Methyl bromide alternatives for protecting post-harvest bagged commodities in shipping containers: pre-loading contact insecticide surface treatments and methoprene-incorporated packaging

by

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Abstract

Stored-product insects damage and contaminate bagged commodities during storage in warehouses and transport in shipping containers, and the phase-out of methyl bromide and the spread of phosphine resistance have increased the need for non-fumigant tools. This thesis evaluated two such tools, residual contact insecticides applied to structural surfaces and methoprene-incorporated packaging. First, beta-cyfluthrin and a liquid combination product containing deltamethrin, (*S*)-methoprene, and piperonyl butoxide (Gravista, hereafter liquid combo) were applied to galvanized steel and plywood arenas held inside shipping containers in Arkansas and Kansas during four seasonal trials. Both insecticides reduced survival of *Tribolium castaneum* (Herbst), red flour beetle, and *Sitophilus oryzae* (L.), rice weevil, through 16 weeks, efficacy was more stable on metal than on plywood, and progeny production was nearly eliminated. Second, the same insecticides were applied to wood, metal, and concrete arenas and held in environmental chambers at five constant temperatures (16, 22, 27, 32, and 37 degrees Celsius), 65 percent relative humidity, and complete darkness over a 12 to 24 week residual period. Metal provided the most persistent efficacy, with 98 to 100 percent mortality of *S. oryzae* and *Rhyzopertha dominica* (F.), lesser grain borer, after 168 h of exposure at all five temperatures and residual weeks, whereas exposure on concrete had the lowest mortality among all species. *Tribolium castaneum* was the most difficult species to control on wood and concrete. Initial mortality generally increased with temperature, and the more effective product depended on the surface, because liquid combo generally caused greater 24 h mortality on metal, whereas beta-cyfluthrin was generally more effective on concrete.

Third, methoprene-incorporated woven polypropylene packaging was evaluated on pallets of bagged milled rice in two simulated warehouses during 182 days of storage, with

repeated releases of five stored-product insect species. Live insects were not detected in sampled bags until 91 days of storage, insects recovered at the final bag opening were concentrated in the bottom layer of each pallet, and no insect-damaged kernels were found in any sample. Pallets in methoprene-incorporated packaging held 57 percent fewer insects than pallets in untreated packaging after 182 days, but this difference was not statistically significant with two replicates, and the packaging did not prevent insects from infesting bags. Together, these studies show that the performance of residual surface treatments depends on surface type, temperature, insect species, and formulation, and that methoprene-incorporated packaging can reduce insect numbers within bags but does not prevent insects from infesting them. Combining pre-loading surface treatments, protective packaging with improved closures, sanitation, and monitoring offers a practical non-fumigant integrated pest management approach for bagged commodities in storage and transport.

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List of Abbreviations

a.i.	active ingredient
ANOVA	analysis of variance
AUD	Australian dollar
CGAHR	Center for Grain and Animal Health Research
df	degrees of freedom
EC	emulsifiable concentrate
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
HSD	honestly significant difference
IDK	insect-damaged kernels
IGR	insect growth regulator
IPM	integrated pest management
LC_{99}	lethal concentration for 99% of the test population
LD_{99}	lethal dose for 99% of the test population
MB	methyl bromide
PBO	piperonyl butoxide
ppm	parts per million
QPS	quarantine and pre-shipment
RH	relative humidity
SC	suspension concentrate
SE	standard error
U.S.	United States
USD	United States dollar
USDA	United States Department of Agriculture
USDA-ARS	United States Department of Agriculture, Agricultural Research Service

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Dedication

To my parents,

whose love, sacrifices, and quiet strength made every step of this journey possible,

and whose life in agriculture first taught me why protecting the food we grow matters.

Chapter 1 - Literature Review

1.1. Introduction

1.1.1. Purpose of the Review

The preservation of stored agricultural commodities is an essential component of global food security and international trade. Effective postharvest storage maintains grain quality, prevents economic loss, and limits the health risks associated with insect infestation and contamination (Hagstrum & Subramanyam, 2009). Stored-product insects nevertheless remain among the most important threats to postharvest commodities worldwide, causing both direct consumption losses and indirect reductions in grain grade and market value (Phillips & Throne, 2010). This chapter synthesizes current knowledge on the biology of the major stored-product insects, the chemical and non-chemical tactics used to manage them, and the specific constraints that arise when commodities are held in warehouses and shipping containers. Attention is given to residual contact insecticides, the insect growth regulator (IGR) (*S*)-methoprene, and insecticide-incorporated packaging, because these tools form the basis of the research presented in this thesis. The review closes by identifying the gaps in knowledge that the subsequent research chapters were designed to address.

1.1.2. Global Importance of Stored Products

Cereal grains and legumes constitute the dietary staple for most of the world's population. Following harvest, these commodities are stored for extended periods to ensure year-round availability, stabilize prices, and facilitate regional and international distribution. During storage, however, grain is vulnerable to infestation by beetles, moths, and other arthropods that reduce both the quantity and the quality of the product (Rees, 2004; Hagstrum & Subramanyam, 2009). Losses during long-term storage take several forms, including direct consumption of kernels,

reduced germination, heating and moisture migration within the grain mass, and contamination with insect fragments, excreta, and webbing (Mason & McDonough, 2012).

The magnitude of loss differs markedly among regions. Postharvest losses caused by stored-product insects have been estimated at up to 9% in developed countries and 20% or more in developing countries (Phillips & Throne, 2010) and estimates of combined direct and indirect losses range from about 10% in temperate regions to almost 50% in humid tropical areas (Wijayaratne et al., 2018). These differences arise because warm climates favor rapid population growth and storage infrastructure is often limited (Kumar & Kalita, 2017). In general the maximum rate of growth and reproduction for most stored-product insects occurs between 25 and 33 °C, and development largely ceases below about 13 °C and above 35 °C (Phillips & Throne, 2010).

Recent global assessments place these losses in a wider economic context. The Food and Agriculture Organization of the United Nations (FAO) estimated that around 14% of the world's food, worth about $400 billion USD per year, is lost after harvest and before reaching the retail level, with losses of 21% in Central and Southern Asia and 16% in Northern America and Europe (FAO, 2019). A further 1.05 billion tons of food, almost one-fifth of the food available to consumers, was wasted in 2022, and food loss and waste together generate 8-10% of global greenhouse gas emissions and cost the global economy approximately $1 trillion USD (UNEP, 2024). For cereals in particular, physical postharvest grain losses in sub-Saharan Africa range from 10% to 20% and are valued at up to $4 billion USD per year, an amount roughly equivalent to the region's annual cereal imports and sufficient to meet the minimum annual food requirements of at least 48 million people (World Bank et al., 2011). Insects are a major contributor to storage losses, with more than 600 beetle, 70 moth, and 355 mite species

associated with stored products, and insect damage accounts for an estimated 10-20% of storage losses (Guru et al., 2022).

Even where absolute losses are lower, as in temperate production systems, infestation carries a substantial economic cost because grain is discounted or rejected on the basis of live insects, insect-damaged kernels, and insect fragments rather than on weight loss alone (Mason & McDonough, 2012). In the United States (U.S.), management of insects in stored wheat has historically combined grain protectants, fumigation, aeration, and sanitation, with the balance among these tactics shifting as regulatory and resistance constraints have changed (Hagstrum et al., 1999). In international trade, the presence of live insects in a consignment can lead to rejection, mandatory treatment at the port of entry, or loss of market access (Myers & Hagstrum, 2012). A cargo of U.S. soft red winter wheat valued at about $84 million USD was rejected at the port of Damietta, Egypt, because insects were detected in the shipment (Nayak et al., 2020). The management of stored-product insects is therefore not only a matter of pest control but also a central component of food system resilience and trade compliance.

1.1.3. Historical Context of Control Methods

Efforts to reduce postharvest losses are as old as agriculture itself. Sun-drying, sanitation of storage structures, and the use of botanical materials were among the earliest methods used to suppress infestation (Rees, 2004). With the intensification of agriculture and the growth of global markets in the twentieth century, chemical control became the predominant strategy. Fumigants, particularly methyl bromide and phosphine, transformed storage pest management because they penetrate bulk commodities and kill all life stages, including the immature stages that develop concealed inside kernels (Bond, 1984; Fields & White, 2002). Methyl bromide controls insects in less than 48 h in space fumigations and has an exceptionally broad spectrum of activity, but it

was identified as an ozone-depleting substance and was banned in developed countries from 2005 except for quarantine and pre-shipment (QPS) uses (Fields & White, 2002), leaving phosphine as the principal fumigant for stored commodities worldwide (Phillips et al., 2012). Under the Montreal Protocol, quarantine applications are treatments that prevent the introduction, establishment, or spread of regulated quarantine pests, or ensure their official control, whereas pre-shipment applications are treatments applied directly before export to meet the official phytosanitary or sanitary requirements of the importing country or the existing official requirements of the exporting country (UNEP, 1995). Because QPS uses are exempt from the phase-out, methyl bromide can still be used for these trade-related treatments. Phosphine came into widespread use in the 1960s, and its low cost, approximately $0.25 USD per ton compared with about $3.00 USD per ton for sulfuryl fluoride, helps to explain its continued dominance (Nayak et al., 2020).

Residual contact insecticides were adopted in parallel as protectants for stored grain and as structural treatments for storage facilities. Organophosphates such as malathion, chlorpyrifos-methyl, and pirimiphos-methyl were used extensively, and pyrethroids were later added because of their rapid knockdown and comparatively low mammalian toxicity (Arthur, 1996; Arthur & Subramanyam, 2012). Heavy reliance on a small number of chemical classes has had predictable consequences. Resistance to malathion became widespread in several key species, and high-level resistance to phosphine has since been documented in *Rhyzopertha dominica* (F.), lesser grain borer, and *Tribolium castaneum* (Herbst), red flour beetle, in Australia, Asia, and North America (Zettler & Cuperus, 1990; Collins et al., 2002; Opit et al., 2012b; Nayak et al., 2020). Resistance reduces the efficacy of existing treatments, encourages higher application rates, and increases

cost and risk, which has increased the need for integrated, multi-tactic approaches to stored-product protection (Opit et al., 2012a; Nayak et al., 2020).

1.1.4. Relevance to Current Agricultural Practices and Trade

Modern supply chains are complex and span continents. Stored-product insects can originate at any point in these chains, from on-farm bins and country elevators to processing facilities, warehouses, and shipping containers (Arthur et al., 2006; Hagstrum & Subramanyam, 2009). The growing reliance on intercontinental trade means that infestations can move readily across borders, increasing the importance of phytosanitary measures (Myers & Hagstrum, 2012).

International trade also creates pathways for the movement of quarantine pests from regions where they are endemic into regions where they are not established. *Trogoderma granarium* Everts, khapra beetle, is among the most serious of these pests. It is regarded as a quarantine pest by major grain-exporting countries, including the U.S., Canada, and Australia, and detections at ports of entry can result in the rejection, treatment, or re-export of consignments and in heightened inspection of commodities from countries where the species is established (Myers & Hagstrum, 2012; Athanassiou et al., 2019). Modeling of the international maritime shipping network has identified container traffic as a plausible pathway for the arrival of *T. granarium* in countries where it is not established (Paini & Yemshanov, 2012), and the cost of such incursions is high. Previous eradication efforts of a large *T. granarium* outbreak in the U.S. took 13 years (1953-1966) and cost an estimated $96-130 million USD in 2016 (Day & White, 2016). The potential establishment of *T. granarium* in Australia has been estimated to cost the Australian economy AUD $15.5 billion over 20 years (DAFF, n.d.), a risk heightened by a sharp rise in border detections, with 16 recorded in 2020 alone (Inspector-General of Biosecurity, 2021). Phytosanitary measures in trade have likewise been developed for other

invasive storage pests, such as *Prostephanus truncatus* (Horn), larger grain borer (Tyler & Hodges, 2002). Beyond regulated species, many importing countries and customers maintain zero tolerance for live insects of any species in grain and finished products (Phillips & Throne, 2010). Australia, for example, has imposed a no tolerance policy for live insects in the grain it exports to other countries, so that consignments must be free of live insects at inspection to be certified for export (DPIRD, n.d.).

Shipping containers have consequently become a focus of stored-product research. Their enclosed but imperfectly sealed structure, the mixture of steel walls and wooden floors, exposure to widely fluctuating temperature and humidity, and their role in long-distance transport of grain and packaged foods present distinctive challenges for insect control (Agrafioti et al., 2021). At the same time, consumer demand for reduced pesticide residues and increasingly strict regulatory requirements continue to narrow the range of available management tools (Phillips & Throne, 2010; Arthur & Subramanyam, 2012).

1.1.5. Objectives of the Literature Review

The overarching objective of this review is to critically evaluate and synthesize research on stored-product insect management, with emphasis on residual chemical control and reduced-risk alternatives relevant to warehouses and shipping containers. The specific aims are:

1. To summarize the biology, ecology, and economic significance of six major stored-product insect pests.
2. To evaluate the history, efficacy, and limitations of fumigants and residual contact insecticides, including the problem of insecticide resistance.
3. To assess IGRs and insecticide-incorporated packaging as reduced-risk management tools.
4. To identify research gaps related to the efficacy of contact insecticides on different storage surfaces under variable environmental conditions, and to the potential of methoprene-

incorporated packaging to suppress infestations in simulated warehouse and shipping container conditions.

1.2. Biology and Importance of Stored-Product Insects

Stored-product insects are a diverse group of beetles and moths that exploit durable postharvest commodities worldwide. Their success is attributed to small body size, high reproductive capacity, cryptic habits, and tolerance of the dry conditions that characterize storage environments (Hagstrum & Subramanyam, 2009). These insects infest raw grain, processed foods, and packaging, and the extent of damage depends on species biology, infestation density, and the storage environment (Mason & McDonough, 2012; Mahroof & Hagstrum, 2012).

This section reviews six species of particular importance to stored grain and processed commodities: *T. castaneum*, *Sitophilus oryzae* (L.), rice weevil, *R. dominica*, *Plodia interpunctella* (Hübner), Indian meal moth, *Trogoderma variabile* Ballion, warehouse beetle, and *Oryzaephilus surinamensis* (L.), sawtoothed grain beetle. Understanding the biology of these species provides the foundation for interpreting their susceptibility to insecticides and for evaluating new management tools.

1.2.1. Primary vs. Secondary Feeders: Biology, Feeding Behavior, and Damage Patterns

Stored-product insects are commonly categorized as primary or secondary feeders based on their ability to attack intact grain (Rees, 2004; Hagstrum & Subramanyam, 2009). Primary feeders can infest sound, undamaged kernels. *Sitophilus oryzae* and *R. dominica,* two of the most important primary pests of stored grain, are two species of internal feeders whose immature stages develop inside the kernel. Secondary feeders cannot penetrate sound kernels and instead feed externally on broken kernels, grain dust, milled products, and kernels previously damaged by primary feeders. Secondary species include *T. castaneum* and *O. surinamensis* (Rees, 2004;

Mason & McDonough, 2012). The two groups differ in oviposition behavior, larval development, feeding ecology, and the type of damage they cause, and these differences largely determine how each group is exposed to control measures.

1.2.1.1. Primary (Internal) Feeders

Primary internal feeders complete their immature development within the kernels of whole grain, from which they emerge as adults. *Sitophilus oryzae* and *R. dominica* differ, however, in how the immature stages gain entry to the kernel. *Sitophilus oryzae* females chew a small cavity into the kernel with the mouthparts at the tip of the rostrum, deposit a single egg, and seal the opening with a gelatinous plug, so that the entire immature period is spent inside the kernel (Sharifi & Mills, 1971; Rees, 2004). In contrast, *R. dominica* females lay their eggs outside the kernel, loosely on the grain surface or in crevices between kernels, and the newly hatched larvae bore into the kernel, often after first feeding on the flour produced by adult feeding (Potter, 1935; Edde, 2012). Once inside, larvae of both species feed on the endosperm and germ and pupate within the kernel.

This concealed development has two important consequences. First, larval feeding hollows out kernels, reducing weight, germination, and milling quality (Rees, 2004; Mason & McDonough, 2012). Second, because infestations are hidden, they often escape detection during inspection and may be transported across regions and international borders before adults emerge (Edde, 2012; Neethirajan et al., 2007). Immature stages within kernels are also largely protected from contact insecticides applied to grain or structural surfaces, which is a recurring limitation of residual treatments discussed later in this review.

1.2.1.2. Secondary (External) Feeders

Secondary feeders, in contrast, oviposit on the surfaces of commodities, residues, or processed foods, and the entire life cycle occurs outside the grain kernel (Rees, 2004; Hagstrum & Subramanyam, 2009). Larvae and adults feed on flour, germ, broken kernels, and grain dust, often in material previously damaged by primary feeders (Rees, 2004). Common secondary feeders include *T. castaneum*, *O. surinamensis*, and *P. interpunctella*. Their damage is primarily qualitative, because they contaminate commodities with frass, cast skins, body fragments, and silk, cause off-odors and discoloration, and in processing facilities can impair product flow and create costly sanitation problems (Mahroof & Hagstrum, 2012). Secondary feeders generally cause less weight loss than primary feeders. Weight loss from primary feeders results mainly from larvae consuming the endosperm inside sound kernels, so that infested kernels are hollowed and lose dry matter, whereas secondary feeders consume mostly broken kernels, dust, and milled material that make up a smaller part of the commodity (Rees, 2004; Mason & McDonough, 2012). Even so, the contamination they produce frequently renders products unfit for sale and leads to consumer complaints or export rejection (Phillips & Throne, 2010). Because larvae and adults move freely over surfaces, secondary feeders are also the group most directly exposed to residual insecticides on structural surfaces and packaging.

1.2.2. Insect Species

1.2.2.1. *Tribolium castaneum* (Herbst) - Red Flour Beetle

Description and Morphology. *Tribolium castaneum* is among the most cosmopolitan and economically significant pests of stored products (Hagstrum & Subramanyam, 2009). Its diagnostic morphological characters were described in detail by early taxonomists (Hinton, 1948;

Halstead, 1963) and are summarized under the adult stage below, and they remain important for accurate identification in storage pest surveys.

Egg Stage. Females are highly fecund and may lay several hundred eggs over a reproductive life that can extend for months (Sokoloff, 1974). Eggs are small, white, and cylindrical to oval, and they are deposited loosely in flour or other fine material, where their sticky surface becomes coated with food particles that camouflage them (Sokoloff, 1974; Rees, 2004). The rate of embryonic development is strongly temperature dependent, and eggs and other life stages are subject to cannibalism by larvae and adults, a density-dependent factor that becomes important in crowded populations (Sokoloff, 1974).

Larval Stage. Larvae are slender, cylindrical, and yellowish white, with a darker head capsule, three pairs of thoracic legs, and a pair of pointed urogomphi at the tip of the abdomen (USDA, 1986; Rees, 2004). They pass through a variable number of instars depending on diet and temperature. Howe (1956) showed that development from egg to adult is fastest at approximately 35 °C, slows progressively at lower temperatures, and is also influenced by relative humidity, with low humidity prolonging development and increasing mortality. Larvae are mobile within the food medium, and diet quality strongly affects development, as finely milled products such as flour support faster development than whole grain (Sokoloff, 1974; Mahroof & Hagstrum, 2012).

Pupal Stage. Pupation occurs in the food medium without a cocoon. Pupae are exarate, meaning that the legs, wings, and antennae are free from the body rather than fused to it, and they are initially white and darken before adult emergence (USDA, 1986). As in the other immature stages, the duration of the pupal stage decreases with increasing temperature up to the optimum (Howe, 1956).

Adult Stage. The adult is a small, reddish-brown beetle, approximately 3-4 mm in length, with a flattened, elongate-oval body (USDA, 1986; Rees, 2004). *Tribolium castaneum* is nearly identical to the closely related *Tribolium confusum* Jacquelin du Val, confused flour beetle, but the two species can be separated by their antennae. In *T. castaneum* the last three antennal segments are abruptly enlarged to form a distinct three-segmented club, whereas in *T. confusum* the antennal segments enlarge gradually toward the tip (Hinton, 1948; USDA, 1986). Males can be distinguished from females by a setiferous patch on the underside of the fore femur (Halstead, 1963; Bousquet, 1990). Adults are long-lived, capable of flight, and readily disperse between facilities (Campbell et al., 2010a). Adults and larvae of both *Tribolium* species are cannibalistic and consume eggs and pupae (Sokoloff, 1974).

Distribution and Ecology. *Tribolium castaneum* is distributed worldwide, with greatest prevalence in warm regions, but it is also common in temperate climates within heated mills, warehouses, and retail environments (Hagstrum & Subramanyam, 2009). Populations persist in residual flour and grain dust that accumulate in cracks, equipment, and structural voids, and long-term monitoring in flour mills has shown that populations rebound after structural fumigation (Campbell & Arbogast, 2004; Campbell et al., 2010a). After 21 fumigations in two flour mills, trap captures of *T. castaneum* took 174 ± 33 days to return to a threshold of 2.5 beetles per trap per 2 weeks, rebound was slower after autumn (248 ± 50 days) than spring (104 ± 21 days) fumigations, and improved integrated pest management (IPM) practices in one mill lengthened the time to rebound from 49 ± 15 to 246 ± 71 days (Campbell et al., 2010b). In a Kansas food-processing facility monitored for 22 months, *T. castaneum* was present year-round and stored-product beetles were nearly always captured in the first trapping interval after methyl bromide fumigation or dichlorvos plus pyrethrin fogging (Toews et al., 2006a). Females exploit

food patches in a way that spreads offspring among small resource patches, which favors persistence in fragmented residues (Campbell & Runnion, 2003). Population growth and damage potential vary among commodities. For example, larval development took 72.8 days on semolina compared with 59.6 and 54.6 days on cracked wheat and cracked maize, respectively (Skourti et al., 2020). However, fecundity was 28.7 eggs per female on semolina and only 2.7 and 1.2 eggs per female on cracked wheat and cracked maize, respectively. Thus, population growth was favored only on semolina (Skourti et al., 2020). In addition to commodity effects on population growth, temperature, humidity, and interspecific competition all influence population size of *T. castaneum* (Papanikolaou et al., 2018). Males produce the aggregation pheromone 4,8-dimethyldecanal, which is used in commercial monitoring traps (Suzuki, 1980). Flight is an important means of dispersal, and population genetic studies indicate substantial gene flow among populations through adult flight (Ridley et al., 2011).

Feeding Behavior and Damage. As a secondary pest, *T. castaneum* primarily infests flour, processed cereals, broken kernels, and grain residues. Infestations cause loss of dry matter, nutrient degradation, and accumulation of frass and cast skins (Rees, 2004). Adults secrete benzoquinones from abdominal and thoracic glands, which impart a pungent odor and discoloration to heavily infested flour and reduce its acceptability (Sokoloff, 1974). In addition to direct losses, the species is an indicator of poor sanitation, and infestations in processing and packaging environments can spread into finished products. Phosphine resistance in *T. castaneum* has been documented in several regions, including the U.S. (Opit et al., 2012b).

1.2.2.2. *Sitophilus oryzae* (L.) - Rice Weevil

Description and Morphology. *Sitophilus oryzae* is one of the most destructive primary pests of stored cereals worldwide (Rees, 2004; Hagstrum & Subramanyam, 2009). It is capable

of flight, a trait that allows it to disperse between storage structures and to infest grain in the field and in transport (Rees, 2004). Unlike secondary feeders such as *T. castaneum*, *S. oryzae* can complete its development inside intact, undamaged kernels, and infestations often remain invisible until adults emerge through characteristic round exit holes (Mason & McDonough, 2012).

Egg Stage. Females chew a small cavity in the kernel, deposit a single egg, and seal the opening with a gelatinous secretion that hardens into a plug, protecting the egg and concealing the oviposition site (Sharifi & Mills, 1971). Lifetime fecundity is typically in the range of several hundred eggs under favorable conditions (Hagstrum & Subramanyam, 2009).

Larval Stage. The larva is legless, fleshy, C-shaped, and creamy white with a brown head capsule (USDA, 1986). It feeds within the kernel throughout development, excavating the endosperm and passing through four instars (Sharifi & Mills, 1971). The rate of development depends on temperature and grain moisture content, and development is slowed or prevented at low grain moisture (Birch, 1945; Hagstrum & Subramanyam, 2009). Larval feeding produces hollowed kernels that lose weight and break easily during handling.

Pupal Stage. Pupation takes place inside the kernel. The pupa is exarate and white, darkening as the adult develops, after which the adult chews a round exit hole through the seed coat (Sharifi & Mills, 1971). Because all immature stages are concealed, they are largely protected from contact insecticides and are difficult to detect by visual inspection.

Adult Stage. Adults are reddish brown when newly emerged and darken to dark brown or nearly black. They are small weevils, approximately 2.5-4 mm in length, with an elongated rostrum bearing chewing mouthparts and geniculate antennae arising from the rostrum (USDA, 1986; Rees, 2004). Four faint reddish to yellowish spots on the elytra help separate *S. oryzae*

from *Sitophilus granarius* (L.), granary weevil, which lacks them, although separation from the closely related *Sitophilus zeamais* Motschulsky, maize weevil, is difficult on external characters alone (Halstead, 1986; Rees, 2004). The pronotum is densely punctate and the elytra are longitudinally striate. Sexual dimorphism is limited, but males and females can be separated by the shape and sculpture of the rostrum (Halstead, 1963). Adults are long-lived, can survive for weeks without food at cool temperatures, and are active fliers, which facilitates dispersal between grain bulks, warehouses, and containers (Rees, 2004).

Distribution and Ecology. *Sitophilus oryzae* occurs worldwide and is particularly abundant in warm, humid regions, although it is also common in temperate storage (Hagstrum & Subramanyam, 2009). It infests rice, wheat, maize, sorghum, and other whole cereals, and also processed products such as pasta that are sufficiently hard and compact for larval development (Rees, 2004). Populations grow most rapidly at warm temperatures and moderate to high grain moisture contents, conditions that may develop within the commodity during storage and long-distance transport (Howe, 1965; Mason & McDonough, 2012).

Feeding Behavior and Damage. Damage begins with oviposition, which breaches the protective seed coat, and continues as larvae consume the endosperm. Infested grain loses weight and germination, produces more broken kernels during milling, and becomes more susceptible to molds and secondary insects (Mason & McDonough, 2012). Because most damage occurs internally, infestation severity is often underestimated, and visually clean samples may still contain substantial hidden infestation. This "hidden infestation" problem complicates the inspection and certification of grain shipments, because hidden immature stages are not detected by visual inspection or sieving and require methods such as X-ray imaging, acoustic detection, or incubation of samples (Neethirajan et al., 2007).

1.2.2.3. *Rhyzopertha dominica* (F.) - Lesser Grain Borer

Description and Morphology. *Rhyzopertha dominica* is a primary pest of stored cereals worldwide and one of the most damaging species in stored wheat and rice (Edde, 2012). Adults are cylindrical, dark reddish brown to black beetles approximately 2-3 mm in length. The head is deflexed beneath a hood-like pronotum that bears rows of tubercles anteriorly, and the antennae terminate in a loose three-segmented club (USDA, 1986; Rees, 2004). Adults are strong fliers, and flight contributes to rapid dispersal among storage facilities and to the colonization of stored grain from external sources (Edde, 2012).

Egg Stage. Females lay several hundred eggs over their lifetime, singly or in clusters, loosely among kernels or in crevices on the grain surface rather than inside the kernel (Potter, 1935; Edde, 2012). This behavior distinguishes *R. dominica* from *S. oryzae* and leaves the egg stage exposed to environmental conditions and to grain protectants applied to the kernel surface.

