendix A.

More traps indicated *T. variabile* beetles avoided the baited traps and preferred the mineral oil trap versus *T. inclusum*. *Trogoderma variabile* chose the mineral oil trap over the X-Lure trap more frequently than with other traps. There was no significant difference in captures among traps for *T. inclusum*. Whereas for *T. variabile* adults, the Trece Dome trap with the Khapra beetle/warehouse beetle lure was the most attractive and X-Lure multi-species trap was the least attractive. There was no significant difference between both Insects Limited All Beetle trap baits and the two Trece Quick Change lures.

For larvae, the *T. variabile* All Beetle traps performed the best with the food pellet being most attractive for both *T. variabile* and *T. inclusum*. This is consistent with the larvae being more attracted to food baits for consumption. *Trogoderma inclusum* larvae had very low capture rates across all six traps. The All Beetle food pellet captured the most larvae and X-Lure captured the second most. None of the Trece traps had larval captures at 72-hours, nor did the Insects Limited All Beetle traps.

More *T. variabile* were captured as both adults and larvae in all traps compared to the Kansas *T. inclusum* population.

Table 3.5. Mean difference in adult and larval captures between commercial bait stations and mineral oil bait stations after 72±2 hours with standard errors. Mean separations are indicated by letter for each species by treatment. (*T. inclusum* adults: F(5,30) = 1.74, P < 0.155; *T. variabile* adults: F(5,30) = 3.31, P < 0.017) (*T. inclusum* larvae: F(5,30) = 1.62, P < 0.1863; *T. variabile* larvae: F(5,30) = 5.38, P < 0.0012).

Treatment	**Lab** ***T. variabile***	**Kansas** ***T. inclusum***
Adults		
All Beetle Trap – Pantry Pest	-2.7 ± 1.3 ab	0 ± 0.3 a
All Beetle Trap – Food Pellet	-0.5 ± 1.2 ab	-0.167 ± 0.2 a
Trécé Dome – KB/WB Lure	4.5 ± 1.1 a	0.33 ± 0.2 a
Trécé Quick Change – KB/WB Lure	-1.8 ± 2.5 ab	-0.5 ± 0.6 a
Trécé Quick Change – MC Lure	-2.0 ± 2.0 ab	0.5 ± 0.4 a
X-Lure R.T.U. MST	-10.8 ± 5.4 b	1.0 ± 0.6 a
Larvae		
All Beetle Trap – Pantry Pest	3.5 ± 1.2 ab	0 ± 0 a
All Beetle Trap – Food Pellet	6.7 ± 1.6 c	0.3 ± 0.2 a
Trécé Dome – KB/WB Lure	-2.3 ± 2.2 bc	0 ± 0 a
Trécé Quick Change – KB/WB Lure	-2.6 ± 1.2 bc	0 ± 0 a
Trécé Quick Change – MC Lure	-0.2 ± 1.6 abc	0 ± 0 a
X-Lure – R.T.U. MST	-4.7 ± 2.7 c	0.2 ± 0.2 a

Experiment 6: Fipronil Gel Bait Bioassay

Figure 3.32 shows 24-hour survival after exposure to fipronil. All six species had a higher survival rate of adults than larvae. *Oryzaephilus surinamensis* had nearly the same survival rate for adults (5.9%) and larvae (4%). At 48-hours (Figure 3.33.), there is more separation in the adult versus larval survival. Three species had no survival of larvae, these are *T. variabile*, *T. castaneum*, and *L. serricorne*. Adult survival of *T. castaneum*, *T. confusum*, and *O. surinamensis* declined significantly after 24-hours. By 72-hours (Figure 3.34.) *T. variabile*, *T. inclusum*, and *L. serricorne* adult survival declined significantly after 48-hours. At 96-hours, only *T. variabile*, *T. inclusum* and *L. Serricorne* had higher rates of survival (+35%).

Adult dermestids had higher survival than adult tenebrionids and *O. surinamensis,* yet was more similar to *L. serricorne*. Table 3.6 shows adults of *T. confusum* adults had the lowest survival rate following 96-hours exposure to fipronil, followed by *O. surinamensis* and *T. castaneum*.

Table 3.6 shows that all six species of larvae were not significantly different from each other after 96-hours post exposure. Two species with 0% survival at 96-hours were lab colonies, *T. variabile* and *L. serricorne*, which had not been exposed to pesticides in over 30 years. The *T. castaneum* population which also had 0% larval survival were field collected, in 2005, and had more field exposure to pesticides than the other two. Two larval populations with survival after 96-hours were also long-term lab populations, *T. confusum* and *O. surnamensis*. *T. inclusum* was field collected in 2012, and was the most recently field collected populations used.

These results are nearly flipped in outcome from chlorfenapyr where the adults have lower survivability and larvae had nearly 100% survival. With fipronil, the adults had more survival and the larvae had less than 4% control for only three species.