Larval Stage. Newly hatched larvae are active and initially feed on flour and grain dust before boring into kernels, where they complete development (Potter, 1935). Mature larvae are C-shaped and creamy white, with a brown head capsule and short thoracic legs (USDA, 1986). Larval feeding hollows out kernels and produces large amounts of fine, powdery frass (Edde, 2012).

Pupal Stage. Pupation occurs within the kernel, and the adult chews an exit hole on emergence (Potter, 1935). Like *S. oryzae*, the concealed pupal stage is protected from contact insecticides.

Adult Stage. Unlike *S. oryzae*, in which damage is caused principally by larvae, adults of *R. dominica* are themselves highly destructive. They bore into kernels to feed, producing irregular holes and copious frass, and they are long-lived, so that a single generation can damage

many kernels (Edde, 2012). The combination of larval and adult feeding makes the species particularly damaging in stored wheat and rice (Edde, 2012).

Distribution and Ecology. *Rhyzopertha dominica* occurs in virtually all cereal-growing regions. It is most prevalent in warm climates but is also established in temperate regions, where flight activity and immigration into storage facilities have been documented throughout the warm season (Edde, 2012). Flight is strongly influenced by temperature and weather (Cox et al., 2007). In central Oklahoma, flight began in late March, ended in mid-November, and no beetles were trapped from December through February, with weekly weather variables explaining about 80-86% of the variation in trap catches (Edde et al., 2006). In mark-release-recapture studies, adults moved mean distances of 261-375 m and some dispersed more than 1,600 m (Mahroof et al., 2010). In northeast Kansas, *R. dominica* was captured in feed mill, grain elevator, and native prairie landscapes (Scheff et al., 2022), and outdoor populations can immigrate into seed wheat warehouses (Toews et al., 2006b). *Rhyzopertha dominica* captures peak in the warmest months (Scheff et al., 2022). Traps containing the male-produced aggregation pheromone, dominicalure (Williams et al., 1981), captured 8.2-fold and 5.4-fold more beetles than food-baited traps alone in 2017 and 2018, respectively, in trapping seasons in Kansas, that is, approximately 9.2-fold and 6.4-fold as many beetles (Scheff et al., 2022). Populations develop rapidly at high temperatures and are tolerant of relatively dry grain, which allows the species to thrive under conditions unfavorable to many other pests (Howe, 1965; Hagstrum & Subramanyam, 2009). High-level resistance to phosphine has been documented in *R. dominica* in Australia, Asia, and North America and is genetically well characterized (Collins et al., 2002; Opit et al., 2012b; Afful et al., 2018; Nayak et al., 2020).

Feeding Behavior and Damage. Because both larvae and adults bore into kernels, infestations produce extensive kernel damage, large amounts of powdery frass, and heating of the grain mass that favors mold development (Edde, 2012). Infested grain has a characteristic sweetish, musty odor and is discounted or rejected at sale (Rees, 2004). The combination of concealed larval development, long-lived destructive adults, strong flight, and phosphine resistance makes *R. dominica* one of the most difficult stored-grain pests to manage.

1.2.2.4. *Plodia interpunctella* (Hübner) - Indian Meal Moth

Description and Morphology. *Plodia interpunctella* is the most important moth pest of stored products in many parts of the world (Mohandass et al., 2007). Early biological studies in the U.S. documented its development on dried fruits (Hamlin et al., 1931), and the species is now known from a very wide range of commodities, including cereals and cereal products, dried fruits, nuts, chocolate, and pet food (Hagstrum et al., 2013). Adults are small moths with a wingspan of about 16-20 mm. The forewings are distinctly bicolored, with the basal portion pale grayish to yellowish and the distal portion reddish brown with a coppery sheen, which readily separates the species from other stored-product pyralids (Rees, 2004).

Egg Stage. Females lay from approximately one hundred to several hundred eggs, singly or in small clusters, on or near suitable food (Mohandass et al., 2007). Development is strongly temperature and diet dependent. When reared on bran diet, almonds, pistachios, and walnuts, development was fastest on bran diet, and at 35 °C the thermal threshold was exceeded: no larvae completed development on walnuts and no progeny were produced on any diet (Johnson et al., 1992).

Larval Stage. Larvae are the damaging stage. They are whitish, sometimes tinged pink, green, or yellow depending on diet, with a brown head capsule, and they pass through five to

seven instars (Mohandass et al., 2007). Development time depends on temperature and diet, and diet quality has a pronounced effect on the rate of development and survival (Johnson et al., 1992). Larvae spin silk as they feed, binding food particles together. Mature larvae typically leave the food to seek pupation sites in cracks, seams, and packaging, and this wandering behavior both spreads infestations and places larvae in concealed sites that are difficult to treat (Mohandass et al., 2007). Larvae are also capable of penetrating many flexible packaging materials, with penetration success depending on film type and thickness (Mullen et al., 2012; Scheff et al., 2018b).

Pupal Stage. Pupation occurs within a silken cocoon, usually in protected sites away from the food (Mohandass et al., 2007). The cocoon and the concealed location provide some protection from desiccation and from contact insecticides (Mohandass et al., 2007).

Adult Stage. Adults do not feed on the commodity and cause no direct damage, and their role is reproduction and dispersal (Mohandass et al., 2007). Adults are short-lived, generally surviving about 7 days, females begin ovipositing within 3 days of emergence, and most eggs are laid at night (Mason, n.d.-a). Females release a sex pheromone that attracts males, and synthetic versions of this pheromone are widely used in monitoring traps and in mating disruption (Burkholder & Ma, 1985; Mohandass et al., 2007).

Distribution and Ecology. *Plodia interpunctella* is cosmopolitan and occurs wherever stored commodities are produced, processed, or sold, including retail stores and homes (Mohandass et al., 2007). Surveys of eight stores of a retail pet store chain in Kansas recorded 30 stored-product insect species, 12-19 species per store, with *P. interpunctella* among the most prevalent and densities of 65-656 adults per kg in infested pet food (Roesli et al., 2003). Its broad

diet, rapid development at warm temperatures, and the dispersal of wandering larvae allow infestations to spread widely within facilities and containers (Mohandass et al., 2007).

Feeding Behavior and Damage. Damage results from larval feeding and, more importantly, from contamination with silk, frass, and cast skins (Mohandass et al., 2007). Webbing binds commodities together, impairs product flow, and makes infestations highly visible to consumers, so that infestations in retail and packaged foods lead to complaints, product returns, and brand damage (Mohandass et al., 2007). Management relies on sanitation, pheromone-based monitoring, aerosol and contact insecticides, IGRs, and insect-resistant packaging (Mohandass et al., 2007; Jenson et al., 2010).

1.2.2.5. *Trogoderma variabile* Ballion - Warehouse Beetle

Description and Morphology. *Trogoderma variabile* is a dermestid beetle closely related to *T. granarium*, one of the most serious quarantine pests of stored products worldwide (Athanassiou et al., 2019). Adults are small, oval beetles, approximately 2-4 mm in length, covered with fine setae that form a variable mottled pattern of brown and pale bands on the elytra (Beal, 1954; Rees, 2004). Larvae are elongate and densely covered with setae, including specialized barbed setae (hastisetae) characteristic of dermestid larvae, and they bear long tufts of hairs at the posterior end (Beal, 1954; Rees, 2004).

Egg Stage. Females lay their eggs in or near food or in cracks and crevices of storage structures (Beal, 1954). The egg stage lasts about 7 days on average, and the rate of egg development, like that of the other life stages, increases with temperature (Hagstrum & Subramanyam, 2009; Mason, n.d.-b).

Larval Stage. Larvae are the feeding stage and pass through a variable number of molts. Under unfavorable conditions, such as low temperature or food shortage, larvae can enter

diapause and survive for long periods without feeding (Beal, 1954; Hagstrum & Subramanyam, 2009). Diapause also enhances cold-hardiness in *T. variabile* (Abdelghany et al., 2015), and diapause-related tolerance of extreme temperatures is a general feature of stored-product dermestids, including *T. granarium* (Hadaway, 1955; Wilches et al., 2016). The cast skins and detached setae contaminate commodities and can cause allergic reactions in sensitive individuals (Rees, 2004).

Pupal Stage. Pupation occurs within the last larval skin, a behavior typical of dermestids, usually in sheltered locations within debris, commodities, or structural crevices (Beal, 1954). Pupae are therefore rarely exposed to surface treatments.

Adult Stage. Adults are relatively short-lived and capable of flight, which facilitates dispersal within and between storage facilities. In a food processing plant, marked adults moved across multiple floors of the facility and from 7 to 216 m through the warehouse (Campbell et al., 2002). Outside a food processing plant, marked males were recaptured an average of 75 m (range 21-508 m) from the release point, and some males marked outside the building were recaptured inside it (Campbell & Mullen, 2004). The daily flight activity of *T. variabile* has been characterized together with that of *R. dominica* (Beal, 1954; Wright & Morton, 1995). Adults contribute little to direct feeding damage, and reproduction begins soon after emergence, because adults mate shortly after emerging and most oviposition occurs during the first few days of adult life (Mason, n.d.-b).

Distribution and Ecology. *Trogoderma variabile* is widely distributed and is a common pest in warehouses, food processing facilities, and retail environments in North America (Hagstrum & Subramanyam, 2009). Its tolerance of starvation, its ability to persist in very small amounts of residual food, and larval diapause make it difficult to eliminate once established.

Larvae are considerably more tolerant of insecticides than adults. In studies on concrete treated with deltamethrin or β-cyfluthrin, adult mortality was 72-100%, whereas larval mortality ranged from about 18% to 97% with deltamethrin and 20% to 72% with β-cyfluthrin (Ghimire et al., 2016). Several characteristics of dermestid larvae probably contribute to this tolerance. Larvae are larger than adults, and in the related *T. granarium*, large larvae were far more tolerant of contact insecticides than small larvae (Kavallieratos et al., 2016), suggesting that a greater body mass dilutes the dose acquired from a treated surface. The dense covering of setae, including the hastisetae, may also hold the body away from the treated surface and reduce the amount of residue that reaches the cuticle. In addition, larvae can enter diapause, during which feeding, movement, and metabolism are reduced, and diapausing dermestid larvae are particularly tolerant of insecticides and other control measures (Wilches et al., 2016; Arthur et al., 2019). The species has also served as a model for evaluating residual pyrethroids and methoprene-treated packaging (Scheff et al., 2017), and its close relatedness to *T. granarium* makes it a useful surrogate in laboratory studies (Scheff et al., 2019).

Feeding Behavior and Damage. Damage is caused mainly by larval feeding and by contamination with cast skins, setae, and frass. Larvae feed on a wide range of cereal products, oilseeds, spices, and animal-based foods (Hagstrum & Subramanyam, 2009). Infestations are associated with inadequate sanitation, residual debris in storage facilities, and defects in packaging, and larvae can persist in trace residues that remain after incomplete cleaning.

1.2.2.6. *Oryzaephilus surinamensis* (L.) - Sawtoothed Grain Beetle

Description and Morphology. *Oryzaephilus surinamensis* is one of the most common secondary pests of stored grain and processed foods (Rees, 2004). Adults are slender, flattened, reddish-brown beetles about 2.5-3.5 mm in length. The most diagnostic feature is the row of six

tooth-like projections on each lateral margin of the pronotum, which gives the species its common name (USDA, 1986; Halstead, 1986).

Egg Stage. Females lay eggs singly or in small groups in crevices of kernels or loosely in the food (Rees, 2004). Fecundity typically amounts to a few hundred eggs over a long reproductive life, and development and reproduction differ substantially among commodities (Hagstrum & Subramanyam, 2009; Nika et al., 2020).

Larval Stage. Larvae are slender, whitish, and active, with three pairs of legs. They feed externally on broken kernels, grain dust, and processed products and cannot attack sound kernels (Rees, 2004). Development is rapid at warm temperatures, and the species is relatively tolerant of dry conditions (Howe, 1965).

Pupal Stage. Mature larvae construct a protective cell from food particles in which they pupate (Rees, 2004).

Adult Stage. Adults are long-lived, often surviving for several months, and are highly active. Although adults have functional wings, dispersal within storage facilities occurs mainly by crawling, and their flattened bodies allow them to penetrate small openings in packaging (Rees, 2004; Mullen et al., 2012).

Distribution and Ecology. *Oryzaephilus surinamensis* is cosmopolitan and abundant in grain storage, flour mills, warehouses, and retail outlets (Rees, 2004; Hagstrum & Subramanyam, 2009). Because it depends on broken grain and processed commodities, population growth is closely tied to sanitation and handling damage. Surveys of residual grain in commercial elevators have shown that secondary feeders such as *O. surinamensis* and *T. castaneum* are common in the spilled grain and dust that accumulate in and around storage structures (Arthur et al., 2006).

Feeding Behavior and Damage. Damage is primarily qualitative and results from contamination with live insects, cast skins, and frass. The species is a frequent problem in dried fruit, nuts, breakfast cereals, and pet food, where even low-level infestations render products unacceptable (Rees, 2004).

1.2.3. Pathways of Container Infestation

Shipping containers are standardized units for moving bulk and bagged commodities, and the maritime network through which they travel has been modeled as a pathway for invasive stored-product pests (Paini & Yemshanov, 2012). Bulk transport in rail cars has similarly been shown to carry detectable infestations (Perez-Mendoza et al., 2004), and network analyses of the postharvest grain system identify transport and storage nodes as key points for the spread of pests (Hernandez Nopsa et al., 2015). Infestation pathways can be grouped into pre-loading contamination, infestation during transit, and post-unloading transfer.

1.2.3.1. Pre-Loading Contamination

Pre-loading contamination is generally regarded as the most important route of infestation. Commodities frequently enter containers already infested from farm storage, elevators, or warehouses (Arthur et al., 2006). Primary feeders such as *S. oryzae* and *R. dominica* are particularly problematic because their immature stages are concealed within kernels and are unlikely to be detected during routine inspection (Edde, 2012). Containers themselves may also act as sources of infestation when residual grain, packaging debris, and dust remain in corners, floor seams, and door channels between shipments. Dermestid larvae, which can survive long periods without food, are especially likely to persist in such residues (Beal, 1954; Athanassiou et al., 2019).

1.2.3.2. Infestation During Transit

Even when commodities are apparently free of insects at loading, populations can develop or spread during transport (Subramanyam et al., 2023). Insects concealed within kernels complete development and emerge, and mobile species disperse through the grain mass, between bags, and into spillage. Container interiors might be expected to be unfavorable for insects, because they are dark and poorly ventilated, and the steel shell can heat well above ambient air temperature in the sun and cool rapidly at night (Agrafioti et al., 2021). However, many stored-product species are adapted to dark, enclosed storage environments and tolerate limited ventilation and a wide range of temperatures (Howe, 1965; Hagstrum & Subramanyam, 2009). Warm periods during transit can permit oviposition and development, and because species such as *P. interpunctella* and *O. surinamensis* can complete a generation in a matter of weeks under warm conditions, long voyages provide time for population growth (Howe, 1965; Mohandass et al., 2007).

1.2.3.3. Post-Unloading Transfer

The third pathway arises after containers are unloaded. Insects that survive transit may disperse into warehouses, silos, and processing facilities at the destination (Paini & Yemshanov, 2012; Stejskal et al., 2020). From a regulatory perspective, this pathway is the most significant, because the detection of live insects at the port of entry may lead to rejection, mandatory treatment, or destruction of the consignment, and because the accidental introduction of quarantine species can trigger costly eradication programs (Myers & Hagstrum, 2012; Athanassiou et al., 2019).

1.2.4. Comparative Life History and Implications for Management

When the six species are considered together, the most important difference for management is where the immature stages develop. *Sitophilus oryzae* and *R. dominica* develop inside kernels, whereas *T. castaneum*, *O. surinamensis*, *P. interpunctella*, and *T. variabile* develop in the commodity or in residues (Rees, 2004; Mason & McDonough, 2012). Surface-active treatments such as pyrethroids and treated packaging are therefore expected to be most effective against secondary feeders and the adults of primary feeders, and much less effective against immatures already inside kernels.

The species also differ in mobility. Adults of *S. oryzae*, *R. dominica*, *T. castaneum*, and *P. interpunctella* fly readily and can recolonize treated structures (Edde et al., 2006; Campbell et al., 2010a), whereas wandering larvae of *P. interpunctella* and *Trogoderma* species contact structural surfaces and packaging as they seek pupation sites (Mohandass et al., 2007; Mullen et al., 2012).

Finally, all six species have high reproductive potential and short generation times at warm temperatures (Howe, 1965; Hagstrum & Subramanyam, 2009), so that small surviving populations can increase rapidly during storage or transit. Tactics that suppress progeny, such as insect growth regulators (IGRs), can therefore contribute to management even without immediate adult mortality, and evaluations should consider progeny production as well as adult mortality. These differences are summarized in Table 1.1.

Table 1.1. Comparative biology of the six stored-product insect species reviewed in this chapter.

Species	Feeding guild	Oviposition site	Site of immature development	Adult dispersal
T. castaneum	Secondary	Loose in food	In food, outside kernels	Flies and walks
S. oryzae	Primary (internal)	Inside kernel, sealed with a plug	Inside kernel	Flies and walks
R. dominica	Primary (internal)	Loose among kernels	Inside kernel after larval entry	Strong flier
P. interpunctella	Secondary	On or near food	In food, pupation in protected sites	Flies, adults do not feed
T. variabile	Secondary	In food or crevices	In food and residues, larval diapause	Flies and walks
O. surinamensis	Secondary	In crevices or loose in food	In food, outside kernels	Mainly walks

Note: Compiled from Beal (1954), Rees (2004), Mohandass et al. (2007), Edde (2012), and Mason and McDonough (2012).

1.3. Conventional Management Strategies and Their Limitations

1.3.1. Fumigation

For most of the past century, fumigation has been the central practice for disinfesting bulk commodities and consignments in trade. Fumigants penetrate commodities and reach all life stages, including the eggs, larvae, and pupae that develop inside kernels and are shielded from contact insecticides (Bond, 1984; Phillips et al., 2012).

Phosphine, generated from aluminum or magnesium phosphide formulations, acts primarily on mitochondrial respiration and oxidative metabolism (Chaudhry, 1997), and is now the principal fumigant for stored commodities worldwide because it is inexpensive, easy to apply, and leaves minimal residues (Phillips et al., 2012). Its efficacy depends on maintaining a lethal concentration for a sufficient exposure period, which in turn depends on the gas-tightness of the enclosure, temperature, and the life stages present (Bond, 1984; Phillips et al., 2012). For most species, the eggs and pupae are the most tolerant life stages (Bond, 1984; Phillips et al.,

2012). Methyl bromide was historically preferred for QPS treatments because of its rapid action, but its phase-out under the Montreal Protocol has restricted it to exempted uses and has increased reliance on phosphine and on alternatives such as sulfuryl fluoride, heat, and modified atmospheres (Fields & White, 2002; Navarro, 2012). Continued reliance on a single fumigant has accelerated the selection of resistance, and strong phosphine resistance is now documented in several key species, including *R. dominica* and *T. castaneum* (Collins et al., 2002; Opit et al., 2012b; Nayak et al., 2020).

Phosphine is slow-acting relative to methyl bromide and requires several days of exposure in a well-sealed enclosure (Bond, 1984; Phillips et al., 2012; Nayak et al., 2020). Inadequate sealing, short exposure periods, and low temperatures all increase the probability that insects survive. Several models have been developed that depict the effect of leakage and sorption on the decline of phosphine concentrations in poorly sealed structures (Plumier et al., 2018), and survival of partially resistant individuals contributes to the selection of resistance (Opit et al., 2012a; Nayak et al., 2020). Alternatives to methyl bromide include sulfuryl fluoride, heat or cold treatment (Fields, 1992), and modified or controlled atmospheres, each of which has advantages and limitations in cost, speed, and compatibility with commodities and structures (Fields & White, 2002; Navarro, 2012; Phillips et al., 2012).

Sulfuryl fluoride has become one of the most widely used replacements for methyl bromide in food-handling structures and for some stored commodities. It was used for many years for structural fumigation, and its use on stored food commodities was registered in the U.S. in 2004, when tolerances were established for sulfuryl fluoride and fluoride residues in treated commodities (Phillips et al., 2012). Compared with phosphine, sulfuryl fluoride acts quickly and penetrates commodities well, and most fumigations require exposure periods of only 24 to 48 h,

which makes it attractive for shipping containers and other transport units that must be treated and released quickly (Fields & White, 2002; Phillips et al., 2012). Phosphine resistance does not confer cross-resistance to sulfuryl fluoride, and sulfuryl fluoride fumigation controlled phosphine-resistant insects infesting stored wheat (Opit et al., 2016; Jagadeesan & Nayak, 2017). Its main limitations are the tolerance of insect eggs, which are generally 5 to 30 times more tolerant than other life stages and therefore determine the dose and exposure period required (Jagadeesan & Nayak, 2017; Hwaidi et al., 2017), and the fluoride residues it leaves in treated commodities. Because its use is limited to commodities for which residue tolerances have been established, and registrations differ among countries, sulfuryl fluoride is not approved for all commodities or for all destinations (Phillips et al., 2012).

1.3.2. Residual Surface Treatments

Contact insecticides are applied to the surfaces of storage structures, processing facilities, and container interiors to kill insects that harbor in structural refugia or move across treated surfaces. Organophosphates such as malathion, chlorpyrifos-methyl, and pirimiphos-methyl were historically the standard residual treatments, but resistance to malathion became widespread in *T. castaneum* and other species, and regulatory reviews have restricted the use of several organophosphates (Zettler & Cuperus, 1990; Arthur, 1996; Arthur & Subramanyam, 2012). Pyrethroids such as deltamethrin, cyfluthrin, and β-cyfluthrin, alone or combined with the synergist piperonyl butoxide (PBO) or with IGRs, are now among the most widely used residual surface treatments (Arthur & Subramanyam, 2012). Other active ingredients, including chlorfenapyr and spinosad, have also been evaluated as surface treatments (Toews et al., 2003; Arthur, 2008b). Aerosol applications of synergized pyrethrins, sometimes combined with

methoprene or pyriproxyfen, are used in processing facilities and warehouses as alternatives or supplements to structural fumigation (Arthur, 2012).

1.3.3. Sanitation

Sanitation is universally regarded as the foundation of stored-product pest management. Removal of spilled grain, dust, and residues from storage structures, equipment, and transport vehicles reduces the food and refugia that sustain residual populations (Heaps, 2012). Grain residues in and around commercial elevators frequently support populations of several stored-product species (Arthur et al., 2006), and monitoring in flour mills has shown that *T. castaneum* populations rebound after fumigation, emphasizing that chemical treatments cannot compensate for persistent residues and refugia (Campbell et al., 2010a). For containers, sanitation includes sweeping and vacuuming the interior, inspecting and replacing infested pallets and dunnage, and checking floor seams and door channels for accumulated residues.

Sanitation is also closely linked to the efficacy of chemical treatments. Food residues provide refugia from insecticides and fumigants, support insects that survive treatment, and can reduce the contact of insects with treated surfaces (Toews et al., 2010; Heaps, 2012). Sanitation programs must therefore be integrated with treatment schedules, with cleaning conducted before residual applications so that insecticides are deposited on clean surfaces. In practice, sanitation can be difficult to apply consistently because of labor costs and time pressure, particularly when containers must be turned around rapidly between shipments (Morrison et al., 2019; Bingham & Hagstrum, 2024).

1.3.4. Limitations of Current Methods in Shipping Containers

Fumigation, residual insecticides, and sanitation are each less reliable in shipping containers than in purpose-built storage structures. Containers are simultaneously closed and

leaky, because gas can escape through door seals, floor seams, and damaged panels, so that fumigant concentration may fall below lethal levels before the exposure period is complete. Studies combining wireless phosphine sensors with computational fluid dynamics in 20- and 40-ft containers found that phosphine concentration was uniform in empty containers but varied considerably within loaded containers, and model predictions agreed well with sensor measurements (Agrafioti et al., 2021). In addition, fumigation during transit raises worker-safety concerns at ports and on vessels (Phillips et al., 2012).

Residual insecticides offer only partial protection (Arthur & Subramanyam, 2012). Their persistence varies with the surface to which they are applied, with temperature and humidity, and with the accumulation of dust and food residues on treated surfaces (Arthur, 1997; Toews et al., 2003; Arthur & Subramanyam, 2012). Contact insecticides also leave internal feeders largely untouched, because the immature stages of *S. oryzae* and *R. dominica* develop inside kernels and are not exposed to treated surfaces (Edde, 2012). Finally, containerized consignments are often infested by several species at once, each with different behavior and susceptibility, so that no single tactic is likely to provide complete control (Phillips & Throne, 2010; Scheff & Phillips, 2020).

1.3.5. Regulatory Constraints in International Trade

Regulatory requirements strongly influence the feasibility of management practices in trade. The Montreal Protocol has restricted methyl bromide to quarantine, pre-shipment, and critical uses, which has increased dependence on phosphine (Fields & White, 2002). The use of residual insecticides is constrained by maximum residue limits that differ among importing countries, by registration status, and by market requirements for low or zero residues, including organic markets in which synthetic insecticides cannot be used (Arthur & Subramanyam, 2012).

At the same time, importing countries apply phytosanitary standards that may require the absence of live insects or of specific quarantine species (Myers & Hagstrum, 2012; Athanassiou et al., 2019). Together, these constraints favor integrated approaches that combine sanitation, monitoring, and targeted, reduced-risk treatments rather than reliance on broad-spectrum chemicals.

Quarantine treatments must achieve very high efficacy, which few alternatives to methyl bromide reliably provide for all life stages and commodities (Fields & White, 2002), and exporters shipping to several markets must satisfy differing requirements for residues, live insects, and approved treatments (Myers & Hagstrum, 2012).

1.3.6. Insecticide Resistance

Insecticide resistance is one of the most important constraints on stored-product protection, and its development is closely linked to the repeated use of a small number of chemical classes in enclosed environments where insect populations are exposed generation after generation (Opit et al., 2012a). Early surveys in the U.S. documented resistance to malathion and other organophosphates in *T. castaneum* and *R. dominica* collected from farm-stored wheat in Oklahoma. All eight *T. castaneum* strains and all 21 *R. dominica* strains collected were resistant to malathion, and 8 of the 21 *R. dominica* strains were also resistant to phosphine (Zettler & Cuperus, 1990). This study demonstrated that use of malathion in stored wheat could be contributing towards resistance in field populations in Oklahoma (Zettler & Cuperus, 1990). An earlier FAO global survey had already shown that resistance to the grain protectants malathion and lindane was widespread among stored-grain pests (Champ & Dyte, 1976). These findings contributed to the decline of malathion as a grain protectant and to the adoption of alternative chemistries (Boyer et al., 2012).