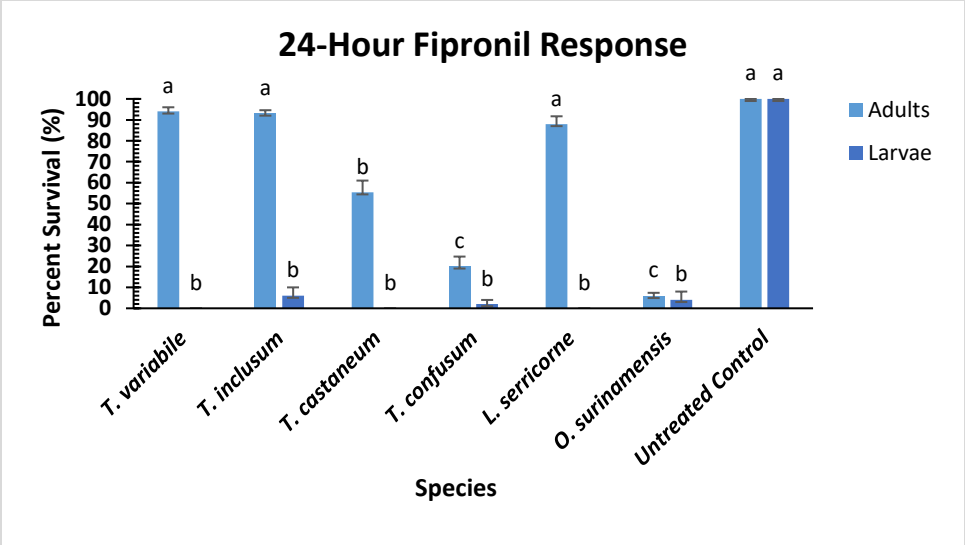


Figure 3.34. Percent survival rate of six species adults and larvae, after 24-hours. (Adults: F(5,114) = 119.34, P < .0001; Larvae: (F11,49) = 908.1, P < .0001).

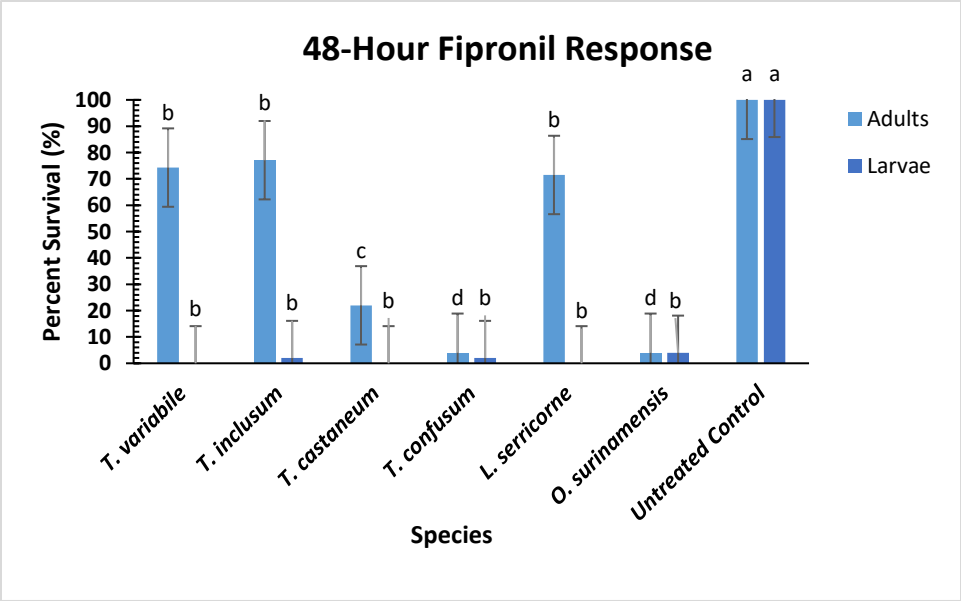


Figure 3.35. Percent survival of six species, adults and larvae, after 48-hours. (Adults: F(5,114) = 118.93, P < .0001; Larvae: F(11,48) = 484.98, P < .0001).

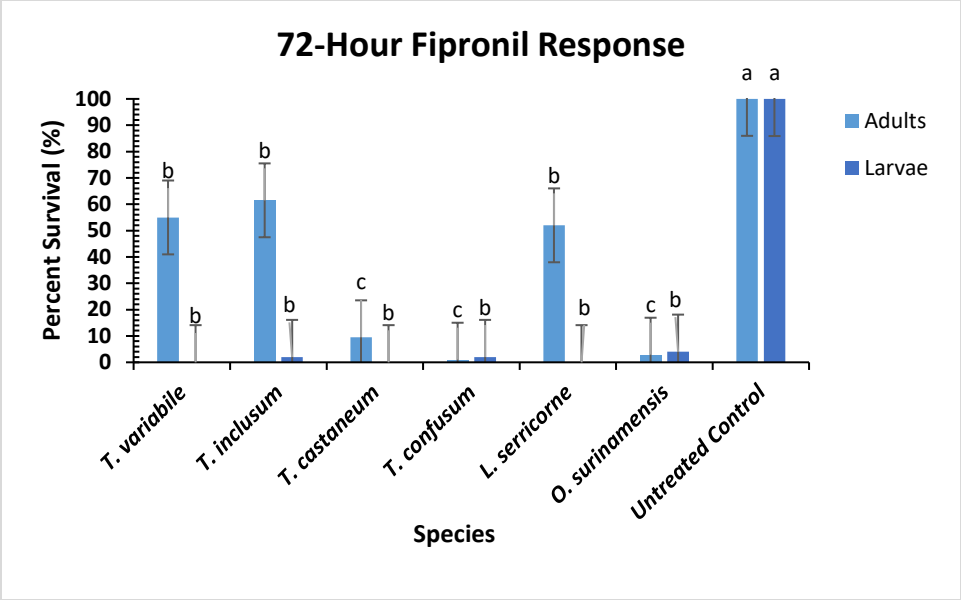


Figure 3.36. Percent survival of six species, adults and larvae, after 72-hours. (Adults: $F(5,114) = 74.79$. $P < .0001$; Larvae: $F(11,48) = 13281.12$, $P < .0001$).

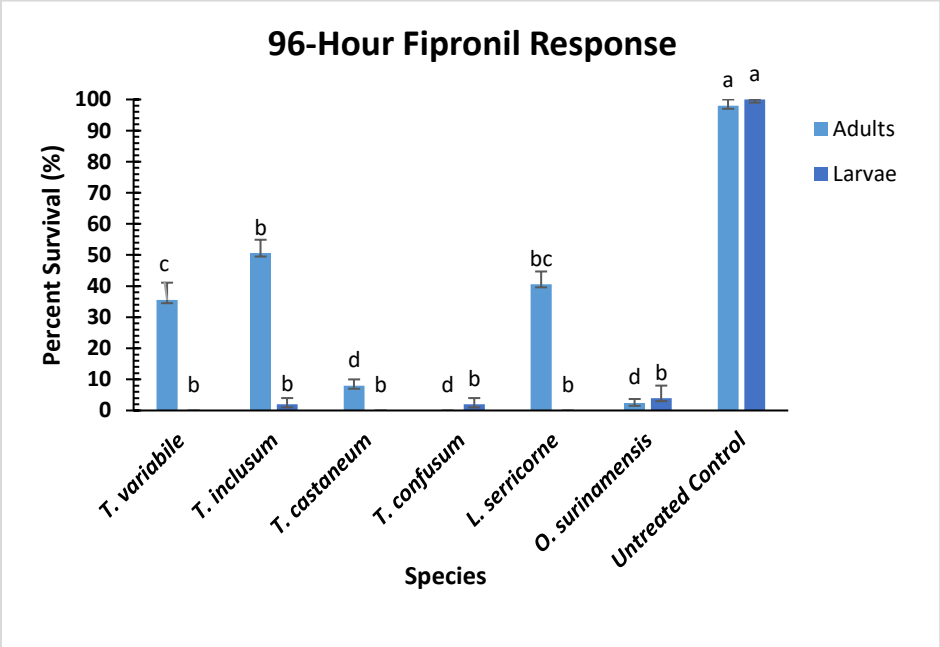


Figure 3.37. Percent survival of six species, adults and larvae, after 96-hours. (Adults: $F(5,114) = 39.23$, $P < .0001$; Larvae: $F(11,48) = 464.97$, $P < .0001$).

Table 3.6. Mean percent survival of six species of stored product pests with standard errors for fipronil gel bait treatment at 96-hours. Mean separations are indicated by letter within formulation for each treatment and species**.** (Adults: $F(5,114) = 36.23$, $P < .0001$; Larvae: $F(11,48) = 464.97$, $P < .0001$).

Fipronil Treatment	***T. variabile***	**Kansas *T. inclusum***	***T. castaneum***	***T. confusum***	***L. serricorne***	***O. surinamensis***
Adults	35.5 ± 5.6 c	50.5 ± 4.4 b	8.0 ± 2.0 d	0.0 ± 0.0 d	40.6 ± 4.1 bc	2.5 ± 1.2 d
Larvae	0.0 ± 0.0 b	2.0 ± 2.0 b	0.0 ± 0.0 b	2.0 ± 2.0 b	0.0 ± 0.0 b	4.0 ± 4.0 b
Untreated Controls	***T. variabile***	**Kansas *T. inclusum***	***T. castaneum***	***T. confusum***	***L. serricorne***	***O. surinamensis***
Adults	100.0 ± 0.0 a	98.0 ± 2.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Larvae	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	98.7 ± 1.3 a	100.0 ± 0.0 a

Fipronil was more successful controlling larvae of six species of stored product insects tested. Adults were less controlled overall after 96-hours compared to larvae. Larvae had a less than 10% survival beginning at 24-hours and was less than 4% at the highest rate for *O. surinamensis* by 96-hours post-treatment exposure. These results show that the fipronil gel bait is successful controlling stored product insects.