Resistance to phosphine is now the most serious resistance problem in stored grain (Opit et al., 2012a; Nayak et al., 2020). Because phosphine is one of the few fumigants registered and widely used on stored commodities, its loss would leave few practical alternatives for disinfesting grain in bulk or in containers (Nayak et al., 2020). In Australian *R. dominica*, weakly and strongly resistant strains were 23.4 and 600 times more resistant than a susceptible strain, resistance was autosomal and incompletely recessive, and the strong phenotype resulted from the genes of the weak phenotype plus an additional major gene (Collins et al., 2002). Autosomal means that resistance is not sex linked, and incompletely recessive means that heterozygous insects are only slightly more tolerant than susceptible insects. In the U.S., resistance to phosphine has been documented in *T. castaneum* and *R. dominica* collected from stored wheat in Oklahoma. In collected adults, 8 out of the 9 *T. castaneum* populations and all 5 *R. dominica* populations contained phosphine-resistant individuals. The resistance frequencies reached 94% and 98% for *T. castaneum* and *R. dominica*, respectively, and the most resistant populations were 119-fold and more than 1,500-fold more resistant than susceptible strains for *T. castaneum* and *R. dominica*, respectively (LC_{99} values of 377 and 3,430 ppm, compared with about 3 and 2 ppm, respectively) (Opit et al., 2012b). Subsequent surveys found phosphine resistance to be geographically widespread in North American populations of *T. castaneum* and *R. dominica*. Resistant individuals were detected in 12 of 25 *T. castaneum* populations from the U.S. and Canada, including populations from Alabama, Arkansas, Florida, Georgia, Kansas, Oklahoma, and Texas, and in 32 of 34 *R. dominica* populations, including populations from Alabama, Arkansas, California, Florida, Kansas, Oklahoma, and Texas (Cato et al., 2017; Afful et al., 2018; Hubhachen et al., 2025). Resistance has also been documented in *T. castaneum* and *P. interpunctella* in California (Gautam et al., 2016). In Australia, national monitoring has tracked

increasing resistance frequencies in *R. dominica* and *S. oryzae* (Holloway et al., 2016; Collins et al., 2017).

Strong resistance results from the synergistic interaction of two genes, *rph1* and *rph2*, the latter encoding the mitochondrial enzyme dihydrolipoamide dehydrogenase (Schlipalius et al., 2012; Nayak et al., 2020). In one Australian survey, 49% of samples carried at least one *rph2* resistance allele even though only 7% expressed the strong resistance phenotype (Nayak et al., 2020). Based on genomic research, with molecular markers based on the *rph1* and *rph2* genes, rapid diagnosis of phosphine resistance in U.S. populations can be achieved (Chen et al., 2015). Globally, phosphine resistance has been reported from Australia, Asia, Africa, and the Americas, and the frequency and strength of resistance have increased in several regions over recent decades (Nayak et al., 2020).

Several factors contribute to the development of phosphine resistance. Fumigations conducted in poorly sealed structures, including leaky storage bins and containers, fail to maintain lethal concentrations for long enough to kill the most tolerant life stages, so that partially resistant individuals survive and reproduce (Mills, 1983; Phillips et al., 2012; Opit et al., 2012a). Simulation models indicate that consistent, effective dosing is the key factor in avoiding the evolution of resistance (Shi et al., 2013). Repeated fumigation of the same commodity and the movement of resistant insects in trade further spread resistance. Resistance management strategies therefore emphasize proper sealing and dosing, adequate exposure periods and monitoring of the fumigation, surveying for resistance in field populations, and rotation or combination of fumigation with tactics that have different modes of action, including aeration cooling, residual protectants, and IGRs (Opit et al., 2012a; Nayak et al., 2015, 2020). Sulfuryl fluoride has been evaluated as an alternative for phosphine-resistant populations because

phosphine resistance has not been shown to confer cross-resistance to sulfuryl fluoride (Jagadeesan & Nayak, 2017).

Resistance is not restricted to fumigants. Reduced susceptibility to pyrethroids has been reported in several stored-product beetles, and the mechanisms identified in other insects, including target-site mutations in the voltage-gated sodium channel and enhanced metabolism by cytochrome P450 enzymes, are expected to operate in stored-product species as well (Davies et al., 2007; Soderlund, 2012). Field strains of stored-grain insects also vary in susceptibility to newer protectants: spinosad did not completely control field strains of *T. castaneum* and *O. surinamensis* from Kansas, and the LD99 of two *O. surinamensis* field strains was about six times that of a laboratory strain, whereas chlorpyrifos-methyl plus deltamethrin controlled all strains (Sehgal et al., 2013), and resistance to methoprene has been reported in a stored-product beetle, *Lasioderma serricorne* (F.), cigarette beetle (Benezet & Helms, 1994), indicating that IGRs are not immune to resistance. The use of synergists that inhibit metabolic enzymes and of combination products that pair adulticides with IGRs is one approach to delaying resistance to residual insecticides (Bernard & Philogène, 1993; Arthur, 2019). For containers, where treatments are applied before loading and cannot easily be repeated, the risk that surviving insects are resistant supports the value of combining tactics with independent modes of action.

1.4. Residual Contact Insecticides in Grain Protection

Residual contact insecticides remain central to protecting stored commodities, particularly as the industry moves away from methyl bromide and faces increasing phosphine resistance (Fields & White, 2002; Nayak et al., 2020). In containers and warehouses, they serve two functions: they suppress surface-active insects in empty or newly loaded structures, and they provide a protective barrier during storage and transit. Their performance depends on the

interaction between the active ingredient, the treated surface, the environmental conditions, and the biology of the target species.

Grain protectants are applied directly to the commodity, whereas contact insecticides are applied to the floors, walls, and equipment of empty storage structures and containers. The two uses of insecticides differ in application rate, substrate, and regulatory requirements, because grain protectants must comply with residue limits in the commodity (Arthur, 1996; Arthur & Subramanyam, 2012).

Residual efficacy is usually evaluated by confining insects on treated arenas of the relevant material for a defined interval, assessing knockdown and then delayed mortality after transfer to food, and repeating the bioassay on surfaces aged for increasing periods (Toews et al., 2003; Arthur et al., 2009). Because knocked-down insects may recover, both measures are needed, and for IGRs the relevant outcome is suppression of progeny or adult emergence (Arthur, 2016b; Wijayaratne et al., 2018).

1.4.1. Pyrethrins and Pyrethroids

Pyrethrins are insecticidal esters obtained from the flowers of *Tanacetum cinerariifolium* (formerly *Chrysanthemum cinerariaefolium*). The natural extract, pyrethrum, contains six related esters, pyrethrins I and II, cinerins I and II, and jasmolins I and II (Casida, 1980). Pyrethrins provide rapid knockdown and have low mammalian toxicity, but they are rapidly degraded by light and air, which limits their residual activity (Casida, 1980). This instability led to the development of synthetic pyrethroids, in which structural modifications increase photostability and insecticidal potency while retaining the same mode of action (Casida, 1980; Davies et al., 2007).

Pyrethrins and pyrethroids act on voltage-gated sodium channels in nerve membranes (Soderlund, 2012). They bind to the channel and slow its inactivation, producing a prolonged sodium current that causes repetitive nerve discharges, hyperexcitation, and, at higher doses, depolarization block and paralysis (Davies et al., 2007; Soderlund, 2012). Pyrethroids are commonly divided into two groups. Type I compounds, such as permethrin and bifenthrin, lack an α-cyano group and produce repetitive firing (Vais et al., 2001; Soderlund, 2012). Type II compounds, such as deltamethrin, cypermethrin, and cyfluthrin, contain an α-cyano group, prolong channel opening to a greater extent, and typically cause more severe intoxication (Vais et al., 2001; Soderlund, 2012). The toxicity of pyrethroids also depends on temperature. Many pyrethroids, particularly Type I compounds, have a negative temperature coefficient of toxicity, meaning that they are more toxic at lower temperatures, whereas the relationship is weaker or variable for some Type II compounds and can differ among species and temperature ranges (Harris & Kinoshita, 1977; Whiten & Peterson, 2016). This relationship is relevant when the efficacy of residual treatments is compared across temperatures. Resistance to pyrethroids arises through target-site mutations in the sodium channel (knockdown resistance) and through enhanced metabolic detoxification (Davies et al., 2007).

In stored-product protection, pyrethroids are used both as grain protectants and as structural treatments. Deltamethrin and β-cyfluthrin are effective against a broad range of external feeders on treated surfaces, but residual activity varies with formulation, surface type, and exposure conditions (Arthur, 1997; Arthur & Subramanyam, 2012). Against dermestids, residual pyrethroids including deltamethrin and β-cyfluthrin on concrete killed 90-100% of *T. granarium* and *Trogoderma inclusum* LeConte, larger cabinet beetle, adults for up to 3 months (Ghimire et al., 2017). However, delayed larval mortality remained below 26%, and field-strain

larvae were more tolerant than laboratory-strain larvae (Ghimire et al., 2017). Arthur (2018) used the rapidity of knockdown of *T. castaneum* on a treated surface to assess the residual efficacy of deltamethrin and found that efficacy varied with temperature and season in field and laboratory settings. Similarly, deltamethrin (Polyzone®) on concrete degraded more quickly when arenas were held outdoors than in a shed, and more quickly in a shed than in the laboratory, as shown by increased survival and adult emergence of *T. granarium* larvae (Arthur et al., 2019). Field studies in pilot-scale warehouses have shown that surface treatments with cyfluthrin can suppress *T. castaneum* activity, but populations were not fully eliminated (Toews et al., 2005b; Toews et al., 2009). Pyrethroids are less effective against internal feeders whose immature stages are protected inside kernels, and the long-term efficacy of deltamethrin as a grain protectant has been improved by combining it with methoprene (Arthur, 2019).

Pyrethrins and pyrethroids are also delivered as aerosols in food processing facilities and warehouses. Aerosols are dispersed into the airspace and settle on exposed surfaces, so their efficacy depends on the distribution of the droplets, the location of the target insects, and the presence of obstructions and food residues. Studies in commercial food storage facilities have shown that pyrethrin aerosol deposition and the resulting mortality of *T. confusum* vary considerably with position within the facility (Arthur & Campbell, 2008; Arthur, 2008a). The presence of flour on treated surfaces reduced the efficacy of aerosolized insecticides against *T. castaneum*, demonstrating that food residues can protect insects from surface deposits (Toews et al., 2010). Combining synergized pyrethrins with methoprene in aerosols extends activity to immature stages. Such combinations reduced the development of *Tribolium* larvae exposed to treated surfaces (Sutton et al., 2011) and suppressed eggs and larvae of *P. interpunctella* (Jenson et al., 2010). Synergized pyrethrin aerosol produced more than 88% mortality of all life stages of

T. castaneum and *T. confusum* when insects were directly exposed, but mortality was significantly lower when insects were treated together with flour or moved onto treated flour, and larvae and adults were more tolerant than eggs and pupae (Kharel et al., 2014). Pyrethrin plus methoprene aerosol provided residual activity against flour beetles in a commercial flour mill (Arthur, 2015b).

Deltamethrin and β-cyfluthrin, the two pyrethroids most relevant to this thesis, are both Type II compounds. Deltamethrin is a single, highly active stereoisomer, whereas β-cyfluthrin is an enriched mixture of the most insecticidal isomers of cyfluthrin (Soderlund, 2012). Both are registered in several countries for use as residual structural treatments in storage facilities, and deltamethrin is also used as a grain protectant, alone or in combination with other active ingredients (Arthur & Subramanyam, 2012; Arthur, 2019). Their high intrinsic activity allows very low application rates, which is advantageous from a residue perspective, but it also means that small reductions in the amount of active ingredient available on a surface, caused by absorption or degradation, can substantially reduce efficacy (Arthur & Subramanyam, 2012; Arthur et al., 2015).

1.4.2. (*S*)-Methoprene

Methoprene is an IGR that mimics juvenile hormone. Exposure of immature insects disrupts metamorphosis, so that larvae fail to pupate or develop into malformed adults that cannot reproduce (Oberlander et al., 1997; Henrick, 2007). The biologically active isomer, (*S*)-methoprene, is used in stored-product protection as a grain protectant, as a surface treatment, as an aerosol, and incorporated into packaging (Wijayaratne et al., 2018). Because it targets an endocrine pathway absent in vertebrates and has very low mammalian toxicity, methoprene is regarded as a reduced-risk insecticide (Henrick, 2007).

As a grain protectant, methoprene is particularly effective against *R. dominica*, preventing the development of progeny at low application rates (Samson et al., 1990; Arthur, 2004; Wijayaratne et al., 2018). Methoprene applied as a layer treatment to the surface of wheat, rice, and maize also controlled *R. dominica* (Athanassiou et al., 2011b). IGRs have been evaluated as grain protectants for other stored product species on wheat and maize (Kavallieratos et al., 2012), and in small-bin trials with wheat treated with methoprene, alone or with controlled aeration. These species included *P. interpunctella*, *T. castaneum*, *Cryptolestes ferrugineus* (Stephens), rusty grain beetle, *R. dominica*, and *S. oryzae*. Results showed that methoprene, alone or with aeration, was very effective in controlling *P. interpunctella*, *T. castaneum*, *C. ferrugineus*, and *R. dominica*, but it did not give good control of *S. oryzae*, and aeration alone was only somewhat effective in suppressing population development (Liu et al., 2016). When wheat, brown rice, rough rice, and corn were treated with 1.25 or 2.5 ppm methoprene and held for 24 months, both rates completely suppressed progeny of *R. dominica*, whereas some progeny of *Sitotroga cerealella* (Olivier), Angoumois grain moth, developed, indicating that additional treatments are needed for complete control of *S. cerealella* (Arthur, 2016a). Efficacy differs among species. Because primary pests develop inside kernels, they tend to be less susceptible to IGRs and may be less completely controlled, and methoprene does not kill adults, so infestations may remain visible even when reproduction is suppressed (Oberlander et al., 1997; Wijayaratne et al., 2018). For this reason, methoprene is often combined with an adulticide such as a pyrethroid or spinosad to broaden the spectrum of control (Athanassiou et al., 2011a; Arthur, 2019).

On structural surfaces and in aerosols, methoprene suppresses the development of immature *T. castaneum* and *P. interpunctella*, but residual efficacy depends on temperature,

surface, and the presence of food (Jenson et al., 2009, 2010; Arthur, 2012). On surfaces stored at 20, 30, or 35 °C for up to 24 weeks, adult emergence of *T. castaneum* exposed as larvae on methoprene-treated concrete increased over time, whereas no adults emerged from larvae exposed on varnished wood even after 24 weeks (Wijayaratne et al., 2012). The addition of flour reduced efficacy on concrete but not on wood, and the authors concluded that persistence was affected more by substrate than by temperature (Wijayaratne et al., 2012). Methoprene can also be transferred horizontally from treated to untreated *Tribolium* adults, which may extend its effects beyond the insects that directly contact a treated surface (Tucker et al., 2014a, 2014b). IGRs have been reviewed extensively as tools for stored-product protection (Oberlander & Silhacek, 2000). A racemic methoprene was introduced for stored products in the 1980s and later replaced by the purified (*S*)-isomer, and recent research has increasingly focused on its sublethal effects (Wijayaratne et al., 2018). The related juvenile hormone analogues hydroprene and pyriproxyfen have also been evaluated as surface treatments, and their residual activity differs among wood, metal, and concrete surfaces (Mohandass et al., 2006a; Arthur et al., 2009). Methoprene is also the active ingredient in commercially available insecticide-incorporated packaging, discussed in Section 1.7 (Arthur, 2016b; Scheff et al., 2017).

The sensitivity of individual life stages to methoprene varies. Eggs and early immature stages exposed to treated surfaces or treated grain often fail to complete development, while exposure of late-instar larvae typically results in deformed pupae or adults (Oberlander et al., 1997; Wijayaratne et al., 2018). These stage-specific effects reflect the role of juvenile hormone in development. Juvenile hormone must be present during larval growth but must decline in the final larval instar before the insect can commit to pupal and adult development (Riddiford, 1994). Exposure of eggs to a juvenile hormone analogue disrupts embryonic development, so

that many eggs fail to hatch, and larvae exposed early in development carry the analogue as they grow, so that metamorphosis is blocked and they die as larvae or at the larval-pupal molt. When late-instar larvae are exposed near the time of pupal commitment, development can proceed only partially, producing larval-pupal or pupal-adult intermediates and deformed adults that cannot reproduce (Oberlander et al., 1997; Henrick, 2007; Wijayaratne et al., 2018). The related juvenile hormone analogue hydroprene prolonged the developmental time and increased the mortality of *P. interpunctella* eggs, illustrating that juvenile hormone mimics can act on the egg stage as well as on larvae (Mohandass et al., 2006b). Combinations of methoprene with other materials have been investigated to compensate for its lack of adulticidal activity. Methoprene combined with diatomaceous earth improved control of *R. dominica* in rough rice compared with either material alone (Chanbang et al., 2007a), and spinosad and methoprene applied alone or in combination were evaluated against six stored-product species, with the combination broadening the range of species controlled (Athanassiou et al., 2011a).

1.4.3. Synergists and Combination Products

Synergists such as PBO have little insecticidal activity alone but enhance the toxicity of insecticides by inhibiting detoxifying enzymes, particularly cytochrome P450 monooxygenases and, to a lesser extent, esterases (Bernard & Philogène, 1993). By blocking metabolic detoxification, synergists can increase the efficacy of pyrethrins and pyrethroids and partially overcome metabolic resistance, allowing lower application rates (Bernard & Philogène, 1993). PBO is a standard component of pyrethrin aerosols used in food facilities (Arthur, 2012).

Combination products that pair a pyrethroid, an IGR, and a synergist aim to kill exposed adults while suppressing the development of progeny. In this thesis, the liquid combination product containing deltamethrin, (*S*)-methoprene, and PBO, marketed as Gravista™ (Central

Life Sciences, Schaumburg, IL, USA), is referred to as liquid combo. Arthur (2019) treated wheat, corn, and brown rice with an existing suspension concentrate (SC) formulation of deltamethrin (Centynal®) at 0.5 ppm or a new emulsifiable concentrate (EC) formulation at 0.5 or 1.0 ppm, each alone or combined with 1.25 or 2.5 ppm methoprene (Diacon® IGR), and bioassayed the commodities every 3 months for 15 months. No *R. dominica* progeny developed on treated brown rice, progeny on treated wheat were nearly absent (≤1.7 adults per sample), and no *T. castaneum* progeny developed on treated corn. However, *S. oryzae* successfully reproduced on wheat in every treatment, although progeny production was lowest with 1.0 ppm EC deltamethrin plus 2.5 ppm methoprene (3.7 adults compared with 154.4 adults on untreated wheat) (Arthur, 2019). Because the EC formulation gave better suppression of *S. oryzae* and *S. zeamais* than the SC formulation in several combinations, the registrant (Central Life Sciences) used these results to replace the SC deltamethrin in its commercial deltamethrin + methoprene grain protectant with the EC formulation, at label rates of 0.5 ppm deltamethrin + 1.25 ppm methoprene or 1.0 ppm deltamethrin + 2.5 ppm methoprene (Arthur, 2019). This deltamethrin + methoprene product is marketed as Diacon® IGR Plus, and the synergist PBO was later incorporated into a new formulation with lower concentrations of deltamethrin and methoprene, which is marketed as Gravista™ (Scheff et al., 2024). As a surface treatment against *T. granarium* larvae, a methoprene + deltamethrin + PBO product reduced survival and adult emergence one month after food had contacted the treated surface (Arthur et al., 2019). The performance of such combinations as residual surface treatments on the materials found in shipping containers has received comparatively little attention.

The rationale for a three-component product such as liquid combo is that each component addresses a different limitation of the others. The pyrethroid provides rapid knockdown and

mortality of exposed adults and mobile larvae. The methoprene suppresses the development of progeny from insects that survive exposure or that are exposed as immatures. The PBO inhibits the metabolic enzymes that contribute to pyrethroid tolerance (Bernard & Philogène, 1993; Arthur, 2019). Combining modes of action in this way may also slow the development of resistance, because insects must overcome more than one mechanism of toxicity. A single pre-formulated product also has practical advantages over tank-mixing separate products, such as a deltamethrin formulation (e.g., Centynal®), a methoprene formulation (e.g., Diacon® IGR), and a PBO synergist. Applicators measure and mix only one product, which simplifies application and reduces the chance of mixing errors. In addition, because PBO increases the activity of the pyrethroid, less active ingredient is needed. Gravista™ contains 1.20% deltamethrin and 2.85% (*S*)-methoprene, compared with 4.75% deltamethrin and 11.40% (*S*)-methoprene in the earlier deltamethrin + methoprene product, Diacon® IGR Plus (Scheff et al., 2024). Whether these complementary effects persist on porous and non-porous surfaces under fluctuating field conditions, and how long they last relative to a single-component pyrethroid such as β-cyfluthrin, are open questions with direct practical relevance to container protection.

1.4.4. Other Reduced-Risk Protectants

Reduced-risk pesticides are those that the U.S. Environmental Protection Agency (EPA) considers to pose less risk to human health and the environment than existing conventional alternatives. Their advantages include low impact on human health, lower toxicity to non-target organisms such as birds, fish, and plants, low potential for groundwater contamination, low use rates, low pest resistance potential, and compatibility with IPM (U.S. EPA, n.d.). Several other active ingredients with some of these characteristics have been developed as alternatives to organophosphates and as partners for pyrethroids and IGRs. Spinosad, a fermentation product of

the soil bacterium *Saccharopolyspora spinosa*, is a naturally derived, reduced-risk insecticide with low mammalian toxicity (Fang et al., 2002; Hertlein et al., 2011). When applied at 1 mg/kg to four classes of wheat, spinosad was highly effective against *R. dominica* and *P. interpunctella*, causing 97.6-99.6% mortality of *P. interpunctella* larvae and suppressing egg-to-adult emergence by 93-100%, but it was effective against *S. oryzae*, *O. surinamensis*, and *T. castaneum* only on durum wheat (Fang et al., 2002). On three Kansas farms, no live *R. dominica* adults and very low densities (≤3 adults/kg) of *T. castaneum*, *C. ferrugineus*, and *O. surinamensis* were found in wheat treated with spinosad at 1 mg/kg (Subramanyam et al., 2007). Spinosad can also be applied as a surface treatment on concrete, galvanized steel, and floor tile and has shown efficacy against most of eight stored-product beetle species, killing 99-100% of adults, although *Tribolium* species were controlled well only on concrete (Toews et al., 2003). Diatomaceous earth, an inert dust composed of fossilized diatoms, kills insects by absorbing cuticular lipids and causing desiccation (Korunic, 1998). Its efficacy is greatest at low relative humidity and is influenced by temperature, exposure interval, and the depth of the treated grain layer (Vardeman et al., 2006; Chanbang et al., 2007b). Chlorfenapyr, a pyrrole insecticide that disrupts mitochondrial energy production, has been evaluated as a residual surface treatment for *Tribolium* species on concrete, vinyl tile, and plywood (Arthur, 2008b). When applied at the maximum label rate of 1.1 g active ingredient (a.i.)/m², no *T. castaneum* adults survived and no progeny were produced in bioassays 0-8 weeks after treatment (Arthur, 2008b, 2009). However, the presence of food greatly reduced mortality at lower rates (Arthur, 2009, 2013). These materials provide additional modes of action that are valuable for resistance management, although each has limitations in speed of action, spectrum, or registration status.

1.5. Influence of Surface Type

The persistence and biological activity of residual insecticides are strongly influenced by the surface on which the residue is deposited. Warehouses and containers combine porous substrates such as wooden pallets, plywood floors, and concrete with non-porous steel and coated metal, which differ in porosity, chemistry, and texture and therefore in the absorption, degradation, and bioavailability of residues (Arthur, 1997; Arthur et al., 2009, 2018).

1.5.1. Porous Surfaces

Porous substrates such as wood and concrete can absorb liquid formulations below the surface, reducing the amount of insecticide available for contact. Concrete is also alkaline, which accelerates the hydrolysis of many insecticides. Comparative bioassays have repeatedly shown that the same insecticide performs differently on porous and non-porous surfaces. Arthur (1997) found that the effectiveness of deltamethrin dust against *T. confusum*, *T. castaneum*, and *R. dominica* differed among plywood, concrete, and tile surfaces. Residual efficacy against *T. confusum* lasted less than 1 week on plywood but about 3 to 4 weeks on concrete and about 6 weeks on tile, whereas *T. castaneum* and *R. dominica* were controlled on all three surfaces (Arthur, 1997). Spinosad applied at 0.05-0.1 mg a.i./cm² killed 99-100% of adults of most of the eight beetle species tested on concrete, galvanized steel, and unwaxed and waxed floor tiles (Toews et al., 2003). The species tested included *T. castaneum*, *T. confusum*, *S. oryzae*, *C. ferrugineus*, *Oryzaephilus mercator* (Fauvel), merchant grain beetle, *O. surinamensis*, and *T. variabile*. However, *Tribolium* spp. recovered after knockdown on floor tile and galvanized steel, resulting in 72-92% mortality, compared with 98-100% on concrete (Toews et al., 2003). Surface effects have also been reported for chlorfenapyr on concrete, vinyl tile, and plywood (Arthur, 2008b), and for the IGRs pyriproxyfen and hydroprene on wood, metal, and concrete (Arthur et

al., 2009). When deltamethrin, cyfluthrin, methoprene, and pyriproxyfen were applied to concrete, wood, painted wood, vinyl tile, and metal and bioassayed over three months against *T. granarium* larvae, the pyrethroids provided a high level of control, the IGRs were less effective, and residues of both classes persisted longest on metal (Arthur et al., 2018). The IGR novaluron likewise significantly reduced adult emergence of *O. surinamensis*, *T. castaneum*, and *T. granarium* for up to 12 weeks on concrete, metal, and wood, with residues most persistent on metal and least persistent on wood (Yasir et al., 2021), and the residual efficacy of methoprene against *T. castaneum* larvae differed between varnished wood and concrete (Wijayaratne et al., 2012). Concrete has received particular attention: alpha-cypermethrin, thiamethoxam, and several other insecticides have been evaluated on concrete against *T. granarium* (Athanassiou et al., 2015; Kavallieratos et al., 2016; Kavallieratos & Boukouvala, 2019), and the residual efficacy of chlorfenapyr differed between sealed and unsealed concrete (Arthur, 2015a). Surface effects have also been reported for methoprene and novaluron on concrete, plywood, and floor tile against *Tribolium* larvae (Arthur & Fontenot, 2012), for malathion, pirimiphos-methyl, and lambda-cyhalothrin on concrete, metal, and plywood against *S. oryzae* (Jankov et al., 2013), and for spinetoram on concrete, ceramic tile, galvanized steel, and plywood (Vassilakos et al., 2014). On concrete, β-cyfluthrin was effective against *R. dominica* but not against field strains of *T. castaneum* and *O. surinamensis* (Sehgal et al., 2014), and combining β-cyfluthrin with imidacloprid was not more effective than β-cyfluthrin alone (Athanassiou et al., 2013). These studies are summarized in Table 1.2. In addition to reducing bioavailability, porous and rough surfaces contain cracks, nail holes, and seams that serve as refugia and accumulate food residues that sustain surviving insects (Heaps, 2012).

Table 1.2. Examples of laboratory studies comparing residual insecticide efficacy among storage surfaces.