Chapter 4 - Discussion

Experiment 1: Bioassay on Crevice Arenas

The PI formulation of chlorfenapyr forms a crystalline structure which sets on the surface of the concrete when dried (visual observation). In contrast, the SC formulation dries without leaving a visible residue. The visible differences may affect how the insecticide interacts with the concrete substrate and the insects. The 96-hour (day 4) post exposure data appeared to be somewhat realistic in terms of pest management, where time was allowed for the treatment to take effect on insects before determining if treatment was effective or if follow-up treatment would be required. In an actual facility, insecticide treatments will continue to affect insects that come into contact with the pesticide residues.

The adult data shows the two lab colonies, *T. castaneum* and *T. variabile*, are more susceptible to both the PI and the SC formulations of chlorfenapyr then the two field populations of *T. inclusum*. These findings are in line with expectations that field populations which have evolved with pesticide pressure would have a higher tolerance to pesticide applications. *Tribolium castaneum* was the most susceptible population to both formulations of chlorfenapyr.

The Kansas *T. inclusum* adult population was the most susceptible to the PI formulation in the crevice only treatment. Both *T. inclusum* adult populations were similar in susceptibility to the SC formulation in the crevice, but are nearly four-times more susceptible than the *T. variabile* lab population which has not been exposed to pesticides for over 30 years. This is important since *Trogoderma* species were found most of the time in the crevice at the time of evaluation. It is important to treat crevices if this is where the insect populations are located when not wandering for food or other resources.

The Kansas *T. inclusum* adult population was also the most tolerant of the PI formulation in the surface only treatment, whereas the Arkansas population was the most tolerant of the SC formulation in the surface only treatment. This data may reflect the behavior of being in the crevices most often at the time of observations. And is important in pest management because it is showing that less control was obtained when crevices are not treated, even more so when crevices are not cleaned and treated.

The food-in-crevice treatment probably most closely duplicates what occurs in food processing facilities and is the area most difficult to control. Dust, grain, other food particles, and debris collect in crevices of floor joints, machinery, storage areas, and manufacturing areas where insects can hide under that debris and not be exposed to pesticide residues. Arthur (2000) showed that when insects have food available after insecticide exposure, the recovery and survival of "knocked down" individuals was significantly higher, whether it was due to the nutritional value in aiding recovery or if the food available was absorbing or masking pesticide residues off the surface of the insect, preventing absorption and exposure. Results in this study were similar, showing that food in the arena crevices increases adult survival of insects in all four populations versus a clean crevice where there was less overall survival.

When evaluating differences between the PI and the SC formulations, in three-fourths of the treatments, there were no significant differences in the formulation for treatments or populations. The exceptions are the crevice treatments for both field collected *T. inclusum* populations and the surface only treatments in the *T. variabile* and the Arkansas *T. inclusum* population.

In the larval studies, the results were very different from the adults. The greatest differences were in the *T. castaneum* exposure to the chlorfenapyr PI formulation at 96-hours

post-treatment. The 'all' treatment and the crevice treatment had the lowest survival at 30.0% and 32.5%, respectively. The surface only treatment was not significantly different from either the food in crevice or the crevice and all treatments. Having the food-in-crevice was not significantly different from the untreated control or the surface only treatment. This is consistent with Arthur, 2013, which showed that a food source present for *T. castaneum* and *T. confusum* increased survival on chlorfenapyr arenas. The larvae, similar to the adults, preferred to hide in the crevices. The crevice and the 'all' treatments statistically resulted in having the fewest survivors reproducing for future generations.

The Kansas *T. inclusum* larvae was the other population where the untreated control and the food-in-crevice were not significantly different in the chlorfenapyr PI formulation treatment. The *T. variabile* and the Arkansas *T. inclusum* populations showed no significant differences to chlorfenapyr PI at 96-hours post exposure.

The 96-hour post exposure larval responses to the chlorfenapyr SC formulation showed no significant differences from the untreated controls in any of the four populations tested. However, *T. castaneum* showed the largest mortality response in the crevice plate with a 72.5% survival rate.

When looking across the larval data for chlorfenapyr exposure, *T. castaneum* had the largest response with the least survival when compared to the three *Trogoderma* populations. Some of the differences in species mortality could be due to the lack of body hairs on *T. castaneum* larvae which would allow more direct body contact to the treated surfaces, allowing for more contact with the pesticide residues. The hairy bodies of the *Trogoderma* spp. larval populations may not have allowed as much direct body contact with the pesticide residues for a lethal dose. No previous studies comparing hairy versus hairless larvae insecticide efficacy were

found to compare with this data. However, even in both *T. inclusum* larval populations, the food-in-crevice arenas had slightly, though not significant, lower survival than the other treatments for the chlorfenapyr SC formulation which could be due to larval feeding activity, exposing the larvae to a higher dose of chlorfenpyr compared to the other treatments which had no food. Arthur, 2013, showed that when there was a food source present with chlorfenapyr treatment, survival was generally higher, which is consistent with these results. This data is also consistent with Ghimire et al. (2016) where *T. variabile* and *T inclusum* larvae were more tolerant to two pyrethroid insecticides, deltamethrin and ß-cyfluthrin. The Ghirmire et al. (2016) findings were consistent with these findings of *T. inclusum* being more susceptible and *T. variabile* in several treatments.

When looking at the two formulations of chlorfenapyr, the PI formulation caused higher mortality for both adults and larvae compared to the SC formulation results. However, the PI formulation is labeled for non-food handling areas only. Chlorfenapyr PI would need to be used in areas adjacent to food processing areas such as offices, control rooms, boiler rooms, janitorial closets, lavatories and other similar areas in a food processing facility. Chlorfenapyr SC is labeled for both non-food and food handling areas, though when used in food handling areas, production needs to be stopped and food needs to be covered. Chlorfenapyr SC is labeled only for crack and crevice treatments in food handling areas against stored product pests.

The implications of this study show the importance of sanitation in crevices when treating for adults. The effects on overall pest management is evident, yet is one of the most difficult areas to treat under real world circumstances. However, the larval mortality was higher when there was some food to feed on for the *T. inclusum* populations, even though it was not significantly different. This highlights the importance of having an ‘at minimum’ annual

maintenance and deep cleaning on facilities during shut down periods to eliminate food sources and harborages in crevices and conduct proper sealing of clean crevices. In a personal communication with Dr. Linda Mason (2017), Purdue Univerisity, she mentioned if crevices are sealed with food debris in them, that she had seen instances in facilities where the emerging larvae chewed through seals to escape after developing. This activity not only compromises the seals, but allows for food, water, and other contaminants to be further introduced, causing the seal to fail sooner than expected. Biocleaners, one of the newer tools for pest management IPM, can also play an important role in keeping crevices cleaned out eliminating barriers to good treatment applications. Biocleaners use proprietary blends of microbes and/or enzymes to digest food debris and organic matter resulting in water and carbon dioxide when digestion of the organic matter is complete (Cisse Spragins, owner and CEO, Rockwell Labs Ltd, personal communication; Douglas ProTech, 2026 website access). They are also recommended to be used after physical crevice cleaning, yet prior to sealing crevices to make sure all food debris is digested and broken down before sealing crevices.

Experiment 2: Barrier to Food

This experiment having a barrier to food, separate from the chlorfenapyr treatment, shows that if there is a food patch, with no insecticide treatment in the area of the food, that there is no significant mortality in larvae to any of the tested species or populations. As was shown in experiment 1, chlorfenpyr PI resulted in the highest mortality in *T. castaneum*, followed by both populations of *T. inclusum,* with the least effect on *T. variabile* at 96-hours post exposure. The mortality that does occur is most likely due to individuals that wandered in the arenas prior to finding the food patch. Larvae were mostly found in the food patch within the first 24-hours of exposure. Adults, could be found huddled in the edges of the arena or up against the barrier. If

compared to experiment 1, where adults were found mostly huddled in the corners of crevices, adults were likely attempting to find hiding places within the open arenas since no crevices existed for them to hide in.

However, retaining those survivors from the 96-hour treatment for one more month, we find that there is nearly 100% mortality in adults of the *Trogoderma* spp., showing they are more tolerant to chlorfenapyr than *T. castaneum*. This is to be expected since the life span of adults averages only two weeks in length. However, the larval survival remains > 90% among the four populations. This indicates that larvae survive to pupate to adults and then reproduce the next generation. Dermestid larvae will also survive in a state of arrested development/diapause (Wilches et al., 2016), especially when they are isolated in Petri dishes, allowing them to survive until more favorable conditions exist, known for up to two years, though that process is not well understood (Rees, 2004; Mason, 2018).

The implications of this study show that having insecticide treatments placed properly, where insects will be exposed to them, is important to control. If insecticide treatments are placed in areas where the insects may never wander, they can avoid them and go directly to a food source, continuing to cause feeding damage and increasing populations numbers. Additionally, there are studies which show the presence of a food source, during or after exposure, increases the survival of *T. castaneum* beetles (Arthur, 1998; Toews et al., 2010).

Experiment 3: Label versus Effective Rate for Chlorfenapyr

In experiment 1 and experiment 2, an "effective rate" of chlorfenapyr SC was used based on data, Arthur (2013), showing a less than label rate of chlorfenapyr is effective against *T. castaneum* and *Tribolium confusum* adults. Since larvae of all four populations were more tolerant to chlorfenapyr in experiment 1, we decided to test the difference between label and

effective rates in larvae. In all trials, chlorfenapyr PI was used at label rate due to it being a ready-to-use aerosol canned product which is not available in any other formulation. The chlorfenapyr PI formulation continued to show the lowest survival rates, especially for *T. castaneum* at 96-hours post-treatment.