Surfaces compared	Active ingredient	Target insects	Reference
Plywood, concrete, tile	Deltamethrin (dust)	*T. confusum*, *T. castaneum*, and *R. dominica* adults	Arthur (1997)
Concrete, galvanized steel	Azamethiphos, fenitrothion, chlorpyrifos-methyl, pirimiphos-methyl	*Liposcelis bostrychophila*, *L. entomophila*, and *L. paeta*	Collins et al. (2000)
Concrete, galvanized steel, unwaxed and waxed floor tile	Spinosad	Adults of eight beetle species, including *T. castaneum*, *T. confusum*, *S. oryzae*, *C. ferrugineus*, *O. surinamensis*, *O. mercator*, and *T. variabile*	Toews et al. (2003)
Concrete, vinyl tile, plywood	Chlorfenapyr	*T. castaneum* and *T. confusum* adults	Arthur (2008b)
Wood, metal, concrete	Pyriproxyfen, hydroprene	Late-instar larvae of *T. confusum*, *T. castaneum*, *O. surinamensis*, *Lasioderma serricorne*, and *P. interpunctella*	Arthur et al. (2009)
Concrete, plywood, floor tile	Methoprene, novaluron	*T. castaneum* and *T. confusum* larvae	Arthur & Fontenot (2012)
Unsealed concrete, varnished wood	Methoprene	*T. castaneum* larvae, surfaces held at 20, 30, and 35 °C	Wijayaratne et al. (2012)
Concrete, metal, plywood	Malathion, pirimiphos-methyl, lambda-cyhalothrin	*S. oryzae* adults	Jankov et al. (2013)
Concrete	β-cyfluthrin + imidacloprid, β-cyfluthrin	*C. ferrugineus*, *O. surinamensis*, *T. castaneum*, *T. confusum*, *Dermestes maculatus*, *L. bostrychophila*, and *L. paeta* adults	Athanassiou et al. (2013)
Concrete, ceramic tile, galvanized steel, plywood	Spinetoram	*R. dominica*, *S. oryzae*, and *T. confusum* adults on all four surfaces, and *Sitophilus granarius*, *O. surinamensis*, and *C. ferrugineus* adults on concrete only	Vassilakos et al. (2014)
Concrete	β-cyfluthrin, chlorpyrifos-methyl + deltamethrin	Laboratory and field strains of *T. castaneum*, *O. surinamensis*, and *R. dominica* adults	Sehgal et al. (2014)
Concrete, plywood, galvanized metal, plastic, ceramic tile	Pirimiphos-methyl (two formulations)	*S. granarius*, *R. dominica*, and *T. confusum* adults	Rumbos et al. (2014)
Sealed and unsealed concrete	Chlorfenapyr	*T. castaneum* adults, with and without flour	Arthur (2015a)
Concrete, galvanized steel	Spinetoram	*S. oryzae*, *T. confusum*, and *O. surinamensis* adults	Vassilakos & Athanassiou (2015)
Concrete, wood, painted wood, vinyl tile, metal	Deltamethrin, cyfluthrin, methoprene, pyriproxyfen	Small and large *T. granarium* larvae	Arthur et al. (2018)
Concrete, plastic	Deltamethrin (two formulations)	*S. oryzae* adults	Quinn et al. (2018)
Concrete, metal, wood	Novaluron	*O. surinamensis*, *T. castaneum*, and *T. granarium*	Yasir et al. (2021)

1.5.2. Non-Porous Surfaces

Steel and coated metals, which form the walls and ceilings of shipping containers, are impermeable and tend to retain residues on the surface, where they remain available to insects. Comparisons among surfaces have generally found greater or more consistent residual activity on non-porous materials such as metal and tile than on concrete or wood, although the magnitude of the difference depends on the active ingredient, formulation, species, and life stage (Toews et al., 2003; Arthur et al., 2009). Non-porous surfaces are not without limitations, because dust and food residues that accumulate on them can reduce insect contact with the insecticide, and condensation can redistribute residues.

1.5.3. Implications for Containers

Because containers combine steel walls and ceilings with wooden floors, pallets, and dunnage, treatments on floors and pallets would be expected to lose activity sooner than those on walls, and wooden components may shelter survivors. Most surface studies, however, have used small treated arenas under constant laboratory conditions (e.g., Ghimire et al., 2016; Arthur et al., 2018), and few have evaluated residual insecticides on commercial container materials under seasonal field conditions.

1.5.4. Food Residues, Refugia, and Insect Behavior

The efficacy of a residual treatment also depends on whether insects actually contact the treated surface for long enough to receive a lethal dose. Food residues on treated surfaces can bind or shield the insecticide and provide an alternative substrate on which insects can rest and feed. In simulated warehouses, the presence of food and structural complexity influenced the capture of *T. castaneum* in traps, indicating that resource distribution affects insect movement and, by extension, exposure to treated surfaces (Toews et al., 2005a). In pilot-scale warehouses,

mortality of *T. castaneum* exposed to pyrethrin or esfenvalerate aerosols declined linearly with increasing flour, never exceeding 60% with 2 g of flour per dish, and never exceeded 75% under pallets compared with at least 80% in the open (Toews et al., 2010). For β-cyfluthrin on concrete, the high label rate (20 mg a.i./m²) remained effective for 8 weeks regardless of flour or sealing, but at the low rate (10 mg a.i./m²) flour absorbed the residue, and accumulated milling debris reduced efficacy in autumn field trials (Arthur et al., 2015). Female *T. castaneum* exploit small patches of food and distribute their offspring among them, a behavior that favors survival in fragmented residues that may be missed during cleaning or treatment (Campbell & Runnion, 2003). These findings emphasize that sanitation is a prerequisite for effective residual treatment, and that bioassays conducted on clean surfaces may overestimate field efficacy.

1.5.5. Quantifying the Decline of Residual Efficacy

Most studies of residual insecticides compare treatments at each residue age (e.g., Ghimire et al., 2016; Arthur, 2018). Fitting decay models to mortality across residue ages would instead provide a rate of decline and a time to a specified loss of efficacy, such as a half-life, allowing comparison among studies and guiding retreatment intervals. Such an approach could be built by fitting a constrained exponential model, $M(t) = M_0 \cdot \exp(-kt)$, to mean mortality at each residue age, where M_0 is the initial mortality (constrained to ≤100%), k is the rate of decline (constrained to ≥0), and the half-life is $\ln(2)/k$. Surface, temperature, humidity, light exposure, and other storage conditions could then be tested as factors that change k, either by fitting the model separately for each condition and comparing the estimated rates and their confidence intervals, or by fitting a single nonlinear model in which k depends on these factors. Alternative forms of decline, such as a Weibull model, could be compared using goodness-of-fit statistics such as the coefficient of determination (R^2), root mean square error, and information criteria,

and predictions of the time to loss of efficacy could be tested against independent bioassays of aged field surfaces. This approach has rarely been applied to stored-product surface bioassays.

1.6. Environmental Factors Affecting Insecticide Performance

Shipping containers experience rapid changes in temperature and humidity as they move between climates and through daily heating and cooling cycles. These fluctuations affect both insect biology and the persistence of insecticide residues (Arthur, 2018).

1.6.1. Temperature

Temperature influences insecticide efficacy in several ways. Higher temperatures generally accelerate the degradation and volatilization of insecticide residues, shortening their residual life. Temperature also alters insect activity and physiology, which can change both the amount of residue an insect picks up from a treated surface and its response to that dose. In addition, temperature determines the rate of insect development (Howe, 1956, 1965). At warm temperatures, development is completed more quickly, which shortens the window during which susceptible immature stages are exposed to IGRs and allows populations to increase rapidly during storage or transit. Methoprene efficacy against *P. interpunctella* applied to surface substrates varied with both temperature and application rate (Jenson et al., 2009), indicating that temperature should be considered when evaluating IGR performance under field conditions.

Field evidence supports these effects. The residual efficacy of deltamethrin against *T. castaneum* differed with temperature and between seasons (Arthur, 2018), and the residual efficacy of methoprene against *T. castaneum* larvae on varnished wood and concrete depended on temperature (Wijayaratne et al., 2012). Because the steel shell of a container exposed to the sun can heat well above ambient air temperature, comparisons of treatments across seasons and locations are needed.

1.6.2. Humidity

Relative humidity inside loaded containers can be high because of limited ventilation, commodity moisture, and temperature cycling, and condensation commonly forms on container walls and ceilings when temperatures fall (Navarro, 2012). High humidity can accelerate the hydrolysis of some insecticides, and condensation can physically redistribute residues on treated surfaces. Humidity also affects insect development and survival. The development of *T. castaneum*, for example, is slower and mortality higher at low relative humidity compared to high relative humidity. When *T. castaneum* was reared on wheatfeed at 15-40 °C and 10, 30, 70, and 90% relative humidity, larval development was fastest at the highest humidity tested at every temperature, eggs failed to hatch at 10% relative humidity at 40 °C, and larval mortality, which was otherwise below 20%, increased at combinations of low humidity and low temperature (Howe, 1956). The combined effects of temperature and humidity on residual insecticides under the fluctuating conditions typical of containers remain poorly characterized, because most efficacy studies are conducted at constant temperature and humidity.

1.6.3. Interactions Among Environmental Factors, Surfaces, and Insects

Temperature, humidity, surface type, and residues interact. The efficacy of chlorfenapyr on concrete depended on whether the concrete was sealed and on the presence of food (Arthur, 2015a), and the effect of temperature on methoprene efficacy differed between wood and concrete (Wijayaratne et al., 2012). Seasonal field studies in which treated surfaces are exposed to ambient conditions inside containers are needed to capture these combined effects.

1.7. Insect Growth Regulators and Treated Packaging

Because containers remain sealed for weeks and offer little opportunity for treatment during transit, there is growing interest in tools that provide long-lasting, low-residue protection.

IGRs and insecticide-incorporated packaging shift the emphasis from treating the storage environment toward protecting the commodity itself (Phillips & Throne, 2010; Arthur, 2016b).

1.7.1. Insect Growth Regulators in Storage and Transit

IGRs interfere with development rather than causing rapid mortality. Juvenile hormone analogues such as methoprene, hydroprene, and pyriproxyfen disrupt metamorphosis and reproduction, whereas chitin synthesis inhibitors interfere with molting (Oberlander et al., 1997). Their delayed but persistent action is well suited to long storage and transit periods, during which suppression of progeny development prevents population growth even when some adults survive. Methoprene has been applied directly to grain, to structural surfaces, as an aerosol, and incorporated into packaging (Arthur, 2012; Wijayaratne et al., 2018). Its low mammalian toxicity and the absence of ozone-depleting properties are important advantages for use in food storage and trade (Henrick, 2007). Its principal limitations are the absence of immediate kill, differences in susceptibility among species and life stages, and reduced exposure of insects that develop inside kernels (Oberlander et al., 1997; Wijayaratne et al., 2018).

Juvenile hormone analogues act by maintaining juvenile hormone-like activity when it should be absent, so that exposed final-instar larvae fail to pupate normally or adults emerge deformed and with reduced reproductive capacity (Oberlander et al., 1997; Mohandass et al., 2006a), and eggs may also fail to hatch (Mohandass et al., 2006b). Because effects depend on the timing and duration of exposure, and appear slowly, juvenile hormone analogues are often combined with fast-acting pyrethroids.

1.7.2. Treated Packaging as an Alternative Control Strategy

Packaging is the final barrier between the commodity and infesting insects. Insects enter packages either by penetrating packaging materials directly or by invading through existing

openings such as seams, closures, and mechanical damage (Mullen et al., 2012). Larvae of *P. interpunctella* and *Trogoderma* species and adults of several beetles are capable of penetrating many flexible films, and the ability to do so depends on the type and thickness of the material (Mullen et al., 2012; Scheff et al., 2018b). The penetration of packaging films by stored-product insects has been studied for decades (Gerhardt & Lindgren, 1954; Highland, 1978; Cline, 1978), and more recent work has examined penetration of polyvinyl chloride, polypropylene, and other films (Bowditch, 1997; Chung et al., 2011). Insect-resistant packaging therefore combines suitable materials, sound package design, and, increasingly, the incorporation of insecticides into the packaging itself (Scheff, 2016).

Packaging-associated insects are often described as either penetrators, which can chew through intact packaging materials, or invaders, which enter through existing openings such as poorly sealed seams, closures, and punctures (Mullen et al., 2012). Larvae of *P. interpunctella* and *Trogoderma* species are among the most capable penetrators, whereas many beetles, including *O. surinamensis* and *Tribolium* species, are primarily invaders. Scheff et al. (2018b) found that third and fifth instars of *P. interpunctella* penetrated multilayer polypropylene energy-bar packages more readily than first instars and that the thickest package was most resistant, indicating that both material selection and seal integrity are important. Effective insect-resistant packaging therefore requires attention to film type and thickness, the quality of seals and closures, and the handling and storage practices that create mechanical damage. Incorporating an insecticide into the packaging adds a further layer of protection by killing or disrupting the development of insects that contact the package surface before they can enter. Methoprene is attractive for this purpose because its low toxicity to mammals is compatible with food-contact applications, and because the suppression of progeny development can prevent populations from

building up around stored packages even when adults are not killed (Arthur, 2016b; Scheff et al., 2017).

Insecticide-incorporated packaging delivers the active ingredient at the boundary between the commodity and the environment. Arthur (2016b) described methods for evaluating methoprene-treated packaging. Exposure of *T. castaneum* and *T. confusum* larvae or parental adults on several treated materials generally resulted in 100% inhibition of adult emergence, the outer surface of treated birdseed (animal feed) bags was more active than the inner surface, and late-stage larvae were the most suitable test stage. Scheff et al. (2017) showed that methoprene-treated woven packaging reduced the development of *T. castaneum* and *T. variabile* larvae exposed on the material, and Scheff et al. (2019) found that methoprene-treated packaging reduced larval development of *T. granarium* and *T. inclusum* and reduced penetration or invasion of food pouches. Packaging incorporating pyrethroids has also been evaluated. On deltamethrin-incorporated storage bags, *T. granarium* adults were knocked down in less than 60 min and all were knocked down or dead within 24 h, whereas larvae had 50% mortality after 4 days of exposure and 97% with continual exposure (Scheff et al., 2020). The continual exposure of larvae to the same bags also allowed less than 7% adult emergence of *T. castaneum* and none of *O. surinamensis* (Scheff et al., 2018a). In another study, deltamethrin-treated storage fabric knocked down 99% of *S. oryzae* and *T. castaneum* in less than 3 h, killed 76.7% and 62.2% of them after 72 h compared with 6% or less on untreated fabrics, and none of three species tested could chew through miniature bags (Paudyal et al., 2017a).

Because the insecticide is confined to the packaging, treated packaging reduces overall chemical use and the potential for residues in the commodity, and it protects for the life of the package without reapplication (Scheff, 2016). Its limitations are higher cost, little effect on

internal feeders already inside kernels, and uncertain stability of active ingredients under prolonged heat and humidity.

Pyrethroid-incorporated storage bags and fabrics have been studied more extensively in field settings than methoprene-treated packaging. Insecticide-incorporated bags reduced infestation by stored-product beetles (Kavallieratos et al., 2017), chlorfenapyr and pirimiphos-methyl applied to different storage bag types gave the highest immediate mortality of *T. granarium*, with delayed mortality exceeding 91% of adults in most combinations (Kavallieratos & Boukouvala, 2018), and efficacy on bag materials varied with bag type and species (Papanikolaou et al., 2021), and deltamethrin-incorporated storage fabric reduced the survival of several stored-product insect species exposed to it (Paudyal et al., 2017a) and acted as a barrier to infestation in field evaluations (Paudyal et al., 2017b). Commercial deltamethrin-treated bags also produced high mortality of *T. granarium* adults and larvae, a result of particular relevance to the prevention of quarantine pest movement in trade (Scheff et al., 2020). Because pyrethroids and methoprene could target different life stages, combining or alternating these active ingredients in packaging could provide broader protection than either active ingredient alone, although these strategies have received limited evaluation.

1.7.3. Methodological Considerations in Evaluating Treated Packaging

Evaluating insecticide-incorporated packaging requires different methods because insects may be exposed on the outer surface, on the inner surface after entry, or only through the packaged food. Arthur (2016b) exposed larvae or parental adults on treated films and recorded adult emergence, Scheff et al. (2017) assessed development after exposure on treated woven packaging, and Scheff et al. (2019) combined developmental assays with tests of package penetration and invasion.

Most evaluations to date have used individual species in small arenas over short periods (Arthur et al., 2019; Scheff et al., 2019). Commercial storage involves mixed-species populations, multiple generations, and exposure periods of weeks to months, so population-level assessments under warehouse conditions are needed to determine whether laboratory effects translate into suppression of field populations.

1.8. Integration in Integrated Pest Management Programs

IPM for stored products emphasizes the coordinated use of multiple complementary tactics, including sanitation, exclusion, monitoring, and physical, biological, and chemical controls, guided by information on pest populations, rather than reliance on a single tool (Phillips & Throne, 2010). IPM is particularly important in shipping containers because of their mixture of porous and non-porous materials, variable microclimates, and the movement of commodities across ecological and regulatory boundaries. Because treatment during transit is rarely feasible, protection must be built in before loading through a layered system that combines sanitation, exclusion, residual surface treatments, treated packaging, and monitoring.

1.8.1. Sanitation and Exclusion

Sanitation forms the foundation of any IPM program, especially in enclosed environments where small amounts of residual food can sustain insect populations (Heaps, 2012). Sanitation must be applied at several levels: in warehouses before goods are staged, in container interiors before loading, and on pallets and dunnage, which can harbor *Tribolium*, *Oryzaephilus*, and dermestid species in cracks and accumulated debris. Exclusion prevents insects from reaching clean commodities and includes sealing structural gaps, maintaining door gaskets and floor seams, and using packaging with sound seals and closures (Heaps, 2012; Mullen et al., 2012).

For containerized commodities, practical sanitation includes inspecting containers for residues and damaged floors or door seals, sweeping and vacuuming floor seams, wall corrugations, and door channels, and replacing or treating infested pallets and dunnage, together with sanitation of the warehouse in which goods are staged before loading (Heaps, 2012).

1.8.2. Surface Treatments as Part of IPM

Residual surface treatments retain strategic value within IPM despite their limitations. Applied to clean surfaces, they kill insects emerging from refugia or moving across treated areas and reduce the initial colonization pressure on the commodity (Arthur & Subramanyam, 2012). Field studies in pilot-scale warehouses have examined how residual treatments interact with monitoring programs and have shown that residual applications and trapping can be used together (Toews et al., 2005b, 2009). Aerosol applications of synergized pyrethrins combined with IGRs are used in food facilities to suppress flying and crawling insects and to reduce the frequency of fumigation (Arthur, 2012; Jenson et al., 2010).

In a warehouse, residual treatments can be reapplied when monitoring indicates declining efficacy, but a treatment applied to a container before loading must remain effective throughout storage and transit. Residual treatments should therefore be regarded as one layer of protection that complements sanitation and packaging rather than replacing them.

1.8.3. Monitoring and Decision Support

Monitoring provides the information needed to determine whether and when intervention is required and to evaluate the outcome of treatments. Pheromone lures are commercially available for approximately 20 species of stored-product insects (Phillips & Throne, 2010). Sex pheromones are effective for moths such as *P. interpunctella*, and aggregation pheromones, such as dominicalure for *R. dominica* and 4,8-dimethyldecanal for *Tribolium* species, attract both

sexes (Suzuki, 1980; Williams et al., 1981; Burkholder & Ma, 1985). Pitfall and probe traps placed on floors or in grain capture walking insects and can detect infestations at low density, and probe-trap captures have been used to estimate beetle populations in wheat stored in concrete silos (Toews et al., 2005c; Toews & Nansen, 2012). Long-term trapping in food facilities has been used to describe seasonal patterns of activity and to assess the effects of fumigation and other interventions (Campbell et al., 2010a; McKay et al., 2017). Outdoor trapping around grain storages has documented seasonal flight activity of several stored-product beetles (Throne & Cline, 1994; Scheff et al., 2022), and farms with high numbers of stored-product insects outside storage structures also had high numbers inside experimental bins (Dowdy & McGaughey, 1994). Temperature and gas sensors installed in storage structures and containers provide additional information on the conditions that influence insect development and treatment efficacy (Agrafioti et al., 2021).

Interpreting trap captures requires caution. Captures reflect not only insect density but also insect activity, which varies with temperature, season, food availability, and the placement of traps relative to resources and structural features (Toews et al., 2005a; Toews & Nansen, 2012). Comparisons of trap captures among treatments or over time are therefore most informative when trap placement and monitoring intervals are standardized and environmental conditions are recorded. Despite these limitations, trapping remains the most practical tool for detecting infestations, evaluating the outcome of interventions, and documenting seasonal patterns of insect activity in warehouses and processing facilities (Campbell et al., 2010a; McKay et al., 2017).

1.8.4. Integration with Non-Chemical Alternatives

Non-chemical tactics are increasingly important components of IPM because of regulatory constraints on fumigants and insecticides. Heat treatment of empty structures and processing facilities, typically at temperatures of 50-60 °C maintained for 24 h or longer, can disinfest facilities without chemical residues (Fields et al., 2012; Tilley et al., 2007a). Hermetic storage and modified or controlled atmospheres reduce oxygen or increase carbon dioxide to levels lethal to insects and have proven particularly valuable in smallholder storage systems (Navarro, 2012; Murdock & Baoua, 2014). In hermetic bags, low oxygen causes larvae to stop feeding, and insects die primarily from desiccation because they can no longer produce metabolic water (Murdock et al., 2012), and triple-layer bags have protected maize as well as cowpea in West Africa (Baoua et al., 2014). Biological control with parasitoids and predators, and with entomopathogens, has been studied for several stored-product species and is used in some markets, although it has not yet been widely adopted in containerized trade (Flinn & Schöller, 2012). When combined with sanitation and insecticide-incorporated packaging, such tactics can provide multiple, independent barriers that are less vulnerable to resistance or to the failure of any single tactic.

1.8.5. Economic Considerations

Economic analysis is an essential but often neglected component of IPM decision-making. The costs of sanitation, monitoring, residual treatments, fumigation, and treated packaging must be weighed against the losses that result from infestation, including price discounts, rejection of consignments, and damage to brand reputation (Adam & Alexander, 2012). Simple methods have been developed to measure the profitability of storage pest management in developing countries (Jones et al., 2014). Comparisons of heat and chemical

treatments for food facilities have shown that the relative cost and risk of each approach depend on facility characteristics and on the frequency of treatment (Tilley et al., 2007b). For containerized commodities, few studies have compared the cost-effectiveness of preventive tactics such as treated packaging with that of repeated fumigation, particularly when the costs of trade rejection and phytosanitary non-compliance are included. Such analyses are needed to support the adoption of reduced-risk tools in commercial practice.

1.9. Gaps in Current Knowledge and Research Needs

Knowledge specific to warehouses and shipping containers remains fragmented. Most of what is known derives from laboratory studies under constant conditions or from studies in mills and warehouses, which do not reproduce the temperature fluctuation, humidity, condensation, and wooden surfaces encountered in containers. Several gaps are evident.

First, the interaction between insecticide formulations and the surfaces found in containers has not been adequately characterized. Laboratory studies have consistently shown that surface type affects residual activity (Arthur, 1997; Toews et al., 2003; Arthur et al., 2009), but few studies have evaluated residual insecticides on the actual wood and metal materials of commercial containers, under seasonal field conditions, and over periods comparable to storage and transit. In particular, the persistence of combination products such as liquid combo on container surfaces has received little attention.

Second, the influence of environmental stressors on residual efficacy remains poorly characterized. Temperature and season affect the residual efficacy of deltamethrin and methoprene (Wijayaratne et al., 2012; Arthur, 2018), but studies that combine temperature fluctuation, humidity, and surface type in a single design are needed to predict performance under container conditions.

Third, treated packaging requires validation beyond the laboratory. Methoprene- and deltamethrin-incorporated packaging have been effective in laboratory studies against several species (Scheff et al., 2017, 2019, 2020; Paudyal et al., 2017a), but trials are usually short relative to storage and transit periods, and their effectiveness in suppressing populations in warehouse or container conditions, where packages are exposed to mixed-species pressure and fluctuating environments, has not been established. Internal feeders that are already present in the commodity also remain largely beyond the reach of packaging treatments.

Fourth, resistance management needs closer attention in this context. Phosphine resistance is widespread in key species (Collins et al., 2002; Opit et al., 2012b; Nayak et al., 2020), and the long-term use of pyrethroids and IGRs in residual and packaging applications could select for resistance unless tactics are rotated or combined.

Finally, integrated programs for containerized commodities have rarely been evaluated. Most studies test single tactics in isolation, and information on the cost-effectiveness of combined tactics relative to repeated fumigation is limited (Adam & Alexander, 2012).

Methodologically, future studies should report both immediate and delayed mortality, record temperature and humidity throughout exposure and aging, extend IGR assessments long enough to measure progeny production, and use decay models to estimate the persistence of residual efficacy.

These gaps define the objectives of the research presented in this thesis. The first objective evaluated the residual efficacy of liquid combo and β-cyfluthrin applied to the wood and metal surfaces of commercial shipping containers in seasonal field trials in Arkansas and Kansas, thereby addressing the need for container-specific data under ambient conditions. The second objective examined, under controlled laboratory conditions, how surface type (wood,

metal, and concrete), temperature, and residue age influence the efficacy of these insecticides against *T. castaneum*, *R. dominica*, and *S. oryzae*, and described the decline of efficacy over time with decay models. The third objective evaluated methoprene-incorporated packaging for the suppression of insect populations in simulated warehouses, providing population-level information on a technology that has so far been evaluated mainly in the laboratory.

1.10. Summary and Conclusions

1.10.1. Main Findings and Trends

Stored-product insect management has shifted from reliance on methyl bromide and phosphine toward integrated programs. The phase-out of methyl bromide and the spread of strong phosphine resistance, with resistance factors exceeding 1,000-fold in some *R. dominica* populations, have exposed the limits of fumigation alone (Fields & White, 2002; Opit et al., 2012b; Nayak et al., 2020).

Residual pyrethroids, alone or combined with synergists and IGRs, remain important, but their persistence depends strongly on the treated surface, on food residues, and on temperature and season, and residues generally persist longest on metal and shortest on wood or unsealed concrete (Wijayaratne et al., 2012; Arthur et al., 2015, 2018; Arthur, 2018; Yasir et al., 2021). Methoprene and insecticide-incorporated packaging shift protection to the commodity itself and have suppressed progeny or package invasion of several species in laboratory studies (Arthur, 2016b; Scheff et al., 2017, 2019, 2020), but evidence from warehouse and container conditions remains limited.

Insecticide resistance runs through all of these trends. Resistance to organophosphates and phosphine has repeatedly reduced the value of once-reliable tools (Zettler & Cuperus, 1990;

Nayak et al., 2020), supporting the use of combination products, rotation of modes of action, and integration of chemical tools with sanitation, monitoring, and non-chemical tactics.

1.10.2. Conclusion

The literature indicates a transition from fumigation-dominated strategies toward integrated programs that combine sanitation, exclusion, monitoring, reduced-risk residual insecticides, and treated packaging. Previous studies consistently show the importance of surface characteristics and environmental conditions in determining the performance of residual treatments, and the advantage of combining tactics over relying on any single intervention. Two questions are particularly relevant to warehouse and container protection: how residual insecticides such as liquid combo and β-cyfluthrin persist on the wood and metal surfaces of shipping containers under seasonal field conditions, and whether methoprene-incorporated packaging can suppress insect populations under simulated warehouse conditions. The research presented in the following chapters was designed to address these questions.