Larvae exposed to the label rate of chlorfenapyr SC versus the 'effective rate' showed no significant differences in the survival rates in most treatments. The most significant difference between the two rates was in the food-in-crevice arenas for the Kansas *T. inclusum* population. All other treatments and populations showed no significant difference between the label rate and the effective rate of chlorfenapyr SC. This result differs significantly from the Arthur (2013) adult results which concluded with label rate adult survival at less than 1%. But this was consistent with our experiment 1 results with adults being more susceptible to chlorfenapyr treatment.

This is a significant finding for larval efficacy. Though not promoted or encouraged to use a less than label rate application, it is not against the label regulations to use less than label rate, though in many cases it may not be as effective as it could be, and in some cases, with repeated use may lead to insecticide resistance. Pesticide applicators cannot, however, apply at greater than label rate. In the broader scope of pesticide application in food processing facilities, it demonstrates that if a pesticide treatment is made at label rate, and some of it is somewhat diluted in the environment to which it is applied, that it can still be effective at not quite full-label rate. Crevices are subject to holding moisture in facilities from wash down and spills, thus, may affect some insecticide applications. Whereas chlorfenapyr is still effective at less than label rate, some pesticides may not be as forgiving in the field setting. Application rates are typically set slightly above the minimum required to be effective so help avoid insecticide

resistance in the field, prolonging the useful lifespan of products. This is important due to the relatively small number of products available for stored product pests compared to larger market categories for pesticides.

Further work could be done in this area by replicating Arthur (2013) work on adults by testing a range of pesticide rates testing larval efficacy. This work was not done as part of this study since the original scope was to compare Arthur's effective rate against the label rate.

Experiment 4: Pyrethrin Aerosol Bioassay

The vertical treatments were set up to try to mimic items stacked in a warehouse type setting where pests and products are stacked at various levels. In evaluating the vertical treatment options, the data shows that not ventilating a treatment after a fogging application for 0.5 hours is not significantly different from leaving it for 1.0 or 2.0 hours. This is significant in the food processing industry where scheduling down time for a treatment is typically deferred until it absolutely has to be done. The treatment time is just a small portion of the down time for a facility which also needs to account for set up and post-treatment clean-up and restart operations. This option for leaving a post-fogging treatment rest time for 0.5 hours as effective is substantiated by work conducted by Scheff et al. (personal communication). Another interesting trend in the vertical data is that there is less survival at the low level especially for the eggs and pupae of *T. castaneum* at 96-hours post-treatment. By week three post-treatment, the Kansas *T. inclusum* population had the least survivors of all stages, egg, larvae, and pupae. *T. castaneum* was most successful at survival, and larvae were most successful at all three heights of low, medium, and high.

The fewest survivors of any life stage were at the lowest level of shelves may be indicative of the aerosol droplets falling and may receive the higher doses of pyrethrin compared

to upper shelves, where the fog, due to the different droplet sizes, may not have had as much insecticide reach the highest shelf level.

The 'no fan' 1.0 hour treatment, did have better efficacy for all three heights and all life stages, except *T. inclusum* pupae on the low shelf and *T. castaneum* larvae on the high shelf which had higher survival than the 0.5 hour treatment. By week three post-treatment on the 'no fan' treatments, there was almost 0% survival at all three treatment times and all three heights. While the 0.5 hour treatment is not statistically significant from the 1.0 hour 'no fan' treatment, the data shows more efficacy at both the 96-hour evaluation and the week three evaluation.

Comparing fan versus no fan treatments on vertical shelving, the no fan treatment had higher efficacy than the fan treatment across all levels. The initial inclination was that the fan would help distribute the pyrethrins more evenly throughout the shed. Instead, the fans may have created a situation where the smaller fogging particles remained airborne and were not able to settle on the insects during the treatment time, resulting in less mortality (personal communications with Dr. Frank Arthur, USDA-ARS-CGAHR, retired).

The horizontal trial tried to mimic conditions that may occur under solid shelves or boxes in a warehouse scenario. The five positions represent different depths to an open area where treatment can spread or reach into those horizonal spaces, with either a 10-cm or 20-cm space above for air movement. Position 1 was in front of the horizontal cover box to represent those insects that may be in the open, near other covered areas. At 96-hours post-treatment, the 0.5-, 1.0- and 2.0-hour fan treatment times all still had greater than 50.0% survival. By week three post-treatment, most were still above 30% survival which would not be considered acceptable control in a warehouse.

The ‘no fan’ treatments, under both the 10-cm and 20-cm boxes, were generally not very effective. Position 1, in front of the box covers, had the best efficacy with *T. inclusum* having the lowest mortality of the two species and the 20 cm box having the lowest number of survivors. For positions under the box the general curve of the survival numbers tends to take on a more normal curve shape with the peak survivorship at positions 2 and 3, then tapering off towards positions 4 and 5. These results are not quite the same as looking at the fan treatment data. This shows that even though the fans may have been moving the fogging particles around, they may not have been getting to the target sites. However, the ‘no fan’ treatments may have allowed some movement of particles into open spaces as they fell.