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Chapter 2 - Evaluating the Efficacy of Contact Insecticides as a Pre-Treatment Strategy for Shipping Containers

Abstract

Stored-product insects can persist and reproduce inside intermodal shipping containers, yet preventive treatments for containers have received little evaluation. This study evaluated β-cyfluthrin (Tempo® SC Ultra) and a liquid combination product containing deltamethrin + (*S*)-methoprene + piperonyl butoxide (Gravista™, hereafter liquid combo) as pre-loading residual treatments against adults of *Tribolium castaneum* (Herbst), red flour beetle, and *Sitophilus oryzae* (L.), rice weevil. Galvanized steel and plywood bioassay arenas were treated at label rates and held inside 6.1-m shipping containers in Arkansas and Kansas, USA, during two seasonal trials per location under widely fluctuating temperatures. At 0, 4, 8, 12, and 16 weeks post-treatment, adults were exposed on arenas for 24 and 168 h, and progeny production was assessed after eight weeks. Both insecticides caused greater adult mortality than untreated control at nearly all residual weeks on both surfaces. Efficacy on metal was stable through 16 weeks, and liquid combo caused 100% mortality of both species after 168 h on metal. On plywood, 24 h mortality of *T. castaneum* declined to control levels by week 8, but 168 h mortality on both insecticides remained significantly greater than the control through week 16. For *S. oryzae*, β-cyfluthrin caused greater 24 h mortality than liquid combo on plywood, whereas both products caused ≥97% mortality on plywood after 168 h. Progeny production on treated surfaces was nearly eliminated for both species (≤1.5 adults per arena vs. 6-28 in controls), even where adult mortality was incomplete. These results support pre-loading residual surface treatments as a non-fumigant integrated pest management tool for shipping containers, particularly when applied close to loading.

Keywords: stored-product insects, residual insecticides, shipping containers, *Tribolium castaneum*, *Sitophilus oryzae*, pyrethroid, integrated pest management, pre-loading treatment

2.1. Introduction

Stored-product insects are a persistent problem in post-harvest systems, causing both quantitative losses and qualitative deterioration of stored commodities. Infestations result in direct feeding damage as well as contamination with frass, insect fragments, and associated microorganisms, reducing commodity quality and marketability (Hagstrum and Subramanyam, 2009; Phillips and Throne, 2010). Global losses attributed to insect infestations are commonly estimated at 10-20%, particularly under suboptimal storage and transportation conditions (FAO, 2011; Agrafioti et al., 2025). These insects are ubiquitous throughout the post-harvest supply chain, colonizing food processing facilities, storage warehouses, and transportation equipment, including railcars and shipping containers (Campbell and Arbogast, 2004; McKay et al., 2017). Shipping containers present a particularly challenging pest management environment because insects harbored within wooden floor planks, structural crevices, and residual commodity debris at the time of loading can persist and reproduce during transit, potentially resulting in heavily infested product at the destination (McKay et al., 2017; Subramanyam et al., 2023). The movement of infested containers through international trade networks also poses a biosecurity risk through the spread of quarantine pest species into new regions (Stejskal et al., 2020).

Shipping containers are widely used for the movement of bagged grain and processed commodities and represent a critical component of modern supply chains. However, these systems can also facilitate the survival and dispersal of stored-product insects. Containers are frequently reused and may harbor residual infestations, while accumulated commodity residues can provide suitable resources for insect persistence. In addition, shipping containers are exposed

to fluctuating environmental conditions during transit, including variation in temperature and humidity, which can influence insect survival and development. In addition, the structural variability and limited gas-tightness of shipping containers can reduce the effectiveness of control measures such as fumigation, resulting in uneven exposure and survival of insect populations (Agrafioti et al., 2021; Nayak et al., 2020). Despite their importance, pest management strategies specifically designed for shipping container environments remain limited compared with those developed for bulk storage systems.

Among the most important stored-product insect species associated with these systems are *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), red flour beetle, and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), rice weevil. These species differ markedly in feeding behavior and, consequently, in their exposure to control measures. *Tribolium castaneum* is a highly mobile external feeder that exploits processed commodities and residual materials, increasing its likelihood of contact with treated surfaces. In contrast, *S. oryzae* is a primary internal feeder that develops within intact grain kernels, limiting exposure during immature stages. Both species are widely distributed and frequently associated with storage and transport systems, including shipping environments, where they can persist despite control efforts (Hagstrum and Subramanyam, 2009; Espino et al., 2014). Their contrasting biology provides a useful framework for evaluating insecticide performance under operational conditions.

Fumigation has historically been the primary management tool for stored-product insects in shipping containers, with methyl bromide (MB) formerly preferred for its rapid, broad-spectrum efficacy against all insect life stages. The international phase-out of MB under the Montreal Protocol has restricted its use to quarantine and pre-shipment (QPS) applications, making phosphine (PH_3) the dominant fumigant used globally (Collins, 2010; Arthur, 2012).

However, effective phosphine fumigation requires sustained temperatures above 15 °C and holding periods of several days to achieve complete mortality of all life stages (Nayak et al., 2020; Studebaker, n.d.). These fumigation parameters can be challenging in northern climates during the winter seasons, where such conditions are frequently incompatible with commercial shipping schedules. Poor container integrity also prevents adequate and sustained phosphine concentrations from building up due to inadequate sealing and holes in the floors, sides, or roof where the gas can escape. This can lead to the lack of spatial uniformity in gas distribution, which can leave localized zones with sublethal concentrations of the fumigant (Agrafioti et al., 2021; Brabec et al., 2022). These structural failures can lead to sublethal phosphine treatments and the potential for the selection of phosphine-resistant individuals. Phosphine resistance has been evaluated in geographically distinct, field-collected populations across North America. Resistant individuals were detected in 12 of 25 *T. castaneum* populations collected from the United States (U.S.) and Canada (Cato et al., 2017) and in 32 of 34 populations of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), lesser grain borer, with resistance ratios of up to approximately 596-fold in a population from Florida (Afful et al., 2018). In Oklahoma, 8 of 9 *T. castaneum* populations and all 5 *R. dominica* populations collected from stored wheat contained phosphine-resistant individuals (Opit et al., 2012). Phosphine resistance has also been documented in *S. oryzae*, for example through national monitoring in Australia (Holloway et al., 2016), and resistance in these species is now reported worldwide (Nayak et al., 2020). Therefore, alternative methods to work in tandem with fumigation are needed to protect bagged commodities during container loading and transit globally.

Residual contact insecticides represent one of the most practical and readily deployable components of integrated pest management (IPM) programs for post-harvest environments

(Arthur, 1996; Phillips and Throne, 2010; Arthur, 2012; Arthur and Subramanyam, 2012; Nayak et al., 2026). Contact insecticides can be used as crack-and-crevice treatments, spot treatments, surface treatments, perimeter treatments, and pre-bin treatments across the grain industry. Extending the pre-bin treatment concept to a pre-container treatment, by applying registered insecticides to container surfaces before loading bagged commodities, offers a logical preventive strategy that could provide residual protection throughout a multi-month transit period. Scheff and Phillips (2020) identified structural surface treatments as a critical IPM hurdle for limiting insect dispersal across facility environments, and Nayak et al. (2026) highlighted residual contact insecticides as key tools for integration into fumigation-dominated programs. Ranabhat et al. (2025) further demonstrated that combining residual contact insecticides with insecticide-incorporated netting significantly reduced stored-product insect dispersal in post-harvest settings, supporting the use of surface treatments as part of a broader multi-tactic IPM framework. However, the performance of surface-applied insecticides in real-world environments can be substantially affected by the accumulation of commodity residues such as grain dust and milling debris, which can absorb the active ingredient (a.i.) and reduce its availability at the treated surface (Arthur et al., 2015). In addition, the efficacy of such pre-loading applications on the substrate types found in shipping container interiors had not been evaluated under realistic field conditions before the present study.

Two commercial formulations were selected for evaluation based on their registered status as pre-bin treatments, their complementary modes of action, and their commercial availability in the U.S. Tempo® SC Ultra (Bayer Environmental Science, Cary, NC, USA) contains the active ingredient β-cyfluthrin (11.8%), a Type II (α-cyano) pyrethroid that acts on voltage-gated sodium channels in insect nerve membranes, prolonging channel opening and

causing membrane depolarization, conduction block, paralysis, and death (Soderlund and Bloomquist, 1989; Arthur, 2012). Gravista™ (Central Life Sciences, Schaumburg, IL, USA) is a liquid combination product containing the active ingredients deltamethrin (1.20%), the insect growth regulator (IGR) (*S*)-methoprene (2.85%), and the synergist piperonyl butoxide (PBO) (33.30%). Methoprene is a juvenile hormone analog that disrupts development and suppresses reproduction, complementing the contact activity of deltamethrin (Wijayaratne et al., 2012; Scheff et al., 2016). PBO inhibits cytochrome P450 monooxygenases, the primary metabolic pathway responsible for pyrethroid detoxification in resistant populations, thereby enhancing the efficacy of deltamethrin against insects with metabolic resistance mechanisms (Hodgson and Levi, 1998).

A critical determinant of residual contact insecticide performance is the physicochemical nature of the treated substrate. Surface porosity governs how much a.i. is absorbed into the substrate matrix rather than retained at the surface where insect contact occurs. Porous materials such as wood absorb insecticide formulations and reduce surface-available concentrations, whereas non-porous surfaces such as metal retain more of the applied residue and generally support faster knockdown and more complete mortality (White and Leesch, 1996; Arthur, 2008; Arthur et al., 2009; Jankov et al., 2013). Yasir et al. (2021) observed a similar pattern for novaluron applied to concrete, metal, and wood against stored-product coleopterans: novaluron reduced adult emergence of three species for the first 12 weeks on all surfaces and remained effective longest on metal. This distinction is directly relevant to shipping containers, which are constructed with wooden flooring and steel walls, roof panels, and framing, creating a mixed-substrate interior in which insecticide performance on the two dominant surface types may differ substantially.

Therefore, the objective of the present study was to determine the initial and residual efficacy of β-cyfluthrin (Tempo® SC Ultra) and liquid combo (deltamethrin + (*S*)-methoprene + PBO, Gravista™) applied to wood and metal arenas held in shipping containers in Jonesboro, Arkansas, and Manhattan, Kansas, during two seasonal trials at each location.

2.2. Materials and Methods

2.2.1. Insect Colonies and Rearing Conditions

Adults of *T. castaneum* and *S. oryzae* were obtained from pesticide-susceptible laboratory colonies maintained at the USDA-Agricultural Research Service, Center for Grain and Animal Health Research (CGAHR), Manhattan, Kansas, USA, for more than 35 years without known exposure to insecticides. *Tribolium castaneum* was reared on a diet of 95% organic unbleached whole-wheat flour and 5% brewer's yeast (wt/wt). *Sitophilus oryzae* was maintained on polished rice. All colonies were held in 0.95 L glass jars fitted with fine-mesh screen and filter paper lids in an environmental chamber 27 ± 1 °C, 60-65% relative humidity (RH), and continuous darkness (Percival, Perry, Iowa, USA), and were sub-cultured on a weekly basis.

2.2.2. Bioassay Arena Preparation

Experimental bioassay arenas were constructed in 23 × 23 cm (529 cm^2) square plastic Petri dishes. The bottom of each dish was fitted with either a 20-gauge galvanized steel panel or a 6 mm plywood panel affixed with adhesive caulking (DAP Products Inc., Baltimore, MD, USA). The inner walls of each arena were coated with polytetrafluoroethylene (Fluon®, Sigma-Aldrich, St. Louis, MO, USA) to prevent insect escape. Treated metal and plywood arenas are shown in Fig. 2.1. A total of 150 arenas per surface type were prepared for each experimental trial period at each test location (Arkansas and Kansas).

2.2.3. Insecticide Formulations and Application

Two commercial insecticide formulations, registered as pre-bin treatments, were evaluated in this study. The first was an 11.8% a.i. β-cyfluthrin formulation (Tempo® SC Ultra, Bayer Environmental Science, Cary, NC, USA), applied at a label rate of 16 mL per 3.8 L of water to treat 93 m², and henceforth referred to as β-cyfluthrin. The second was a liquid combination formulation containing 1.2% deltamethrin, 2.85% methoprene, and 33.31% PBO (Gravista™, Central Garden & Pet, Schaumburg, IL, USA), applied at a label rate of 177 mL per 3.8 L of water to treat 93 m², and henceforth referred to as liquid combo. Both insecticides were applied at their respective registered label rates. Water applied at the same volume served as the untreated control.

Insecticide applications at the Arkansas location were performed using a hand-held multipurpose pump sprayer fitted with an adjustable sprayer (Model 1501HDX, HDX Company, Atlanta, GA, USA) pressurized to 40 psi. Applications at the Kansas location utilized a battery-powered hand sprayer fitted with a TeeJet 8001EVS flat fan nozzle (Green Gorilla, Chicago, IL, USA) pressurized to 40 psi. All 150 arenas per surface type were treated on the same day under ambient indoor conditions (Fig. 2.1B) and allowed to air dry for 24 h prior to the initiation of bioassay trials.

2.2.4. Experimental Design and Bioassay Procedure

Two seasonal trials were conducted at each location: May to September 2024 and October 2024 to February 2025 in Arkansas, and July to December 2024 and January to May 2025 in Kansas. Following the 24 h drying period, treated arenas were transported to a 6.1 m (20-foot) intermodal shipping container and randomly distributed across the container floor (Fig. 2.1A). Temperature and RH inside each container were recorded throughout each trial with

HOBO data loggers (Onset Computer Corporation, Bourne, MA, USA). Ten arenas per insecticide treatment and surface combination were randomly selected after 0 (1 d post-treatment), 4, 8, 12, and 16 weeks of storage in the container. Of those selected arenas at each interval, half were designated for *T. castaneum* bioassays and the remainder for *S. oryzae* bioassays (n = 5 per insecticide treatment, surface, and insect combination).

Ten 1- to 2-week-old *T. castaneum* or *S. oryzae* adults of mixed sexes were introduced into individual arenas along with approximately 1 g of flour diet (*T. castaneum*) or 2 g of rice (*S. oryzae*). Arenas at the Arkansas location were held under ambient laboratory conditions on a benchtop, while those at the Kansas location were held in an environmentally controlled chamber set at 27 °C and 65% RH. Insect mortality assessments were conducted 1, 2, and 7 d (24, 48, and 168 h) after the start of exposure. Each adult was assigned to one of three mutually exclusive categories: alive (actively moving or walking normally), affected (alive but exhibiting abnormal posture, uncontrolled twitching of antennae or legs, or loss of coordination), or dead (no detectable movement after gentle probing with a fine-tipped brush) (Scheff et al., 2019). Only dead adults were counted as mortality.

Following the 7-day mortality assessment, all adults were discarded. The remaining diet from each arena was carefully collected, transferred into a 7-dram (25.88 mL) plastic vial, fitted with a mesh screen and held at 27 °C and 65% RH for 8 weeks to monitor for progeny development as an indicator of sublethal reproductive effects. This complete test cycle was repeated every four weeks for a total experimental storage duration of 16 weeks.

2.2.5. Statistical Analysis

Mean daily temperature (°C) and RH (%) were calculated as the mean ± standard error (SE) of all logger readings recorded within each 24 h period. The numbers of dead adults after 24

and 168 h of exposure were converted to percentages and means ± SE were calculated for each species and surface separately. Mortality data were transformed to angular values to normalize treatment variances (Zar, 2010). Before pooling, mortality data from each location were analyzed separately, and location (Arkansas and Kansas) was then included as a factor in a preliminary analysis of variance (ANOVA) with insecticide treatment and residual week for each species, surface, and exposure time (24 and 168 h). Location had no significant main effect and did not interact significantly with insecticide treatment or residual week, and the ranking of treatments was the same at both locations. Data from both locations and both seasonal trials at each location were therefore pooled for the final analysis. Pooling increased the number of replicates for each treatment, surface, and residual week combination. It also matched the objective of the study, which was to evaluate how the treatments performed across the range of seasonal conditions found in shipping containers, rather than to compare the two sites. The same arena preparation, application rates, insect cultures, and bioassay procedures were used at both locations. Data for each species and surface combination were analyzed using a two-way ANOVA in R (version 4.4.1; R Core Team, 2024) to determine the main and interactive effects of insecticide treatment and residual week at each observation time (24 and 168 h). When treatment effects were significant, treatments were compared within each residual week using Tukey's honestly significant difference (HSD) test on estimated marginal means from the two-way model, computed with the *emmeans* package (Lenth, 2023) at $\alpha = 0.05$. When neither residual week nor the interaction was significant, data were pooled across residual weeks and treatments were compared using Tukey's HSD test.

In some treatment replicates, there were zero adult progeny counts. Therefore, the data on the total number of adult progeny produced were transformed to log $(x + 1)$ scale to normalize

treatment variances prior to analysis (Zar, 2010). The adult progeny data were analyzed separately by species and surface type using a two-way ANOVA to determine significant differences ($P \leq 0.05$) among the main and interactive effects of insecticide treatment and residual week. If ANOVA was significant, means among treatments were separated using Tukey's HSD test via the *emmeans* package (Lenth, 2023) with a family-wise significance level of $\alpha = 0.05$.



Figure 2.1. (A) Treated metal and plywood bioassay arenas distributed on the floor of a 6.1-m shipping container. (B) Insecticide application to plywood bioassay arenas before placement in the container.

2.3. Results

Because location did not significantly affect adult mortality or interact with insecticide treatment or residual week, results are presented for data pooled across both locations and both seasonal trials.

2.3.1. Environmental Conditions

Temperature and RH inside the shipping containers varied considerably within and among the four seasonal trials (Fig. 2.2). During the Arkansas summer trial (May to September 2024), daily mean temperature was 28.0 ± 0.3 °C (mean ± SE; range, 19 to 37 °C) and daily mean RH was 60.9 ± 0.4% (range, 50 to 72%). During the Arkansas winter trial (October 2024 to February 2025), daily mean temperature was 8.5 ± 0.6 °C (range, −7.6 to 24.2 °C) and fell below 16 °C on 111 days, whereas daily mean RH remained relatively stable at 59.2 ± 0.4% (range, 48 to 67%). Temperatures below 16 °C are near the lower developmental limit of *S. oryzae* and below the developmental range of *T. castaneum* (Hagstrum and Subramanyam, 2009).

In Kansas, daily mean temperature during the fall trial (July to December 2024) was 24.9 ± 0.3 °C (range, 13 to 34 °C) and daily mean RH was 41.7 ± 1.1% (range, 15 to 67%). Temperatures declined from summer highs approaching 34 °C to approximately 13 °C in mid-October and then remained between approximately 20 and 27 °C from November to December, when RH was lowest (15 to 30%). During the Kansas spring trial (January to May 2025), daily mean temperature was 10.9 ± 0.7 °C (range, −13.3 to 26.1 °C) and fell below 16 °C on 70 days, and daily mean RH was 54.5 ± 0.9% (range, 35.3 to 72.7%). Temperatures warmed gradually toward the end of the spring trial, and the lowest RH values occurred during the coldest weeks.

Overall, the two locations experienced overlapping but distinct environmental regimes. The Arkansas summer and Kansas fall trials were the warmest (28.0 ± 0.3 and 24.9 ± 0.3 °C, respectively), but RH was higher and more stable in Arkansas (60.9 ± 0.4%) than in Kansas (41.7 ± 1.1%). In contrast, the Arkansas winter and Kansas spring trials both included extended periods below 16 °C (111 and 70 days, respectively), and the Kansas spring trial recorded the

lowest daily mean temperature of the study (−13.3 °C) (Fig. 2.2). Across all four trials, mean RH was consistently higher in the Arkansas container (59.2 to 60.9%) than in the Kansas container (41.7 to 54.5%).

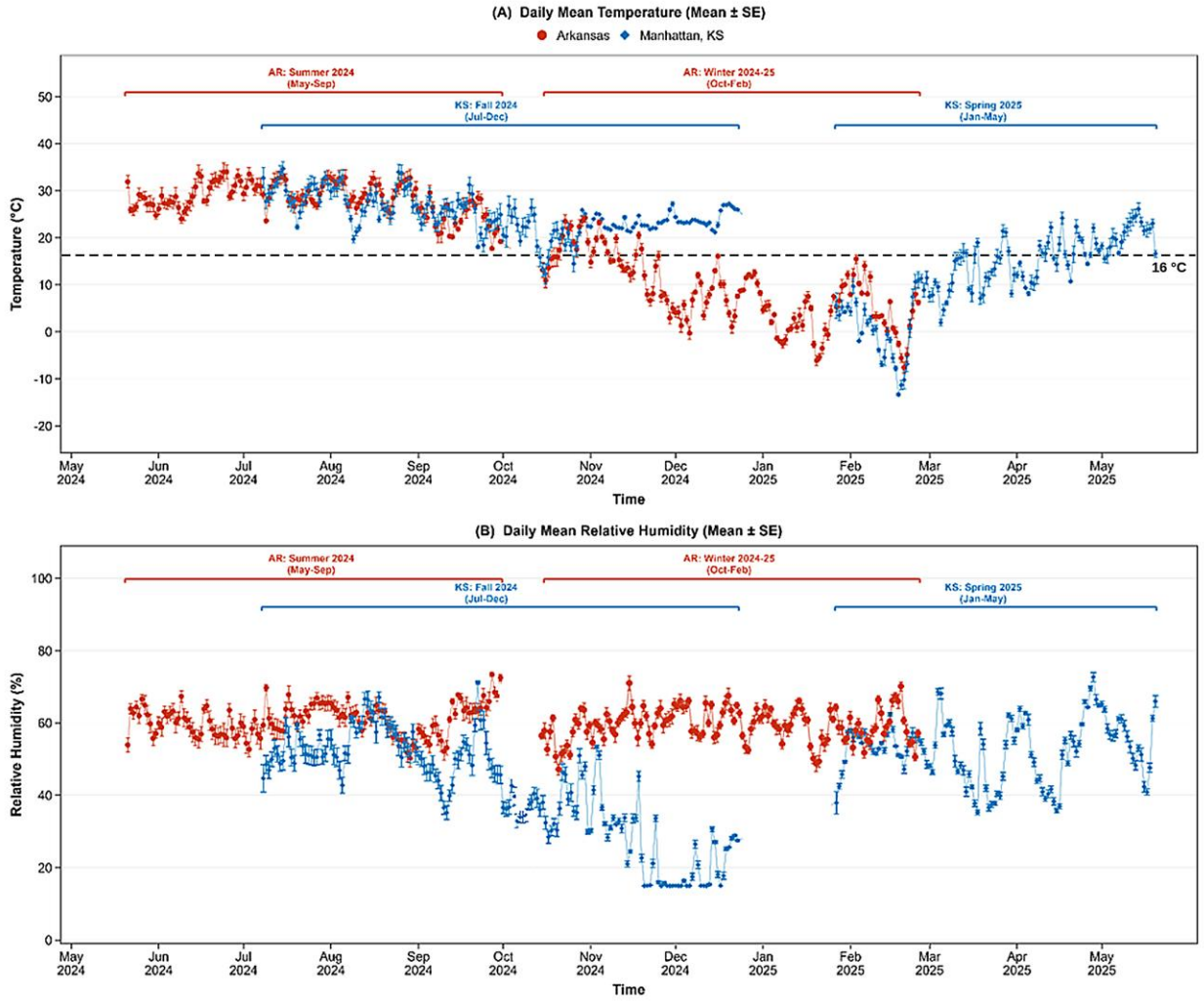


Figure 2.2. Mean (± SE) daily (A) temperature (°C) and (B) relative humidity (%) recorded by HOBO data loggers inside shipping containers in Jonesboro, Arkansas (red circles) and Manhattan, Kansas (blue diamonds) during four seasonal trials: Arkansas summer 2024 (May-September), Kansas fall 2024 (July-December), Arkansas winter 2024-25 (October-February), and Kansas spring 2025 (January-May). The dashed line in (A) marks 16 °C, which is near the lower developmental limit of *S. oryzae* and below the developmental range of *T. castaneum*.

2.3.2. Efficacy on *Tribolium castaneum*

Control mortality of *T. castaneum* was ≤2% after 24 h and ≤17% after 168 h on both surfaces across residual weeks, therefore we did not correct for control mortality. The percentages of *T. castaneum* adults classified as live, affected (alive but impaired), or dead are shown in Figure 2.3. Both insecticides substantially reduced the percentage of live adults compared with the control, and the shift from live to dead was greatest after 168 h and on metal. On metal, virtually no adults exposed to either insecticide were classified as live at either observation time. After 168 h, all adults on liquid combo were dead, and 75-95% of adults on β-cyfluthrin were dead with the remainder affected. On wood, the percentage of live adults on both insecticide treatments increased at later residual weeks, reaching 18-48% after 24 h at weeks 8-16, whereas ≥94% of control adults remained live at every week.

2.3.2.1. Wood Bioassay Arenas

Mortality of *T. castaneum* adults after 24 h on wood was significantly affected by treatment ($F = 26.39$; df = 2, 283; $P < 0.001$), residual week ($F = 14.98$; df = 4, 283; $P < 0.001$), and the treatment × residual week interaction ($F = 4.52$; df = 8, 283; $P < 0.001$) (Fig. 2.4A). At week 0, mortality differed among all three treatments: Liquid combo (49%) caused greater mortality than β-cyfluthrin (28%; $P = 0.0016$), and both caused greater mortality than the control ($P < 0.0001$). At week 4, both liquid combo ($P = 0.0002$) and β-cyfluthrin ($P = 0.0007$) caused greater mortality than the control but did not differ from each other ($P = 0.949$). There were no significant differences among treatments at weeks 8, 12, or 16 ($P = 0.170$-0.985), when mortality on both insecticides was ≤10%. Mortality on both insecticides declined with residual age (Fig. 2.4A): mortality on liquid combo decreased from 49% at week 0 to 4-10% at weeks 8-16, and

mortality on β-cyfluthrin decreased from 28% at week 0 to 4-6% at weeks 8-16. Control mortality remained <2% at every residual week.

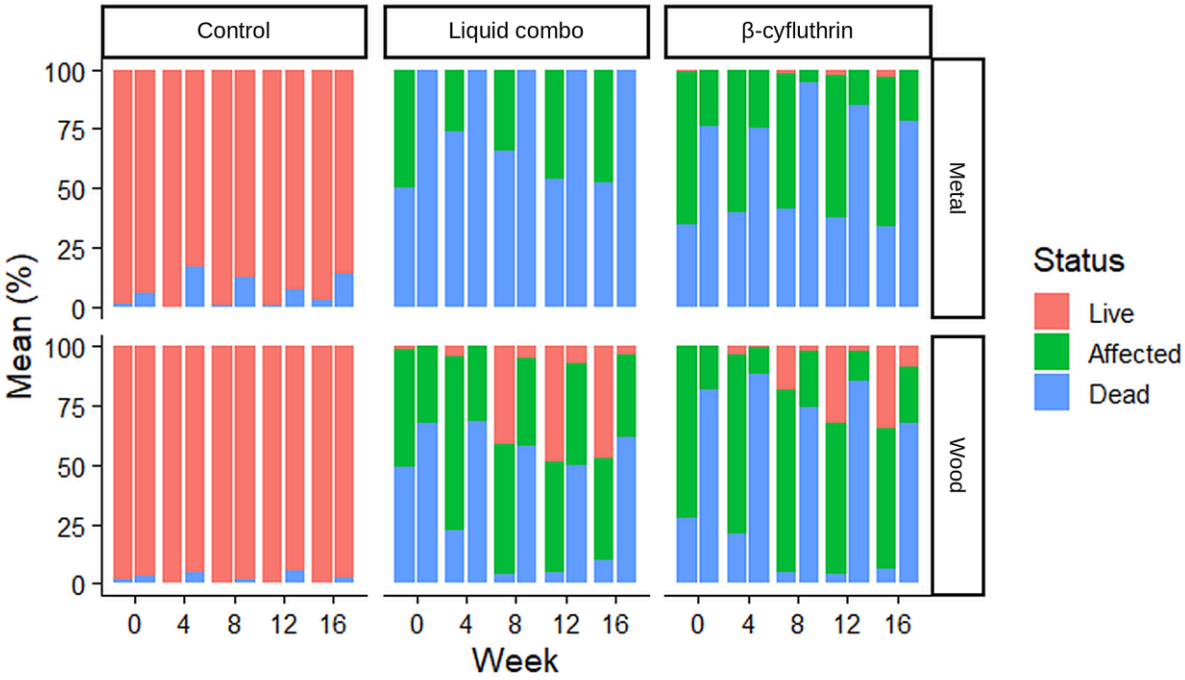


Figure 2.3. Mean percentage of *Tribolium castaneum* adults classified as live (red), affected (green), or dead (blue) on metal (upper panels) and wood (lower panels) bioassay arenas treated with water (control), liquid combo, or β-cyfluthrin after 24 h (left bar within each week) and 168 h (right bar within each week) of exposure at 0, 4, 8, 12, and 16 weeks post-treatment. Data are pooled across both locations (Arkansas and Kansas) and both seasonal trials at each location.

Increasing the exposure time to 168 h increased *T. castaneum* mortality on wood. Treatment ($F = 346.86$; df = 2, 284; $P < 0.001$) and residual week ($F = 2.71$; df = 4, 284; $P = 0.031$) were significant, but the treatment × residual week interaction was not ($F = 1.50$; df = 8, 284; $P = 0.158$) (Fig. 2.4B). Both insecticides caused significantly greater mortality than the control at every residual week ($P < 0.0001$), with mortality of 50-69% on liquid combo and 68-88% on β-cyfluthrin compared with ≤6% on the control. Mortality did not differ between liquid combo and β-cyfluthrin at week 0 ($P = 0.168$) or week 16 ($P = 0.918$), but β-cyfluthrin caused greater mortality than liquid combo at weeks 4 ($P = 0.020$), 8 ($P = 0.024$), and 12 ($P < 0.0001$).

Mortality on β-cyfluthrin was greatest at week 4 (88%) and lowest at week 16 (68%), whereas mortality on liquid combo ranged from 50% at week 12 to 69% at week 4.

Control wood arenas produced 7.4 to 13.8 adult *T. castaneum* progeny over the 16-week residual period, whereas no progeny were produced on liquid combo or β-cyfluthrin arenas at any residual week (Table 2.1). Treatment ($F = 126.15$; df = 2, 285; $P < 0.001$), residual week ($F = 2.90$; df = 4, 285; $P = 0.022$), and the treatment × residual week interaction ($F = 2.90$; df = 8, 285; $P = 0.004$) were all significant.

Table 2.1. Mean (± SE) number of adult *Tribolium castaneum* progeny produced on wood bioassay arenas treated with water (control), liquid combo, or β-cyfluthrin at 0, 4, 8, 12, and 16 weeks post-treatment.

Residual Week	Mean (± SE) number of adult progeny per arena		
	Control[a]	Liquid combo[b]	β-cyfluthrin[b]
0[c]	13.8 ± 2.0 Aa	0.0 ± 0.0 b	0.0 ± 0.0 b
4[d]	10.5 ± 2.5 Aa	0.0 ± 0.0 b	0.0 ± 0.0 b
8[e]	7.4 ± 3.0 Ba	0.0 ± 0.0 b	0.0 ± 0.0 b
12[f]	12.5 ± 3.7 Aa	0.0 ± 0.0 b	0.0 ± 0.0 b
16[g]	12.9 ± 5.2 Aa	0.0 ± 0.0 b	0.0 ± 0.0 b

[a] Means within the control column followed by different uppercase letters are significantly different among residual weeks ($F = 2.90$; df = 4, 285; $P = 0.022$; Tukey's HSD). Within each row, means followed by different lowercase letters are significantly different among treatments (Tukey's HSD, $P < 0.05$).

[b] No progeny were produced on liquid combo or β-cyfluthrin arenas at any residual week, and because these treatments had no variance among residual weeks, they were not compared among weeks.

[c] There were significant differences among the treatments at week 0 (control vs. liquid combo and control vs. β-cyfluthrin: $t = 9.29$; df = 285; $P < 0.0001$; Tukey's HSD).

[d] There were significant differences among the treatments at week 4 (control vs. liquid combo and control vs. β-cyfluthrin: $t = 6.44$; df = 285; $P < 0.0001$; Tukey's HSD).

[e] There were significant differences among the treatments at week 8 (control vs. liquid combo and control vs. β-cyfluthrin: $t = 3.52$; df = 285; $P = 0.0015$; Tukey's HSD).

[f] There were significant differences among the treatments at week 12 (control vs. liquid combo and control vs. β-cyfluthrin: $t = 6.12$; df = 285; $P < 0.0001$; Tukey's HSD).

[g] There were significant differences among the treatments at week 16 (control vs. liquid combo and control vs. β-cyfluthrin: $t = 5.40$; df = 285; $P < 0.0001$; Tukey's HSD).

2.3.2.2. Metal Bioassay Arenas

Mortality of *T. castaneum* after 24 h on metal was significantly affected by treatment (F = 76.41; df = 2, 278; $P < 0.001$), but not by residual week (F = 1.03; df = 4, 278; P = 0.390) or the treatment × residual week interaction (F = 0.72; df = 8, 278; P = 0.676). Data were therefore pooled across residual weeks. Pooled across weeks, liquid combo caused greater mortality (59%) than β-cyfluthrin (37%), and both caused greater mortality than the control (1%) (Tukey's HSD, $P < 0.05$; Fig. 2.5A).

After 168 h on metal, treatment was significant (F = 681.27; df = 2, 279; $P < 0.001$), but residual week (F = 1.86; df = 4, 279; P = 0.118) and the treatment × residual week interaction (F = 1.68; df = 8, 279; P = 0.102) were not. Pooled across residual weeks, mortality differed among all three treatments: Liquid combo caused 100% mortality, β-cyfluthrin 82%, and the control 11% (Tukey's HSD, $P < 0.05$; Fig. 2.5B).

Control metal arenas produced 6.0 to 18.8 adult *T. castaneum* progeny, whereas no progeny were produced on liquid combo or β-cyfluthrin arenas at any residual week (Table 2.2). Treatment was significant (F = 100.41; df = 2, 285; $P < 0.001$), but residual week (F = 1.83; df = 4, 285; P = 0.123) and the treatment × residual week interaction (F = 1.83; df = 8, 285; P = 0.071) were not.

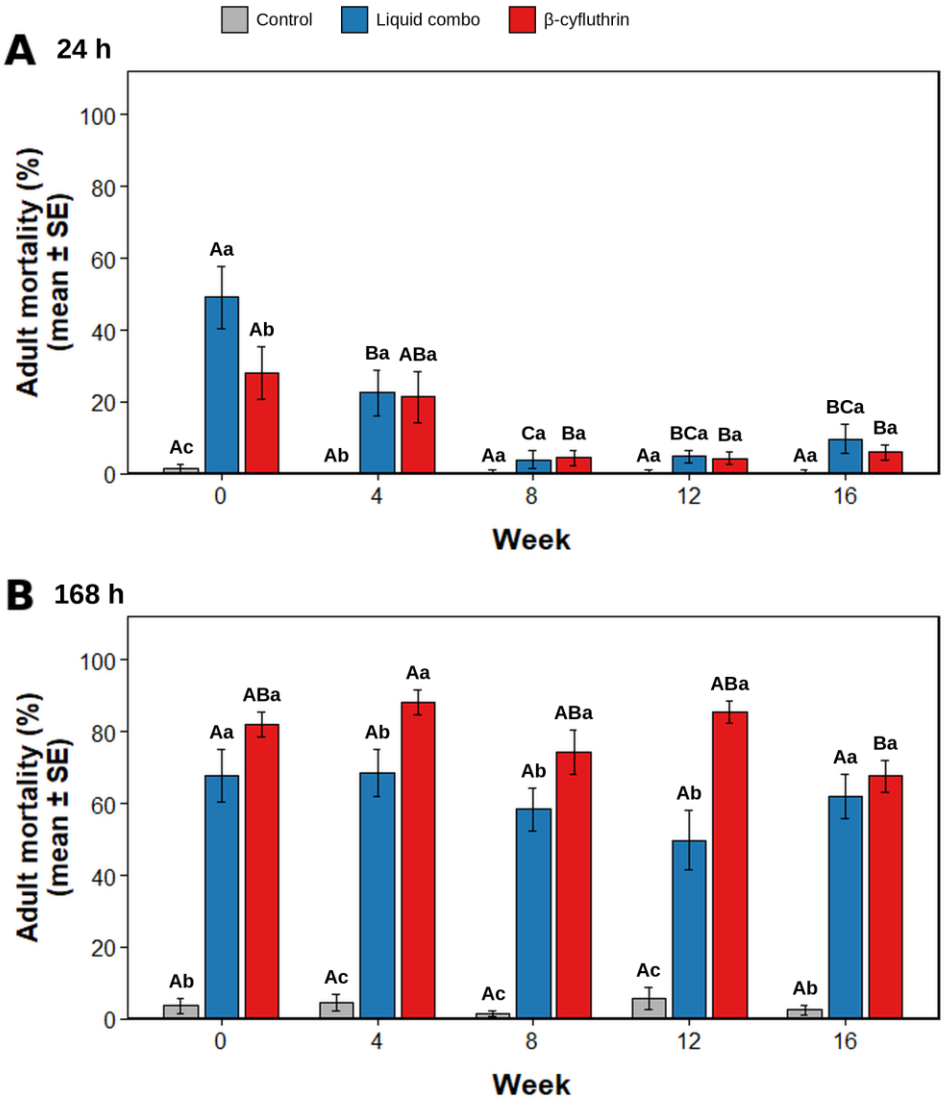


Figure 2.4. Mean (± SE) percent mortality of *Tribolium castaneum* adults exposed on wood bioassay arenas treated with water (control, gray bars), liquid combo (blue bars), or β-cyfluthrin (red bars) after (A) 24 h and (B) 168 h at 0, 4, 8, 12, and 16 weeks post-treatment. Within each residual week, bars with different lowercase letters are significantly different among treatments, and within each treatment, bars with different uppercase letters are significantly different among residual weeks (Tukey's HSD, $P < 0.05$).

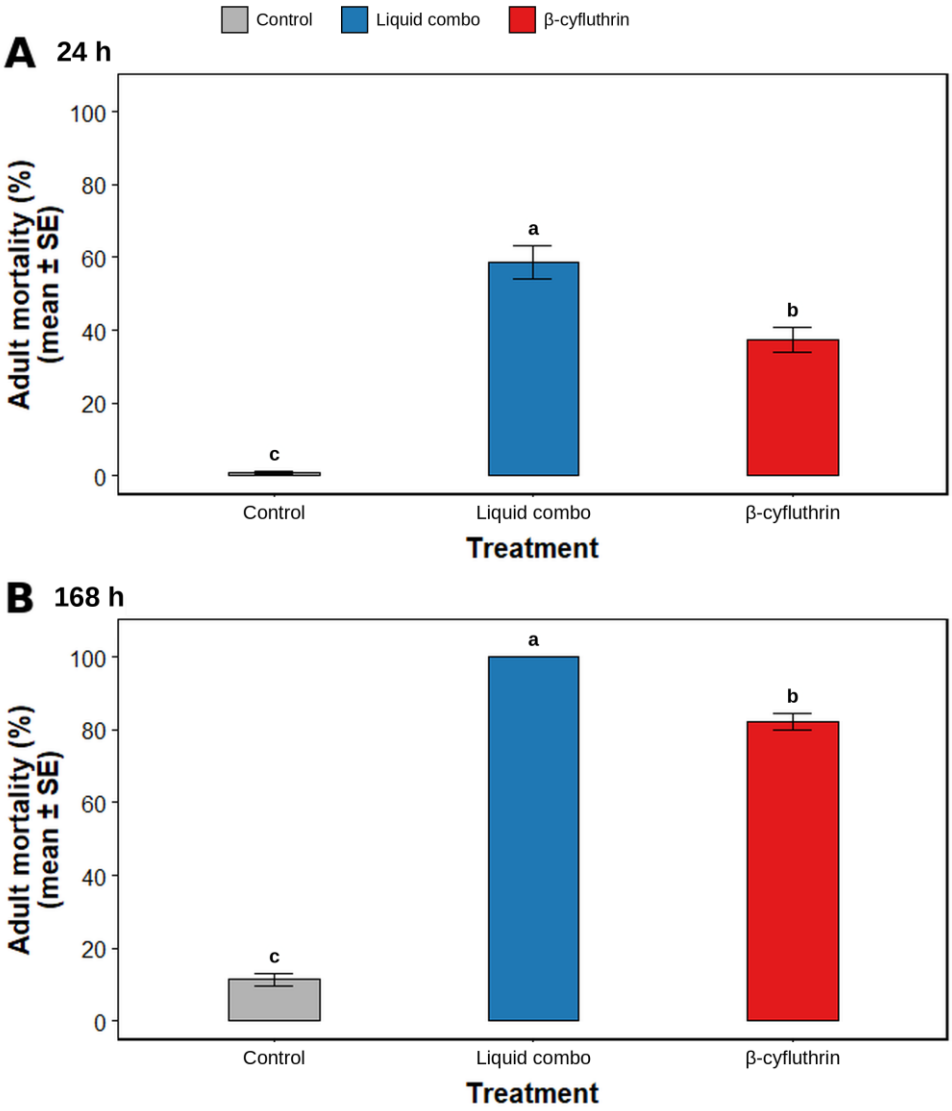


Figure 2.5. Mean (± SE) percent mortality of *Tribolium castaneum* adults exposed on metal bioassay arenas treated with water (control), liquid combo, or β-cyfluthrin after (A) 24 h and (B) 168 h, pooled across residual weeks 0-16 because neither residual week nor the treatment × residual week interaction was significant. Bars with different lowercase letters are significantly different (Tukey's HSD, $P < 0.05$).

2.3.3. Efficacy on *Sitophilus oryzae*

The mean control mortality of *S. oryzae* ranged from 0 to 5.0% after 24 h and from 1.6 to 30.5% after 168 h across surfaces and residual weeks, therefore mortality data were not corrected for control mortality. The percentages of *S. oryzae* adults classified as live, affected (alive but impaired), or dead are shown in Figure 2.6. After 24 h, most adults on treated surfaces that were

not dead were classified as affected, except on wood treated with liquid combo, where 20-33% of adults remained live at weeks 4-16. After 168 h, nearly all adults exposed to either insecticide were dead on both surfaces, except on metal treated with β-cyfluthrin at week 4, where approximately one-quarter of adults remained live.

Table 2.2. Mean (± SE) number of adult *Tribolium castaneum* progeny produced on metal bioassay arenas treated with water (control), liquid combo, or β-cyfluthrin at 0, 4, 8, 12, and 16 weeks post-treatment.

Residual Week	Mean (± SE) number of adult progeny per arena		
	Control[a]	Liquid combo[b]	β-cyfluthrin[b]
0[c]	13.7 ± 2.5 a	0.0 ± 0.0 b	0.0 ± 0.0 b
4[d]	11.9 ± 3.3 a	0.0 ± 0.0 b	0.0 ± 0.0 b
8[e]	11.6 ± 4.8 a	0.0 ± 0.0 b	0.0 ± 0.0 b
12[f]	6.0 ± 5.9 a	0.0 ± 0.0 b	0.0 ± 0.0 b
16[g]	18.8 ± 4.8 a	0.0 ± 0.0 b	0.0 ± 0.0 b

[a] Means within a row followed by different letters are significantly different among treatments (treatment: $F = 100.41$; df = 2, 285; $P < 0.001$; Tukey's HSD, $P < 0.05$). Control progeny did not differ among residual weeks ($F = 1.83$; df = 4, 285; $P = 0.123$), and the treatment × residual week interaction was not significant ($F = 1.83$; df = 8, 285; $P = 0.071$).

[b] No progeny were produced on liquid combo or β-cyfluthrin arenas at any residual week, and because these treatments had no variance among residual weeks, they were not compared among weeks.

[c] There were significant differences among the treatments at week 0 (control vs. liquid combo and control vs. β-cyfluthrin; Tukey's HSD, $P < 0.05$).

[d] There were significant differences among the treatments at week 4 (control vs. liquid combo and control vs. β-cyfluthrin; Tukey's HSD, $P < 0.05$).

[e] There were significant differences among the treatments at week 8 (control vs. liquid combo and control vs. β-cyfluthrin; Tukey's HSD, $P < 0.05$).

[f] There were significant differences among the treatments at week 12 (control vs. liquid combo and control vs. β-cyfluthrin; Tukey's HSD, $P < 0.05$).

[g] There were significant differences among the treatments at week 16 (control vs. liquid combo and control vs. β-cyfluthrin; Tukey's HSD, $P < 0.05$).

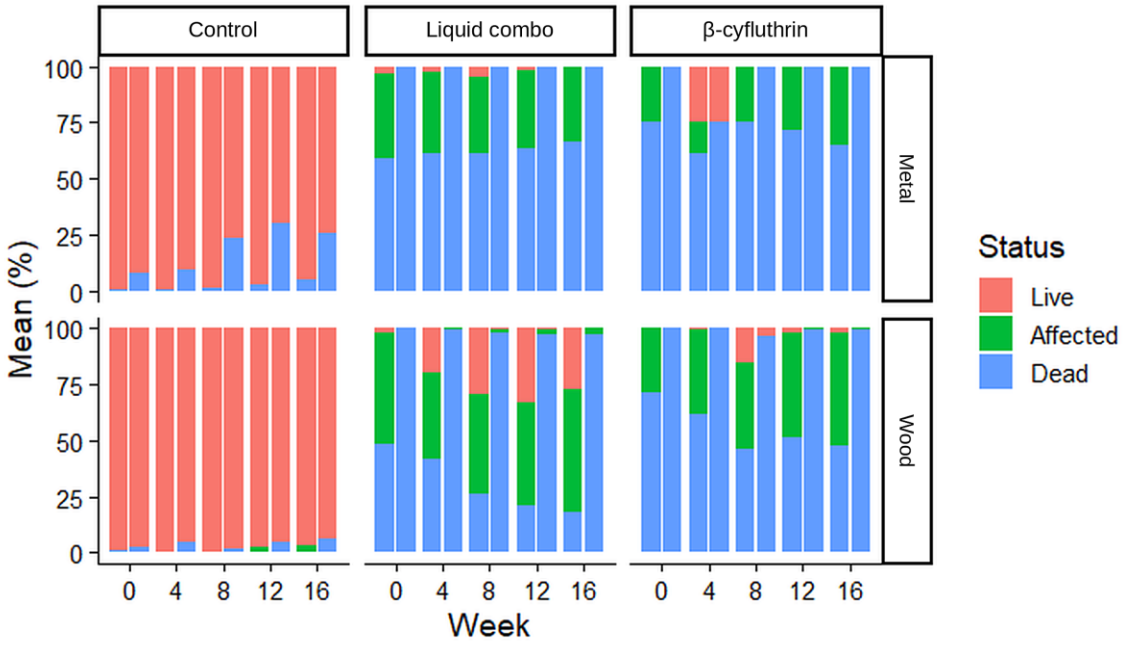


Figure 2.6. Mean percentage of *Sitophilus oryzae* adults classified as live (red), affected (green), or dead (blue) on metal (upper panels) and wood (lower panels) bioassay arenas treated with water (control), liquid combo, or β-cyfluthrin after 24 h (left bar within each week) and 168 h (right bar within each week) of exposure at 0, 4, 8, 12, and 16 weeks post-treatment. Data are pooled across both locations (Arkansas and Kansas) and both seasonal trials at each location.

2.3.3.1. Wood Bioassay Arenas

Mortality of *S. oryzae* after 24 h on wood was significantly affected by treatment (F = 162.65; df = 2, 276; $P < 0.001$) and residual week (F = 7.84; df = 4, 276; $P < 0.001$), but not by the treatment × residual week interaction (F = 1.94; df = 8, 276; P = 0.054) (Fig. 2.7A). At every residual week, β-cyfluthrin caused greater mortality than liquid combo (P = 0.0002-0.035), and both insecticides caused greater mortality than the control ($P \leq 0.016$). Mortality on β-cyfluthrin declined from 71.4% at week 0 to 46.0-51.3% at weeks 8-16, and mortality on liquid combo declined from 48.8% at week 0 to 18.1% at week 16 (Fig. 2.7A). Control mortality was ≤1.1% and did not differ among residual weeks.

After 168 h on wood, treatment was significant (F = 3822.63; df = 2, 275; $P < 0.001$), but residual week (F = 1.35; df = 4, 275; P = 0.253) and the treatment × residual week interaction (F

= 1.20; df = 8, 275; $P = 0.300$) were not (Fig. 2.7B). Pooled across residual weeks, mortality on liquid combo (98.6%) and β-cyfluthrin (99.2%) did not differ ($P \geq 0.341$), and both were greater than mortality on the control (3.8%) ($P < 0.0001$).

Control wood arenas produced 11.6 to 15.8 adult *S. oryzae* progeny, whereas liquid combo and β-cyfluthrin arenas produced no more than 1.5 and 0.5 adults per arena, respectively (Table 2.3). Treatment ($F = 308.74$; df = 2, 285; $P < 0.001$) and the treatment × residual week interaction ($F = 2.06$; df = 8, 285; $P = 0.040$) were significant, but residual week was not ($F = 1.38$; df = 4, 285; $P = 0.242$). Both insecticides produced fewer progeny than the control at every residual week ($P < 0.0001$) and did not differ from each other at any residual week ($P \geq 0.538$).

Table 2.3. Mean (± SE) number of adult *Sitophilus oryzae* progeny produced on wood bioassay arenas treated with water (control), liquid combo, or β-cyfluthrin at 0, 4, 8, 12, and 16 weeks post-treatment.

Residual Week	Mean (± SE) number of adult progeny per arena		
	Control[a]	Liquid combo[b]	β-cyfluthrin[b]
0[c]	15.8 ± 1.9 a	0.0 ± 0.0 b	0.0 ± 0.0 b
4[d]	14.1 ± 3.4 a	0.1 ± 0.1 b	0.0 ± 0.0 b
8[e]	13.4 ± 3.6 a	1.5 ± 0.7 b	0.5 ± 0.4 b
12[f]	12.6 ± 2.3 a	0.0 ± 0.0 b	0.0 ± 0.0 b
16[g]	11.6 ± 3.0 a	0.2 ± 0.1 b	0.1 ± 0.1 b

[a] Means within a row followed by different letters are significantly different among treatments (Tukey's HSD, $P < 0.05$). Progeny counts were $\log(x + 1)$-transformed for analysis, and untransformed means are shown. Residual week was not significant ($F = 1.38$; df = 4, 285; $P = 0.242$).

[b] liquid combo and β-cyfluthrin did not differ from each other at any residual week ($P \geq 0.538$; Tukey's HSD).

[c] There were significant differences among the treatments at week 0 (control vs. liquid combo and control vs. β-cyfluthrin: $t = 12.13$; df = 285; $P < 0.0001$; Tukey's HSD).

[d] There were significant differences among the treatments at week 4 (control vs. liquid combo: $t = 9.64$; control vs. β-cyfluthrin: $t = 9.79$; df = 285; $P < 0.0001$; Tukey's HSD).

[e] There were significant differences among the treatments at week 8 (control vs. liquid combo: $t = 7.44$; control vs. β-cyfluthrin: $t = 8.50$; df = 285; $P < 0.0001$; Tukey's HSD).

[f] There were significant differences among the treatments at week 12 (control vs. liquid combo and control vs. β-cyfluthrin: $t = 10.22$; df = 285; $P < 0.0001$; Tukey's HSD).

[g] There were significant differences among the treatments at week 16 (control vs. liquid combo: $t = 7.94$; control vs. β-cyfluthrin: $t = 8.19$; df = 285; $P < 0.0001$; Tukey's HSD).

2.3.3.2. Metal Bioassay Arenas

Mortality of *S. oryzae* after 24 h on metal was significantly affected by treatment (F = 152.59; df = 2, 276; $P < 0.001$), but not by residual week (F = 0.49; df = 4, 276; P = 0.747) or the treatment × residual week interaction (F = 0.33; df = 8, 276; P = 0.952) (Fig. 2.8A). Pooled across residual weeks, mortality differed among treatments (F = 156.60; df = 2, 288; $P < 0.001$): mortality on liquid combo (62.1%) and β-cyfluthrin (69.8%) did not differ ($P \geq 0.283$), and both were greater than mortality on the control (2.1%) ($P < 0.0001$).

After 168 h on metal, treatment (F = 402.43; df = 2, 277; $P < 0.001$), residual week (F = 4.88; df = 4, 277; $P < 0.001$), and the treatment × residual week interaction (F = 2.53; df = 8, 277; P = 0.012) were all significant (Fig. 2.8B). Both insecticides caused greater mortality than the control at every residual week ($P < 0.0001$). Liquid combo caused 100% mortality at every residual week, and β-cyfluthrin caused 100% mortality at every week except week 4 (75.5 ± 9.8%), when it was significantly lower than liquid combo (P = 0.0013), and the two insecticides did not differ at the other weeks (P = 1.000). Control mortality varied among residual weeks, increasing from 8.1% at week 0 to 30.5% at week 12 (Fig. 2.8B).

Control metal arenas produced 6.4 to 28.1 adult *S. oryzae* progeny, whereas liquid combo and β-cyfluthrin arenas produced no more than 0.3 and 0.1 adults per arena, respectively (Table 2.4). Treatment (F = 161.90; df = 2, 285; $P < 0.001$) and the treatment × residual week interaction (F = 2.13; df = 8, 285; P = 0.033) were significant, but residual week was not (F = 1.93; df = 4, 285; P = 0.105). Both insecticides produced fewer progeny than the control at every residual week ($P < 0.0001$) and did not differ from each other at any residual week ($P \geq 0.941$).