These treatments show that using no fan may result in higher mortality and better fogging coverage regardless of whether the target pest is on the vertical or horizontal planes during treatment. Our treatments were also made from a handheld fogger held at 1 meter above the floor. Better coverage may be achieved if foggers were mounted above the shelves where the pyrethrin particles would move downwards and have complete interior coverage versus spraying outward from the fogging machine and expecting the entire height of the shed to be covered from a single point.

Experiment 5: Bait Station Bioassay

The results in experiment 5 were very interesting. Even though we opened the pheromones to let them vent in an air hood for 24±1 hours, the Trécé, Inc. pheromones were most likely still at concentrations which were too high for the size of our test arenas. According to James Miller (personal communication), when this happens, the pheromone stations can act as a repellant rather than an attractant. This action may have caused the beetles to seek refuge from the repellant pheromones into the untreated mineral oil traps. This could help explain the beetle

data where more adults were found in the mineral oil compared to the actual baited traps. This may be useful information in a food facility where bait stations are placed in tight or enclosed areas such as electrical boxes and no beetles are trapped. It may take a week or more to see results when the overpowering new pheromone trap or lure has time to air out and weaken somewhat from a new pheromone deployment.

However, we did see that the Trécé Dome trap with the KB/WB lure performed the best in attracting *T. variabile* with the pheromone that was specific to *T. variabile*, whereas the best bait trap to attract the Kansas *T. inclusum* population was the X-Lure R.T.U MST trap. The Trécé Quick Change MC (multi-combi) Lure is designed to attract over 23 species (Trécé pheromone instruction sheet). It may have performed lower at attracting adult *Trogoderma* because it is designed for many species to be attracted to it. The X-Lure trap is also a multi-species trap (MST). What was not answered by this study, since multiple species are attracted to these multi-combi or multi-species traps, is what species might be most attracted to these traps. Future research could explore additional work to find the optimum pheromone 'airing out' time for the pheromones to be at the best levels for smaller spaces such as the tub arenas used in this study. In food processing facilities, smaller areas such as electrical boxes and enclosed mechanical casings for motors have pheromone traps placed inside for monitoring in those protected areas. Knowing how long it may take before pheromones are actually attracting insects, and not repelling them, would be useful. Pest control technicians who may not realize that high levels of the pheromones can repel may assume there are no insects in those enclosed areas when it could be temporary due to the higher pheromone levels.

When larvae were exposed to the different bait stations a different pattern occurred. The All Beetle bait stations performed the best for *T. variabile* and *T. inclusum*. This makes sense as

these are food driven traps which would naturally be more attractive to larvae as the most active feeding stage of *Trogoderma* species. All four pheromone baited traps resulted in being not attractive to larvae for *T. inclusum*, whereas *T. variabile* was negatively attracted. This is not surprising as pheromones are designed to attract adults through food aggregation and/or mating pheromones which larvae would not be attracted to.

Pheromone traps and bait stations are an important component of an integrated pest management program. Many decisions to deploy other pest control methods are made based on numbers attracted to bait and pheromone traps. When traps are not working as assumed, and are in fact possibly repelling the insects, timely pest control decisions may be delayed, causing a significant pest problem to increase, making it more difficult to bring under control.

Experiment 6: Fipronil Gel Bait Bioassay

There were no previous studies looking at the effects of fipronil gel bait on stored product pests, however, Kavallieratos et al. (2010) conducted a study on the efficacy of fipronil on four stored product pests which is the first fipronil study that was found on stored product pests. Kavallieratos et al. (2010) used a fipronil SC formulation (Termidor® SC) and determined that fipronil was very effective against stored product pest beetles. The gel bait used in this study is commercially sold as MaxForce® FC Magnum, a cockroach gel bait, but does list adult "pantry pests" on the label for control by crack and crevice only treatments. All species tested were specifically listed on the MaxForce® label.

All six species adults showed continuous decline from 24-hours post-treatment through 96-hours post-treatment. After 48-hours *T. confusum* and *O. surinamensis* had 4.0% adult survival, but only *T. confusum* reached 0.0% survival by 96-hours post-treatment and *O. surinamensis* had 2.5% survival. Overall, results show adult *T. variabile*, *T. inclusum*, and *L.*

serricorne were least affected by fipronil, with survival over 35%, even when it was the only food source available. This could also be a result of the species which feed less as adults compared to those with higher adult feeding rates.

Larvae were much more susceptible to the fipronil gel bait. By 24-hours post-treatment all species were at or below 6.0% survival, *T. variabile*, *T. castaneum*, and *L. serricorne* had 0.0% survival. At 48-hours through 96-hours post-treatment *T. inclusum*, *T. confusum* plateaued with 2.0% survival and *O. surinamensis* with 4.0% survival for the same time frame. Larvae were not left longer than 96-hours post-treatment to evaluate. Longer exposure times may have brought those survival numbers lower and would be interesting to explore in future studies.