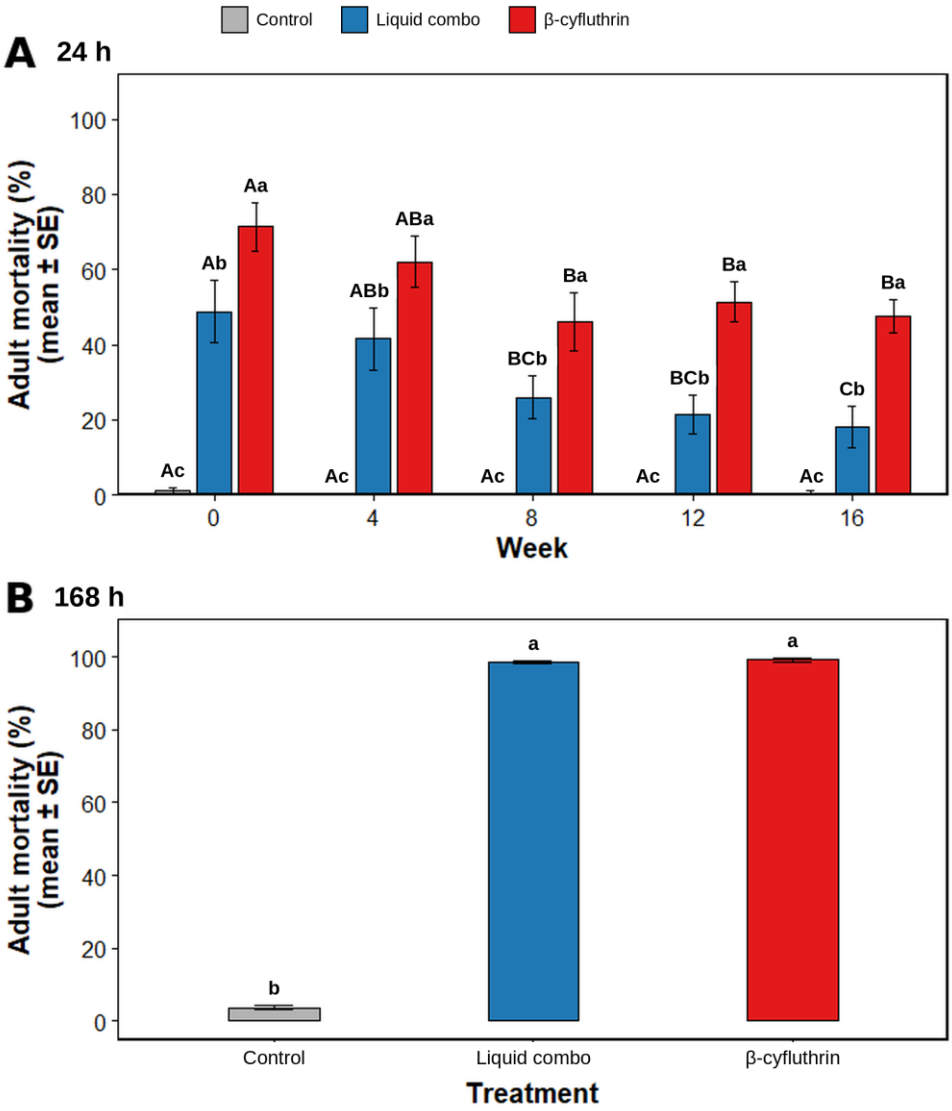


Figure 2.7. Mean (± SE) percent mortality of *Sitophilus oryzae* adults exposed on wood bioassay arenas treated with water (control, gray), liquid combo (blue), or β-cyfluthrin (red) after (A) 24 h at 0, 4, 8, 12, and 16 weeks post-treatment and (B) 168 h, pooled across residual weeks. In (A), bars within a residual week with different lowercase letters are significantly different among treatments, and bars within a treatment with different uppercase letters are significantly different among residual weeks. In (B), bars with different lowercase letters are significantly different (Tukey's HSD, $P < 0.05$).

Table 2.4. Mean (± SE) number of adult *Sitophilus oryzae* progeny produced on metal bioassay arenas treated with water (control), liquid combo, or β-cyfluthrin at 0, 4, 8, 12, and 16 weeks post-treatment.

Residual Week	Mean (± SE) number of adult progeny per arena		
	Control[a]	Liquid combo[b]	β-cyfluthrin[b]
0[c]	15.7 ± 2.7 a	0.0 ± 0.0 b	0.0 ± 0.0 b
4[d]	16.4 ± 5.4 a	0.0 ± 0.0 b	0.0 ± 0.0 b
8[e]	28.1 ± 7.7 a	0.0 ± 0.0 b	0.0 ± 0.0 b
12[f]	6.4 ± 1.8 a	0.0 ± 0.0 b	0.0 ± 0.0 b
16[g]	10.4 ± 2.9 a	0.3 ± 0.2 b	0.1 ± 0.1 b

[a] Means within a row followed by different letters are significantly different among treatments (Tukey's HSD, $P < 0.05$). Progeny counts were $\log(x + 1)$-transformed for analysis, and untransformed means are shown. Residual week was not significant ($F = 1.93$; df = 4, 285; $P = 0.105$).

[b] liquid combo and β-cyfluthrin did not differ from each other at any residual week ($P \geq 0.941$; Tukey's HSD).

[c] There were significant differences among the treatments at week 0 (control vs. liquid combo and control vs. β-cyfluthrin: $t = 8.50$; df = 285; $P < 0.0001$; Tukey's HSD).

[d] There were significant differences among the treatments at week 4 (control vs. liquid combo and control vs. β-cyfluthrin: $t = 6.74$; df = 285; $P < 0.0001$; Tukey's HSD).

[e] There were significant differences among the treatments at week 8 (control vs. liquid combo and control vs. β-cyfluthrin: $t = 8.96$; df = 285; $P < 0.0001$; Tukey's HSD).

[f] There were significant differences among the treatments at week 12 (control vs. liquid combo and control vs. β-cyfluthrin: $t = 4.65$; df = 285; $P < 0.0001$; Tukey's HSD).

[g] There were significant differences among the treatments at week 16 (control vs. liquid combo: $t = 5.83$; control vs. β-cyfluthrin: $t = 6.17$; df = 285; $P < 0.0001$; Tukey's HSD).

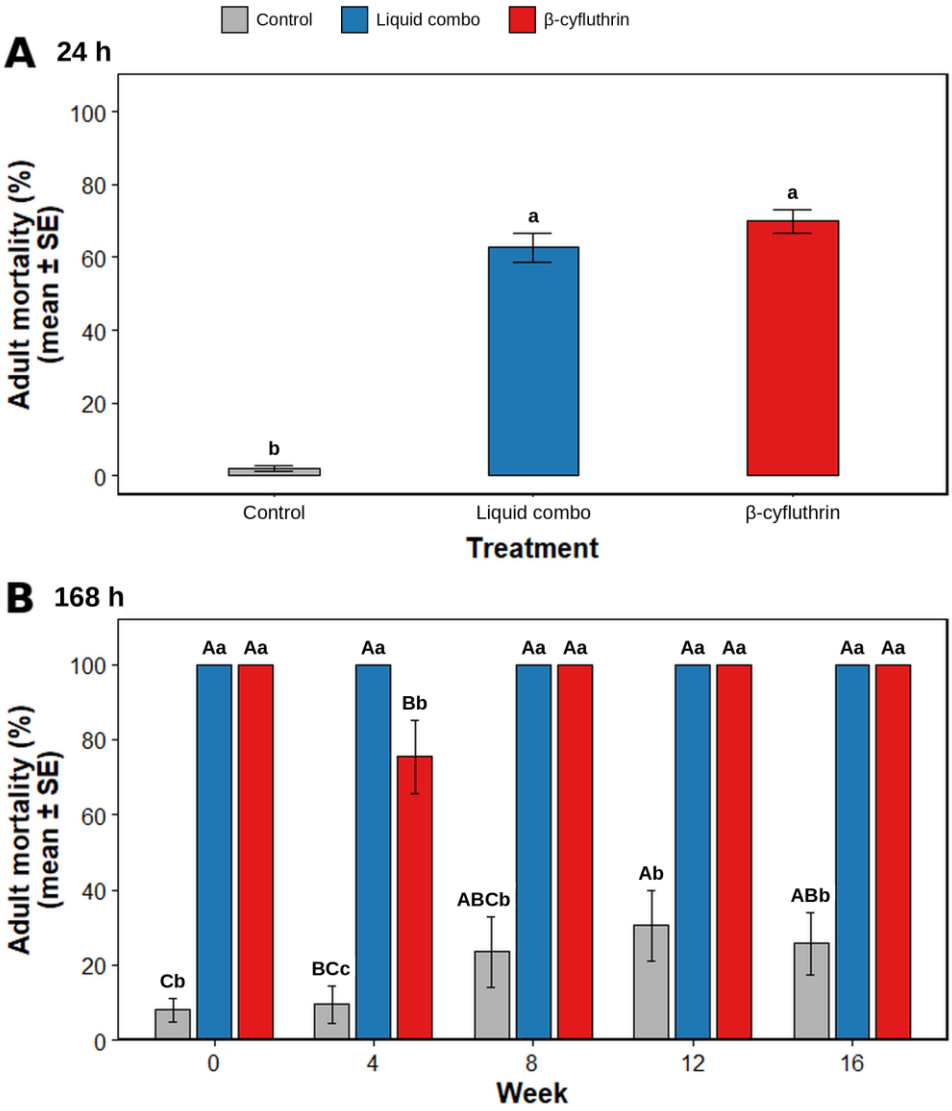


Figure 2.8. Mean (± SE) percent mortality of *Sitophilus oryzae* adults exposed on metal bioassay arenas treated with water (control, gray), liquid combo (blue), or β-cyfluthrin (red) after (A) 24 h, pooled across residual weeks, and (B) 168 h at 0, 4, 8, 12, and 16 weeks post-treatment. In (A), bars with different lowercase letters are significantly different. In (B), bars within a residual week with different lowercase letters are significantly different among treatments, and bars within a treatment with different uppercase letters are significantly different among residual weeks (Tukey's HSD, $P < 0.05$).

2.4. Discussion

The present study evaluated the initial and residual efficacy of liquid combo and β-cyfluthrin applied to wood and metal bioassay arenas against *T. castaneum* and *S. oryzae* adults at two geographic locations across four seasonal trials. Both insecticides significantly reduced

adult survival and progeny production compared with the control, but their efficacy depended strongly on insect species, surface type, exposure interval, and residual period. These results are consistent with previous surface-treatment studies showing that residual contact insecticides are useful structural IPM tools, but that their efficacy cannot be generalized across surfaces, a.i., formulations, exposure intervals, or target species (Arthur, 2012; Nayak et al., 2026; Scheff et al., 2026). To our knowledge, this is the first study to evaluate residual contact insecticides as pre-loading treatments inside shipping containers under field conditions.

On wood, 24 h mortality of *T. castaneum* was greatest at weeks 0 and 4 and declined to levels that did not differ from the control by week 8. This progressive loss of short-term efficacy is consistent with the absorption of insecticide into porous substrates over time. Porous materials such as plywood retain applied formulations within the substrate matrix rather than at the surface, reducing the amount of a.i. available for tarsal contact with walking insects (White and Leesch, 1996; Arthur, 2008). Jankov et al. (2013) evaluated capsule suspension and wettable powder formulations of lambda-cyhalothrin applied to concrete, metal, and plywood against *S. oryzae* under laboratory conditions, exposing adults for 24 h to deposits aged up to 180 days. Efficacy on plywood declined after 120 days and was 55% at 180 days, whereas efficacy on metal remained 100% throughout the 180-day period (Jankov et al., 2013). The faster decline observed for *T. castaneum* on wood in the present study, in which 24 h mortality no longer differed from the control by week 8, likely reflects the combined effects of substrate absorption, fluctuating container conditions, and the relatively short 24 h exposure, which may be insufficient for insects to acquire a lethal dose when surface residues are reduced.

Extending the exposure to 168 h showed that both formulations remained significantly more effective than the control at every residual week on wood, confirming that a.i. residues

persisted in sufficient quantity to cause mortality when insects remained in contact with treated surfaces for longer periods. This difference between the 24 h and 168 h responses is biologically important because short exposures to pyrethroids often produce knockdown or affected insects that later either die or recover, depending on species, dose, food availability, and environmental conditions (Arthur et al., 2020; Gerken et al., 2025). Sublethal exposure can also reduce insect movement and, therefore, further contact with treated surfaces (Arthur et al., 2020; Quellhorst et al., 2022). Gerken et al. (2025) exposed *T. castaneum* adults to deltamethrin (CentynalEC, 4.75% a.i.) and found that recovery increased with food availability, temperature (18 to 32 °C), and relative humidity (30 to 60%). *Tribolium castaneum* adults that were provided even a light dusting of flour recovered at rates above 90%, and most recovery occurred between 24 and 48 h after exposure (Gerken et al., 2025). Yasir et al. (2021) similarly found that residues of the IGR novaluron applied to concrete, metal, and wood reduced adult emergence of three stored-product coleopteran species for 12 weeks on all surfaces and remained effective longest on metal. Surface type has also influenced the residual efficacy of the IGRs pyriproxyfen and hydroprene applied to wood, metal, and concrete (Arthur et al., 2009). Thus, short-term mortality alone may underestimate the value of residual surface treatments in shipping containers, where insects can remain in contact with treated surfaces for the duration of transit.

In contrast to wood, residual week did not significantly affect *T. castaneum* mortality on metal at either observation interval. This stability is consistent with the retention of pyrethroid residues on non-porous surfaces. In the study by Jankov et al. (2013), both lambda-cyhalothrin formulations remained 100% effective against *S. oryzae* on metal 180 days after treatment. Scheff et al. (2026) found that deltamethrin and the high rate of pyrethrin caused significantly lower survival of six stored-product insect species on metal than on concrete and attributed this

difference to the non-absorbent character of metal. Ghimire et al. (2016) also reported residual activity of deltamethrin and β-cyfluthrin, the same pyrethroids evaluated in the present study, against *Trogoderma variabile* Ballion, warehouse beetle, and *Trogoderma inclusum* LeConte, larger cabinet beetle. Arthur et al. (2018) evaluated pyrethroid insecticides and IGRs applied to different surfaces for control of *Trogoderma granarium* Everts, khapra beetle, a quarantine pest of particular concern in international trade, and Kavallieratos et al. (2023) compared five surfaces treated with d-tetramethrin plus acetamiprid against *Tenebrio molitor* L., yellow mealworm, and *Alphitobius diaperinus* (Panzer), lesser mealworm. The absence of a significant residual week effect on metal indicates that both β-cyfluthrin and liquid combo retained functional residual activity on galvanized steel for 16 weeks under field conditions inside shipping containers, a practically important finding for the pre-loading treatment concept.

The relative performance of the two formulations against *T. castaneum* depended on surface and exposure interval. Liquid combo caused greater mortality than β-cyfluthrin on metal after both 24 h (59% vs. 37%) and 168 h (100% vs. 82%) and on wood at week 0 after 24 h, whereas β-cyfluthrin caused greater mortality than liquid combo on wood after 168 h at weeks 4, 8, and 12. Both a.i. act on voltage-gated sodium channels in insect nerve membranes (Soderlund and Bloomquist, 1989). The advantage of liquid combo on metal is consistent with the synergistic role of PBO, which inhibits the cytochrome P450 monooxygenases through which insects detoxify pyrethroids (Hodgson and Levi, 1998). Arthur et al. (2020) exposed *R. dominica*, *Cryptolestes ferrugineus* (Stephens), rusty grain beetle, and *S. oryzae* to a formulation containing deltamethrin (12 mg a.i./mL), methoprene (27 mg a.i./mL), and PBO (320 mg a.i./mL) at concentrations up to the proposed label rate for 1 to 72 h and found that movement decreased with increasing concentration and exposure time, with *R. dominica* the most

susceptible and *S. oryzae* the least susceptible species. Despite the differences in adult mortality, the two formulations did not differ in *T. castaneum* progeny production on either surface, because no progeny were produced on either treatment.

For *S. oryzae*, the pattern was reversed. β-cyfluthrin caused significantly greater 24 h mortality than liquid combo on wood at every residual week ($P \leq 0.035$) and after 48 h at every residual week except week 4. This reversal is consistent with the low susceptibility of *S. oryzae* to the deltamethrin, methoprene, and PBO combination reported by Arthur et al. (2020) and suggests that the two formulations interact differently with the *S. oryzae* cuticle or with the plywood substrate. Athanassiou et al. (2004) likewise evaluated the insecticidal and residual effects of three pyrethroids against *S. oryzae* on stored wheat. One possible explanation is formulation-related: Tempo® SC Ultra is a suspension concentrate, whereas Gravista™ contains PBO as a major component (33.3% by weight), and the physical interaction among PBO, the plywood substrate, and the *S. oryzae* cuticle may differ from that of β-cyfluthrin alone. Athanassiou et al. (2013) found that β-cyfluthrin alone provided strong contact activity against stored-product insects on concrete, another porous surface, suggesting that the availability of the a.i. from a suspension concentrate may contribute to early efficacy on porous substrates independent of synergist interactions. After 168 h on wood, however, the two insecticides did not differ for *S. oryzae* ($P \geq 0.341$), indicating that with extended contact the formulation difference was eliminated and both products caused near-complete mortality.

The slower 24 h response of *S. oryzae* on both surfaces and the lower efficacy of β-cyfluthrin against this species on metal at week 4 may reflect the biological characteristics of *S. oryzae* relative to *T. castaneum*. *Sitophilus oryzae* has a more heavily sclerotized cuticle and a distinctive rostrum that reduces the ventral surface area in contact with treated substrates during

locomotion (Hagstrum and Subramanyam, 2009). As a result, the rate of pyrethroid uptake per unit of contact time may be lower in *S. oryzae* than in *T. castaneum*, making *S. oryzae* mortality more sensitive to variation in surface residue concentrations as the formulations aged in the field.

The shipping containers in this study experienced daily mean temperatures from −13.3 to 37 °C and daily mean RH from 15 to 73%, conditions far more variable than the constant temperatures typically used in laboratory residual studies. Because arenas were retrieved from the containers and bioassayed under laboratory conditions, these environmental extremes influenced efficacy primarily through their effects on the persistence and availability of surface residues rather than on insect activity during exposure. Even though the analysis combined trials conducted under very different seasonal conditions, residual week had no significant effect on mortality on metal, suggesting that residues on non-porous surfaces remained stable under these fluctuations. Temperature also influences the toxicity of contact insecticides and the recovery of affected insects. In laboratory bioassays with the same two formulations on wood, metal, and concrete at 16 to 37 °C, efficacy generally increased with temperature (Boddepalli et al., 2025), and recovery of *T. castaneum* after deltamethrin exposure increased at warmer temperatures and higher humidity (Gerken et al., 2025). Temperature also affected the residual efficacy of a pyrethrin + methoprene aerosol against two dermestid species (Lanka et al., 2019). Cold periods are also operationally important. Daily mean temperatures were below 16 °C on 111 days during the Arkansas winter trial and on 70 days during the Kansas spring trial. At these temperatures, insect development and activity are reduced, and phosphine fumigation requires substantially longer exposure periods, for example 4 to 5 days at 12 to 15 °C and 8 to 10 days at 4 to 12 °C for pellet and tablet formulations, and fumigation is not recommended below 4.4 °C (Studebaker, n.d.). Residual surface treatments may therefore provide a protective option during cooler

months when fumigation is least practical, although insects that move less at low temperatures will also contact treated surfaces less frequently.

The most consistent result of this study was the near-complete suppression of progeny. Control arenas produced 6.0 to 28.1 adult progeny, whereas no *T. castaneum* progeny and no more than 1.5 *S. oryzae* progeny were produced on treated arenas at any residual week on either surface. Progeny suppression remained complete for *T. castaneum* on wood at weeks 8 to 16, when 24 h mortality did not differ from the control and many adults were classified as affected rather than dead after 168 h. This indicates that adults surviving exposure to treated surfaces did not produce a detectable next generation. For *T. castaneum*, which oviposits externally on commodities and in crevices, newly hatched larvae would contact residues on the treated surface and on the diet placed on it (Hagstrum and Subramanyam, 2009). Wijayaratne et al. (2012) showed that methoprene residues on varnished wood, concrete, and wheat reduced the survival of *T. castaneum* larvae across temperatures, methoprene affects the fecundity, egg hatchability, and egg-to-adult emergence of *T. castaneum* (Scheff et al., 2016), and residual pyrethrin + methoprene treatments have been evaluated for *Tribolium* control in commercial flour mills (Arthur, 2015). In the present study, however, progeny suppression was equally strong with β-cyfluthrin, which contains no IGR, indicating that the methoprene component of liquid combo did not provide a detectable additional benefit beyond the effects of the pyrethroid under the conditions of this study. For *S. oryzae*, an internal feeder whose larvae develop within intact kernels, suppression more likely reflects reduced oviposition by intoxicated females than direct exposure of immature stages. Liba et al. (2026) noted that *S. oryzae* females bore into kernels before ovipositing, which limits the exposure of developing larvae to treated surfaces. From a transport perspective, these results suggest that an infestation entering a treated container is

unlikely to establish a new generation during a multi-week transit, reducing the risk of heavily infested product arriving at its destination even when adult mortality is incomplete.

The results of the present study have direct practical relevance for the management of stored-product insects in intermodal shipping containers. Phosphine fumigation is the dominant tool for container pest management globally, but it requires adequate temperatures and exposure periods of several days, and container seal integrity is frequently insufficient to maintain lethal concentrations (Nayak et al., 2020; Agrafioti et al., 2021). Fumigant concentrations in transport units such as shipping containers and railcars have been characterized with wireless monitoring and modeling (Agrafioti et al., 2021; Brabec et al., 2022), and *T. castaneum* populations can rebound after fumigation (Campbell et al., 2010). Phosphine resistance has been confirmed in field populations of *T. castaneum* in the U.S. (Collins, 2010; Opit et al., 2012; Cato et al., 2017), further limiting the reliability of fumigation. Nayak et al. (2026) highlighted the strategic integration of contact insecticides into fumigation-dominated stored-product protection programs, and Scheff and Phillips (2020) identified surface treatments as a critical hurdle for limiting insect dispersal within facilities. The present study demonstrates that both β-cyfluthrin and liquid combo can function as this complementary surface-treatment hurdle in shipping containers, maintaining significant adult mortality and progeny suppression of both *T. castaneum* and *S. oryzae* for 16 weeks. The greater durability of both formulations on metal and the decline in short-term efficacy on plywood over time suggest that pre-loading treatments should be applied as close to loading as possible and should target the wooden container floor, where insects harbor and residues are least persistent. Effective sanitation to remove commodity residues and debris before application is also important, because flour and milling debris reduce the efficacy of β-cyfluthrin against *T. castaneum* (Arthur et al., 2015), and sanitation improves

the efficacy and longevity of stored-product insect control programs by reducing refuges and food resources (Morrison et al., 2019; Bingham and Hagstrum, 2024). Integration of residual surface treatments with long-lasting insecticide-incorporated netting may further reduce insect dispersal and damage as part of a multi-hurdle IPM strategy (Ranabhat et al., 2025).

2.5. Conclusion

Both β-cyfluthrin (Tempo® SC Ultra) and liquid combo (deltamethrin + (*S*)-methoprene + PBO, Gravista™) demonstrated strong and persistent residual efficacy against *Tribolium castaneum* and *Sitophilus oryzae* when applied as pre-loading surface treatments inside intermodal shipping containers. Although efficacy varied with surface type, insect species, and exposure interval, both formulations consistently reduced adult survival and suppressed progeny production relative to untreated controls throughout the 16-week evaluation period. Metal surfaces provided more stable residual performance than plywood, while longer exposure intervals revealed that biologically active residues persisted on wood far longer than indicated by short-term observations alone. Importantly, both products maintained efficacy across diverse environmental conditions and seasonal periods representative of commercial shipping environments in the south-central and central U.S.. These findings demonstrate the practical value of residual surface treatments as an operationally feasible, non-fumigant management strategy that can complement existing sanitation and fumigation programs within IPM systems for post-harvest protection. Overall, this study supports the incorporation of residual contact insecticides as an additional protective barrier during commodity transport and provides a foundation for future evaluations under commodity-loaded conditions and against resistant insect populations.

Future research should investigate the efficacy of contact insecticides on additional surface types commonly encountered throughout the postharvest supply chain, evaluate longer residual periods across a broader range of pest species and developmental stages, and determine compatibility with other stored-product pest prevention tactics, including IGRs, packaging modifications, and pheromone-based monitoring systems. Additional research should evaluate these formulations against field-collected insect populations with documented pyrethroid or phosphine resistance, under commodity-loaded container conditions, and in combination with sanitation and long-lasting insecticide-incorporated netting to fully characterize their role within an integrated protection framework.

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Chapter 3 - Interactive Effects of Temperature and Surface Type on the Residual Efficacy of Two Contact Insecticides Against *Tribolium castaneum*, *Sitophilus oryzae*, and *Rhyzopertha dominica*

Abstract

Residual contact insecticides applied to structural surfaces are an important component of integrated pest management for stored-product insects, but their performance across the temperatures found in storage and transport facilities is poorly understood. We evaluated β-cyfluthrin (Tempo® SC Ultra) and deltamethrin + (*S*)-methoprene + piperonyl butoxide (Gravista™, liquid combo) applied to wood, metal, and concrete against adults of *Tribolium castaneum* (Herbst), red flour beetle, *Sitophilus oryzae* (L.), rice weevil, and *Rhyzopertha dominica* (F.), lesser grain borer. Treated arenas were held at 16, 22, 27, 32, and 37 °C and bioassayed at 0, 4, 8, and 12 weeks after application (and at 24 weeks for *T. castaneum*), and mortality was assessed after 24 h and 168 h of exposure. Insecticide, residual week, and temperature interacted significantly in most analyses. Metal provided the greatest and most persistent efficacy. After 168 h of exposure, mortality of *S. oryzae* and *R. dominica* on metal was 98-100% at every temperature and residual week combination. Mortality was lowest on concrete, where no *T. castaneum* mortality occurred from week 12 onward. On wood and concrete, *T. castaneum* mortality declined to ≤44% within 4 weeks at most temperatures, and mortality at week 0 generally increased with temperature. The liquid combo treatment generally caused greater 24 h mortality than β-cyfluthrin on metal, whereas β-cyfluthrin was generally more effective than liquid combo on concrete. These results show that surface type, temperature, target

species, and formulation must be considered together when selecting residual treatments and re-treatment intervals for storage and transport structures.

Keywords: β-cyfluthrin, deltamethrin, piperonyl butoxide, structural surfaces, stored-product beetles, integrated pest management

3.1. Introduction

Residual contact insecticides applied to floors, walls, and other structural surfaces are a central component of integrated pest management (IPM) for stored-product insects in warehouses, mills, food processing facilities, and transport units. These treatments are intended to intercept adults as they walk across treated surfaces and to suppress populations harboring in cracks, crevices, and accumulated debris, particularly before new commodities are brought into a structure (Arthur, 1996; Hagstrum and Subramanyam, 2009). Pyrethroids such as β-cyfluthrin and deltamethrin are among the most widely labeled active ingredients for this purpose because of their rapid knockdown, low application rates, and relatively low mammalian toxicity. Insecticide formulation and active ingredients influence residual efficacy. In an emulsifiable concentrate, the active ingredient is dissolved in an organic solvent with emulsifiers and forms an emulsion when mixed with water. In a wettable powder and a suspension concentrate, the active ingredient remains as fine solid particles, which are supplied as a dry powder or as a suspension in water, respectively (Knowles, 2008). Arthur (1994) found emulsifiable concentrate and wettable powder formulations of cyfluthrin differed in persistence on concrete, and a suspension concentrate of β-cyfluthrin alone was at least as effective on concrete as a combination of β-cyfluthrin and imidacloprid (Athanassiou et al., 2013). Recently registered products now combine a pyrethroid with the insect growth regulator (*S*)-methoprene and the synergist piperonyl butoxide (PBO). The PBO inhibits cytochrome P450 monooxygenases that

detoxify pyrethroids, and methoprene extends activity to immature stages (Arthur et al., 2020). Regardless of formulation, the performance of a residual treatment depends on application rate, the material surface, the presence of food material, the target species, the age of the residue, and the environmental conditions under which insects are exposed (Arthur, 2000a, 2013).