O. surinamensis had the least tolerant adults by the end of the study, but were the most tolerant larvae by this study. Larvae, while not listed on the label for the fipronil gel bait responded faster than adults to exposure. This may be due to the nature of larvae being the actively feeding life stage. If the larvae consumed more bait than the adults, it could have affected the observations. Fipronil is also a contact insecticide, so it is hard to say if all of the exposure was through feeding or by general contact rubbing against the bait product.

In practice, through personal communications with the Industrial Fumigant Company (IFC) employees who have deployed this gel bait in warehouse and milling facilities (Chris Mueller, VP Marketing and Steve Snow, IFC California Territory Manager) the gel bait is effective in controlling stored product pests, however, very strict integrated pest management and sanitation strategies must accompany gel bait use in the field for it to be effective. In facilities with poor sanitation, IFC has experienced gel bait failure, attributing this to the abundance of food resources for the insects and no need to feed on the bait for food. Questions still remain as to whether the fipronil gel bait can have secondary mortality effects as it does in

cockroaches where the adults feed on the product and when defecation back in the harborage, nymphs feed on the fecal materials containing the fipronil from the adults and controls the cockroaches at their source. This secondary mortality would not apply to all stored product pest species. It may apply only to those species with carnivorous tendencies such as the *Trogoderma* species or *Tribolium* larvae which may consume smaller larvae. Also, finding harborages for stored product pests is more difficult in a food processing facility. Typically when visually inspecting for pests in facilities, larvae are the most commonly found since they are directly in the food sources, actively feeding. Finding adults in facilities, and where adults are hiding, can be more challenging, even when larvae are readily found.

Fipronil is not widely used in the stored product pest area, however, it is widely used in other areas of pest control such as structural pest control and for pets flea and tick control. This gel bait is unique in that it can add an additional mode of action group for insecticide rotation if needed.

Overall Conclusions

This research is very important for pest control companies working in grain and food processing facilities. All pesticides used in this research are commercially available, but several have primary markets other than grain and food processing facilities. They are typically structural pest products with much broader labels for several uses and sites for application. For example, the chlorfenapyr SC label is Phantom® Termiticide-Insecticide, stored product pest usage is a small market for this product and is mostly marketed as a termiticide product. Therefore, in-depth testing of these products for efficacy against stored product pests is not the primary focus industry.

Each type of insecticide application, whether a contact insecticide, aerosol fogging, or gel bait, have challenges in the field. Pheromone bait stations can help remove some individual insects from the general population, but they are not control devices. Having them work accurately in all spaces they are placed in is critical in understanding the pest control needs in a food facility. These studies help us understand the importance of treating cracks and crevices for stored product pests, in particular *Trogoderma* species and *T. castaneum*. Understanding how pyrethrin aerosol treatments work and how long they need to set before ventilating those for best treatment. This will help facilities manage treatment times with their PCOs. Floor bait traps perform best when in an appropriate sized space which allows for the strength of the pheromone. Results may take a few days to over a week to be positive in freshly deployed lure traps. And when looking at newer pesticide applications such as using cockroach gel bait for stored product pests, especially an active ingredient which is not widely used for stored product pests such as fipronil, it can be a useful tool for hard to reach areas or an alternative active ingredient to deploy in the facility.

Cracks and crevice features are some of the most difficult to deal with in the industry. They are everywhere. In the structure of the facility itself, the machinery in the facility, and the interface between the machinery, the structure, and food products. Since insects can hide in any of these spaces, good exclusion and sanitation are the beginning of a quality integrated pest management program for food facilities. Having tools to help control these pests in difficult areas is critical. While the contact insecticides tested in these studies are not for food contact areas, protecting the areas around the actual food processing sites is just as important to prevent insects entering food products, causing recalls, loosing consumer trust, and loss of finished

product which is more costly in the long run than controlling stored product pests before they are a problem.

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Appendix A - Experiment 5: Pheromone/Bait Station Evaluation

Appendix Figure A.1. Actual numbers of adult and larval-captures over six replicates between the commercial bait stations and mineral oil bait stations at 72 ±2 hours with standard errors. Mean separations are indicated by letter within formulation for each treatment and species.

Treatment	**Lab** ***T. variabile*** **Bait Station**	**Lab** ***T. variabile*** **Mineral Oil**	**Kansas** ***T. inclusum*** **Bait Station**	**Kansas** ***T. inclusum*** **Mineral Oil**
Adults				
All Beetle Trap – Pantry Pest	5	21	1	1
All Beetle Trap – Food Pellet	9	12	0	1
Trece Dome – KB/WB Lure	31	4	3	1
Trece Quick Change – KB/WB Lure	32	43	1	4
Trece Quick Change – MC Lure	21	33	4	1
X-Lure R.T.U. MST	46	111	7	1
Larvae				
All Beetle Trap – Pantry Pest	24	3	0	0
All Beetle Trap – Food Pellet	46	6	2	0
Trece Dome – KB/WB Lure	17	31	0	0
Trece Quick Change – KB/WB Lure	15	31	0	0
Trece Quick Change – MC Lure	24	25	0	0
X-Lure – R.T.U. MST	15	43	1	0