Surface type is one of the best-documented sources of variation in residual efficacy. Early comparisons established that porous substrates generally reduce insecticide activity relative to non-porous ones. Giga and Canhão (1991) demonstrated pyrethroid deposition on glass was more toxic to *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), larger grain borer, and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), maize weevil, compared to deposition on wood, mud, or jute. Collins et al. (2000) also demonstrated four organophosphates applied to galvanized steel remained effective against liposcelid psocids considerably longer compared to concrete. Concrete is porous and alkaline, where it may retain part of insecticide deposition within its matrix, making it unavailable to insects walking on the surface (Chadwick, 1985; Lampiri et al., 2023). Sealing concrete with a commercial waterproofing sealant before application has been shown to substantially extend cyfluthrin activity (Arthur, 1994). Deltamethrin dust was differentially effective on plywood, concrete, and tile against three stored-product beetles (Arthur, 1997), and the insect growth regulator hydroprene was least persistent on concrete and most persistent on metal (Arthur et al., 2009). Likewise, deltamethrin and cyfluthrin applied to concrete, wood, painted wood, vinyl tile, and metal, and methoprene and pyriproxyfen applied to concrete, metal, and wood, were most persistent on metal against larvae of *Trogoderma granarium* Everts (Coleoptera: Dermestidae), khapra beetle (Arthur et al., 2018). Novaluron, an insect growth regulator, was also most persistent on metal, followed by concrete and then wood, 12 weeks after application against *Oryzaephilus surinamensis* (L.) (Coleoptera:

Silvanidae), sawtoothed grain beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), red flour beetle, and *T. granarium* (Yasir et al., 2021). Formulation strongly modifies the concrete effect. An emulsifiable concentrate of deltamethrin caused complete mortality of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), rice weevil, adults on plastic but no mortality on concrete at any of three rates, whereas a suspension concentrate caused 100% mortality on both surfaces, and the same pattern held in a commercial warehouse (Quinn et al., 2018). When deltamethrin was applied to four surfaces in the same laboratory study, it caused 24.2% mortality of *S. oryzae* adults on concrete after 3 d of exposure, compared with 92.5% on metal, and only 4.2-16.7% mortality of *O. surinamensis* on concrete over 14 d, compared with 90.8-100% on metal (Lampiri et al., 2023). However, surface effects are not universal. Lambda-cyhalothrin was initially more effective against *S. oryzae* on concrete than on metal or plywood, whereas malathion and pirimiphos-methyl remained fully effective on metal for 180 d (Jankov et al., 2013). Chlorfenapyr was more effective on concrete than on other substrates against *Tribolium* spp. (Arthur, 2008), and the efficacy of spinetoram differed among concrete, ceramic tile, galvanized steel, and plywood only for *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), confused flour beetle (Vassilakos et al., 2014). Spinosad killed 98-100% of *Tribolium* adults on concrete but allowed recovery on steel and floor tile (Toews et al., 2003), and two pirimiphos-methyl formulations showed no consistent surface effect across five substrates (Rumbos et al., 2014). These contrasts indicate that the surface × insecticide relationship must be established for each active ingredient and formulation of interest. Published studies that compared residual insecticides among storage surfaces are summarized in Table 1.2 (Chapter 1).

Temperature is a second, less understood driver of residual efficacy, and it acts through several routes at once. Inside unconditioned warehouses, mills, and transport units, surface temperatures can range from below 16 °C in winter to above 35 °C in summer. Temperature first governs insect behavior as walking activity of stored-product beetles declines sharply as temperature approaches species-specific lower activity thresholds, and these thresholds differ among species (Stejskal et al., 2019). Fewer and shorter contacts with a treated surface at low temperatures may result in sublethal exposure. Temperature also alters insect physiology, including the uptake and metabolic detoxification of active ingredients, and it changes the rate at which residues degrade on the substrate. Pyrethroids commonly exhibit a negative temperature coefficient, becoming more toxic at lower temperatures (Sparks et al., 1982). Consistent with this, *T. castaneum* exposed to aging cyfluthrin residues on concrete recovered from knockdown at 25, 30, and 35 °C, whereas survival remained low at 20 °C (Arthur, 1999). Gerken et al. (2025) confirmed this pattern for deltamethrin, where *T. castaneum* adults knocked down on treated concrete recovered far more readily at 27 and 32 °C than at 18 and 22 °C, and almost none recovered at the cooler temperatures when no food was available. In contrast, chlorfenapyr was more effective at 32 °C than at 27 °C (Arthur, 2013), and the efficacy of spinosad against *S. oryzae* and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), lesser grain borer, increased from 20 to 30 °C (Athanassiou et al., 2008). Temperature can also act on the residue itself. Deltamethrin deposits on concrete held in a grain bin that accumulated more hours above 32 °C produced slower knockdown than deposits held in a constant 27 °C chamber (Arthur, 2018). Yet methoprene residues were largely unaffected by temperature from 20 to 35 °C on concrete and varnished wood, and from 25 to 40 °C as aerosol deposits (Wijayaratne et al., 2012; Lanka et al.,

2019). The net effect of temperature therefore depends on the balance between insect-mediated and residue-mediated processes, which is likely to differ among active ingredients and surfaces.

The target species adds a further layer of complexity. *Tribolium castaneum* is a highly mobile secondary pest of milled products (Hagstrum and Subramanyam, 2009; Gerken et al., 2025) and is one of the species most frequently used in residual surface studies (Arthur, 2000a; Arthur et al., 2015; Gerken et al., 2025). In contrast, *S. oryzae* and *R. dominica* are primary pests whose immature stages develop inside whole kernels (Rees, 2004; Hagstrum and Subramanyam, 2009), and they differ from *T. castaneum* in thermal requirements (Stejskal et al., 2019) and insecticide susceptibility (Toews et al., 2003; Sehgal et al., 2014). Adults of all three species are rapidly knocked down by deltamethrin, but some individuals recover from knockdown at lower deposits (Paudyal et al., 2016). β-cyfluthrin applied to concrete was effective against *R. dominica* but failed to control field strains of *T. castaneum* even at four times the labeled rate (Sehgal et al., 2014), and *T. confusum* was among three of seven species that tolerated a β-cyfluthrin + imidacloprid formulation on concrete, with mortality of 57% or less after 7 d (Athanassiou et al., 2013). Exposure to a deltamethrin + methoprene + PBO combination reduced the movement of *R. dominica* more strongly than that of other grain beetles (Arthur et al., 2020), and the presence of food after exposure can allow knocked-down *T. castaneum* to recover and reproduce (Arthur, 2000a; Scheff and Arthur, 2018). Even a light dusting of flour allowed more than 90% of beetles to recover after deltamethrin exposure (Gerken et al., 2025), and flour can absorb β-cyfluthrin from treated concrete within 24 h (Arthur et al., 2015). Because species also differ in their activity thresholds and thermal optima (Stejskal et al., 2019), a treatment that performs well against one species at one temperature may fail against another species, or against the same species at a different temperature.

Despite this body of work, including studies on recovery after exposure (Gerken et al., 2025), most residual surface studies have tested one or two temperatures, one or two surfaces, a single species, or residual periods of 8-12 weeks. Lampiri et al. (2023) noted that because most studies examine only a small subset of these parameters, results are often not transferable among studies. In Chapter 2, residual efficacy of β-cyfluthrin and deltamethrin + (*S*)-methoprene + PBO (liquid combo) inside commercial shipping containers differed between wood and metal and across seasons, but temperature could not be separated from humidity, dust, light, and other uncontrolled field variables. To our knowledge, no study has crossed a realistic storage temperature gradient with the three dominant structural substrates, multiple stored-product beetle species, and a residual period long enough to follow efficacy to failure. Such data are needed to predict when residual treatments will lose effectiveness under the thermal conditions found in warehouses and transport units, and to set evidence-based re-treatment intervals.

Therefore, the objective of this study was to determine the effect of temperature on the initial and residual efficacy of two contact insecticides, Tempo® SC Ultra (β-cyfluthrin) and Gravista™ (liquid combo), applied to wood, metal, and concrete surfaces against adults of *T. castaneum*, *S. oryzae*, and *R. dominica*. Treated arenas were held at five constant temperatures (16, 22, 27, 32, and 37 °C) and bioassayed at 0, 4, 8, and 12 weeks after application, with an additional evaluation at 24 weeks for *T. castaneum*. We hypothesized that (1) efficacy would differ significantly with surface type and temperature, with the greatest and most persistent mortality on metal and the most rapid loss on concrete, (2) temperature and surface would interact, with porous substrates losing efficacy fastest at high temperatures, and (3) liquid combo would provide more consistent efficacy than β-cyfluthrin across surfaces, temperatures, and species.

3.2. Materials and Methods

3.2.1. Insects

Adults of *T. castaneum*, *S. oryzae*, and *R. dominica* were obtained from pesticide-susceptible laboratory colonies maintained at the USDA-ARS Center for Grain and Animal Health Research (CGAHR), Manhattan, KS, USA. Colonies were reared in environmental chambers at 27 ± 1 °C and 60-65% relative humidity (RH) in complete darkness on species-appropriate diets. *Tribolium castaneum* was reared on organic whole-wheat flour with 5% (w/w) brewer's yeast. *Rhyzopertha dominica* was reared on organic whole hard red winter wheat kernels. *Sitophilus oryzae* was reared on polished rice. Mixed-sex adults of mixed age were taken directly from the colonies for all bioassays.

3.2.2. Insecticides

Two commercial formulations labeled for structural surface treatment were evaluated. Tempo® SC Ultra (11.8% β-cyfluthrin active ingredient [a.i.], Bayer Environmental Science, Cary, NC, USA) was diluted at the label rate of 16 mL formulated product in 3.8 L of water to treat 93 m². Gravista™ (1.2% deltamethrin a.i., 2.85% (*S*)-methoprene a.i., and 33.3% piperonyl butoxide a.i., Central Life Sciences, Schaumburg, IL, USA), hereafter referred to as liquid combo, was diluted at 177 mL formulated product in 3.8 L of water to treat 93 m². Spray solutions were prepared in potable water immediately before application. Untreated control arenas received potable water only.

3.2.3. Preparation of Surfaces

Three structural substrates representative of storage and transport facilities were used, 1) plywood (6 mm thick), 2) galvanized steel (20 gauge), and 3) concrete. Plywood and steel were cut into discs sized to fit the base of a 100 × 20 mm plastic Petri dish (approximately 62 cm²)

with a circular saw and metal punch, respectively. Each disc was secured to the base of the Petri dish with a thin bead of adhesive caulk (DAP Products Inc., Baltimore, MD, USA) around the edge to secure the material to the bottom of the Petri dish. Concrete arenas were made by mixing cement (Rockite®, Hartline Products Co., Inc., Cleveland, OH, USA) with water at a 1:2 ratio, pouring the mixture directly into the base of the Petri dishes, and allowing it to cure for at least 2 weeks under ambient laboratory conditions. The inner wall of every dish was coated with polytetrafluoroethylene (Fluon®, Sigma-Aldrich, St. Louis, MO, USA) to prevent escape. The three types of arenas are shown in Fig. 3.1A.

Arenas were treated individually with an artist's airbrush inside a fume hood (Fig. 3.1B), applying the spray solution evenly across the entire surface at the volume corresponding to the label rate for the arena area. Control arenas were sprayed with potable water in the same manner using a separate airbrush. All arenas were allowed to dry for 24 h at ambient conditions before the first bioassay.

3.2.4. Experimental Design

The experiment was a full factorial of three surfaces (plywood, steel, concrete) × three treatments (β-cyfluthrin, liquid combo, untreated control) × five temperatures (16, 22, 27, 32, and 37 °C) × three species (*T. castaneum*, *R. dominica*, *S. oryzae*), with five replicate arenas per combination, giving 225 arenas per surface and 675 arenas in total. Each arena was assigned to one species and one temperature for the duration of the study. Arenas were held in five environmental chambers set at the assigned temperature and 65% RH. Temperature and RH in each chamber were recorded continuously with HOBO® data loggers (Onset Computer Corp., Bourne, MA, USA).

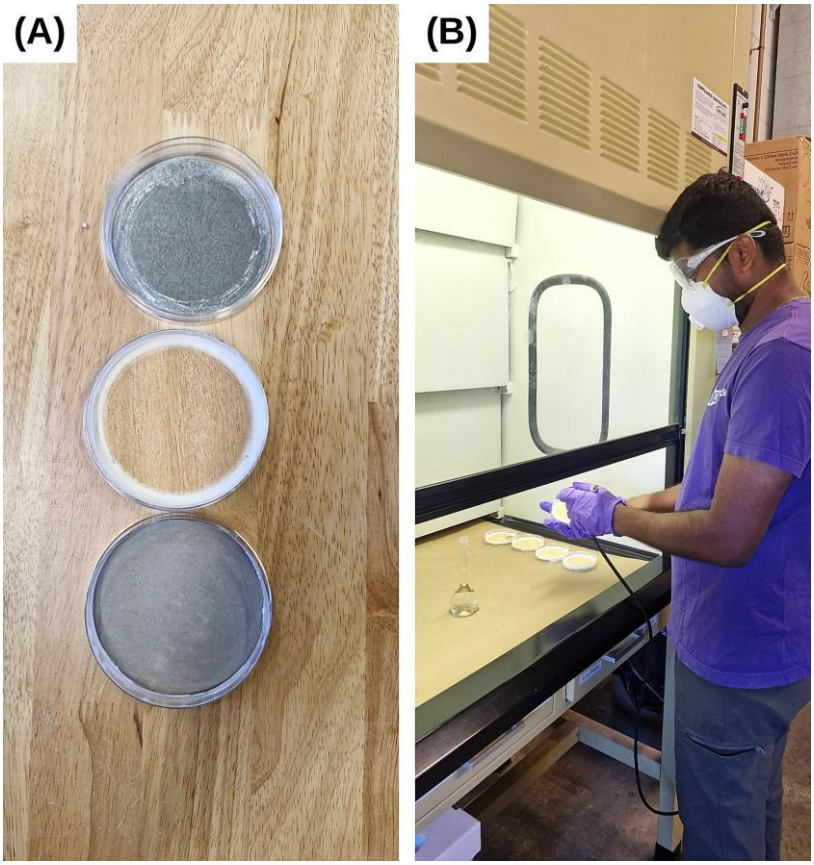


Figure 3.1. Bioassay arenas and application of residual insecticides. (A) Arenas of the three structural surfaces evaluated, galvanized steel (top), plywood (middle), and concrete (bottom), each prepared in the base of a 100 × 20 mm Petri dish with the inner wall coated with Fluon® to prevent escape. (B) Application of liquid combo, β-cyfluthrin, or potable water (control) at the label rate with an artist's airbrush inside a fume hood. Arenas were dried for 24 h before the first bioassay.

3.2.5. Bioassay Procedure

For each bioassay, 10 mixed-sex adults of the assigned species were introduced into each arena together with 500 mg of their rearing diet. Adults were assessed 1, 3, and 7 d after introduction and classified as alive (normal, coordinated movement), affected (lying on the dorsum, unable to walk, or twitching abnormally), or dead (no movement in response to gentle probing with a fine brush) (Scheff et al., 2019). After the 7 d assessment, all adults and diet were removed and the arenas were returned to their respective chambers.

Residual efficacy was evaluated by repeating the bioassay on the same arenas with new adults at 0, 4, 8, and 12 weeks after application for all three species, with an additional evaluation at 24 weeks for *T. castaneum*. Arenas were held at their assigned temperature between bioassays, so that treated surfaces aged under the same thermal conditions in which insects were exposed.

3.2.6. Statistical Analysis

For each arena, the number of dead adults after 24 h and 168 h of exposure was expressed as percentage mortality, and means ± standard error (SE) were calculated separately for each species. Mortality in the untreated control arenas remained below 5% throughout the study, therefore no correction for control mortality was applied (Abbott, 1925), and control arenas were excluded from the statistical analyses so that the two insecticides could be compared directly. Percentage mortality data were transformed to angular values before analysis to stabilize variances (Zar, 2010), and untransformed means ± SE are presented. Normality of residuals and homogeneity of variances were evaluated with the Shapiro-Wilk test and Levene's test, respectively. Residuals departed from normality in most data sets, and variances were heterogeneous in some. Because the design was balanced with equal replication, analysis of variance (ANOVA) was retained, as it is robust to moderate departures from these assumptions.

Transformed mortality data were analyzed separately for each species, surface, and exposure interval (24 h and 168 h) using a three-way ANOVA with insecticide (liquid combo or β-cyfluthrin), residual week, and temperature as fixed factors, together with all two- and three-way interactions. Type III sums of squares with sum-to-zero contrasts were computed with the car package (Fox and Weisberg, 2019). Data sets in which every arena had the same mortality (0% or 100%) contained no variation and were not analyzed. Because the three-way interaction

was significant in most analyses, means were compared within each temperature using Tukey's honestly significant difference (HSD) test with the emmeans package (Lenth, 2023) at $\alpha = 0.05$. Residual weeks were compared within each insecticide, and the two insecticides were compared within each residual week. All analyses were conducted in R (version 4.4.1, R Core Team, 2024).

3.3. Results

Overall, mortality depended on the surface, the insecticide, the temperature, and the species. Metal was the surface with the greatest and most persistent mortality, and *T. castaneum* was the least susceptible species. Liquid combo was generally the more effective insecticide on metal and, against *T. castaneum*, on wood, whereas β-cyfluthrin was generally the more effective insecticide on concrete. The results for each species and surface are presented below.

3.3.1. *Tribolium castaneum*

Tribolium castaneum was the least susceptible of the three species tested. Mortality after 24 h did not exceed 24.0% on any surface, and mortality after 168 h was generally greatest on metal. Liquid combo was the more effective insecticide on wood and metal, whereas β-cyfluthrin was the more effective insecticide on concrete. On wood and concrete, mortality was greatest and persisted longest at 37 °C. No mortality occurred on concrete from week 12 onward, and mortality was ≤4.0% on all three surfaces at week 24 (Tables 3.1-3.3).

3.3.1.1. Wood Bioassay Arenas

Mortality of *T. castaneum* after 24 h on wood was significantly affected by residual week ($F = 18.41$; df = 4, 200; $P < 0.001$), temperature ($F = 2.62$; df = 4, 200; $P = 0.036$), the insecticide × temperature interaction ($F = 4.75$; df = 4, 200; $P = 0.001$), the residual week × temperature interaction ($F = 2.62$; df = 16, 200; $P < 0.001$), and the insecticide × residual week ×

temperature interaction ($F = 4.75$; df = 16, 200; $P < 0.001$), but not by insecticide ($F = 0.61$; df = 1, 200; $P = 0.435$) and the insecticide × residual week interaction ($F = 0.61$; df = 4, 200; $P = 0.654$) (Table 3.1). Mortality after 24 h was low for both insecticides and it occurred only at week 0, where it did not exceed 12.0% (liquid combo at 37 °C), and no mortality was recorded from week 4 onward at any temperature. Liquid combo and β-cyfluthrin did not differ at any temperature or residual week.

Mortality of *T. castaneum* after 168 h on wood was significantly affected by insecticide ($F = 150.92$; df = 1, 200; $P < 0.001$), residual week ($F = 347.20$; df = 4, 200; $P < 0.001$), temperature ($F = 118.96$; df = 4, 200; $P < 0.001$), the insecticide × residual week interaction ($F = 12.69$; df = 4, 200; $P < 0.001$), the insecticide × temperature interaction ($F = 9.56$; df = 4, 200; $P < 0.001$), the residual week × temperature interaction ($F = 13.30$; df = 16, 200; $P < 0.001$), and the insecticide × residual week × temperature interaction ($F = 4.83$; df = 16, 200; $P < 0.001$) (Table 3.1). At week 0, mortality after 168 h increased with temperature for β-cyfluthrin, from 24.0% at 16 °C to 100.0% at 32 and 37 °C, whereas liquid combo caused 62.0-70.0% mortality at 16-22 °C and 94.0-100.0% at 27-37 °C. By week 4, mortality had declined to ≤44.0% for both insecticides at 16-27 °C and to 12.0% for β-cyfluthrin at 32 °C, whereas liquid combo caused 56.0% mortality at 32 °C and 100.0% at 37 °C. At 37 °C, liquid combo mortality remained 70.0-84.0% at weeks 8 and 12, compared with 12.0-32.0% for β-cyfluthrin. No mortality occurred on wood at week 24 for either insecticide at any temperature. Liquid combo caused greater mortality than β-cyfluthrin at weeks 0 and 8 at 16 °C, at week 12 at 27 °C, and at weeks 4-12 at 32 and 37 °C. β-cyfluthrin did not cause greater mortality than liquid combo at any temperature or residual week.

3.3.1.2. Metal Bioassay Arenas

Mortality of *T. castaneum* after 24 h on metal was significantly affected by insecticide (F = 14.72; df = 1, 200; $P < 0.001$), residual week (F = 41.97; df = 4, 200; $P < 0.001$), the insecticide × residual week interaction (F = 19.80; df = 4, 200; $P < 0.001$), the insecticide × temperature interaction (F = 3.40; df = 4, 200; P = 0.010), and the insecticide × residual week × temperature interaction (F = 1.82; df = 16, 200; P = 0.030), but not by temperature (F = 0.49; df = 4, 200; P = 0.742) and the residual week × temperature interaction (F = 1.24; df = 16, 200; P = 0.242) (Table 3.2). At week 0, liquid combo caused 4.0-24.0% mortality after 24 h and β-cyfluthrin caused 0-6.0%, and liquid combo caused greater mortality than β-cyfluthrin at 16, 22, and 37 °C. From week 4 onward, 24 h mortality was ≤5.2% for both insecticides at every temperature.

Mortality of *T. castaneum* after 168 h on metal was significantly affected by insecticide (F = 63.74; df = 1, 200; $P < 0.001$), residual week (F = 675.13; df = 4, 200; $P < 0.001$), temperature (F = 44.98; df = 4, 200; $P < 0.001$), the insecticide × residual week interaction (F = 7.53; df = 4, 200; $P < 0.001$), the insecticide × temperature interaction (F = 2.77; df = 4, 200; P = 0.028), the residual week × temperature interaction (F = 11.37; df = 16, 200; $P < 0.001$), and the insecticide × residual week × temperature interaction (F = 3.63; df = 16, 200; $P < 0.001$) (Table 3.2). At week 0, liquid combo caused 100.0% mortality after 168 h at every temperature, and β-cyfluthrin caused 80.0-100.0% mortality, which was lower than liquid combo at 22 and 27 °C. Residual efficacy on metal was longest at 16 °C, where liquid combo caused ≥98.0% mortality through week 8 and 42.7% at week 12, compared with 90.0, 48.7, and 16.0% for β-cyfluthrin at weeks 4, 8, and 12, respectively. Liquid combo caused greater mortality than β-cyfluthrin at each of these weeks. At 22-32 °C, mortality declined to ≤36.9% by week 8 and ≤36.0% by week 12

for both insecticides, and liquid combo exceeded β-cyfluthrin only at week 0 at 22 and 27 °C and at week 4 at 27 °C. At 37 °C, liquid combo caused ≥98.0% mortality through week 8 and greater mortality than β-cyfluthrin at weeks 4 and 8. By week 24, mortality on metal was ≤4.0% at every temperature.

3.3.1.3. Concrete Bioassay Arenas

No *T. castaneum* mortality occurred after 24 h on concrete for either insecticide at any temperature or residual week, therefore these data were not analyzed and comparisons were done among the insecticide treatments only (Table 3.3).

Mortality of *T. castaneum* after 168 h on concrete was significantly affected by insecticide ($F = 141.29$; df = 1, 200; $P < 0.001$), residual week ($F = 400.79$; df = 4, 200; $P < 0.001$), temperature ($F = 243.71$; df = 4, 200; $P < 0.001$), the insecticide × residual week interaction ($F = 24.80$; df = 4, 200; $P < 0.001$), the insecticide × temperature interaction ($F = 2.81$; df = 4, 200; $P = 0.027$), the residual week × temperature interaction ($F = 47.06$; df = 16, 200; $P < 0.001$), and the insecticide × residual week × temperature interaction ($F = 8.94$; df = 16, 200; $P < 0.001$) (Table 3.3). At week 0, mortality after 168 h was lowest at 16-22 °C (2.0-6.0% for liquid combo and 39.3-52.0% for β-cyfluthrin) and reached 100.0% for both insecticides at 37 °C. β-cyfluthrin caused greater mortality than liquid combo at week 0 at 16-32 °C, at week 4 at 22 and 27 °C, and at week 8 at 27 and 37 °C. Liquid combo did not cause greater mortality than β-cyfluthrin at any temperature or residual week. Mortality declined rapidly. By week 4, it was ≤42.0% for both insecticides at 16-32 °C, and from week 12 onward no mortality occurred on concrete at any temperature. At 37 °C, β-cyfluthrin caused 94.0-100.0% mortality through week 8, whereas liquid combo mortality declined to 34.0% at week 8.

Table 3.1. Mean (± SE) percent mortality of *Tribolium castaneum* adults exposed for 24 h and 168 h on wood arenas treated with liquid combo or β-cyfluthrin and held at 16, 22, 27, 32, or 37 °C, for 0, 4, 8, 12, and 24 weeks after application.

Temperature (°C)	Week	Mean (± SE) percent mortality[a,b]			
		24 h		168 h	
		Liquid combo[c]	β-cyfluthrin[d]	Liquid combo[c]	β-cyfluthrin[d]
16	0	2.0 ± 2.0 Aa	9.8 ± 3.2 Aa	70.0 ± 7.1 Aa	24.0 ± 6.8 Ab
	4	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba	8.0 ± 5.8 Ba	2.0 ± 2.0 Ba
	8	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba	20.0 ± 3.2 Ba	0.0 ± 0.0 Bb
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba	0.0 ± 0.0 Ba	0.0 ± 0.0 Ba
	24	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba	0.0 ± 0.0 Ba	0.0 ± 0.0 Ba
22	0	0.0 ± 0.0 Aa	2.0 ± 2.0 Aa	62.0 ± 5.8 Aa	52.0 ± 9.2 Aa
	4	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	10.0 ± 4.5 Ba	2.0 ± 2.0 Ba
	8	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	0.0 ± 0.0 Ca	0.0 ± 0.0 Ba
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	0.0 ± 0.0 Ca	0.0 ± 0.0 Ba
	24	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	0.0 ± 0.0 Ca	0.0 ± 0.0 Ba
27	0	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	94.0 ± 4.0 Aa	76.0 ± 9.3 Aa
	4	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	44.0 ± 9.3 Ba	24.0 ± 10.3 Ba
	8	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	12.0 ± 5.8 Ba	0.0 ± 0.0 Ba
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	22.0 ± 3.7 Ba	6.0 ± 4.0 Bb
	24	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba	0.0 ± 0.0 Ba
32	0	6.6 ± 4.6 Aa	0.0 ± 0.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	56.0 ± 9.3 Ba	12.0 ± 5.8 Bb
	8	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	40.0 ± 10.5 Ba	0.0 ± 0.0 Cb
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	36.0 ± 5.1 Ba	0.0 ± 0.0 Cb
	24	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	0.0 ± 0.0 Ca	0.0 ± 0.0 Ca
37	0	12.0 ± 5.8 Aa	0.0 ± 0.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	100.0 ± 0.0 Aa	44.0 ± 15.7 Bb
	8	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	84.0 ± 5.1 Ba	32.0 ± 10.7 Bb
	12	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	70.0 ± 4.5 Ba	12.0 ± 7.4 Bb
	24	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	0.0 ± 0.0 Ca	0.0 ± 0.0 Ba

[a] Within each temperature and column, means followed by different uppercase letters are significantly different among residual weeks (Tukey's HSD, $P < 0.05$).

[b] Within each temperature, residual week, and exposure time (24 h or 168 h), means followed by different lowercase letters are significantly different between insecticides (Tukey's HSD, $P < 0.05$).

[c] Liquid combo = deltamethrin + (*S*)-methoprene + piperonyl butoxide (Gravista™).

[d] β-cyfluthrin = Tempo® SC Ultra.

Table 3.2. Mean (± SE) percent mortality of *Tribolium castaneum* adults exposed for 24 h and 168 h on metal arenas treated with liquid combo or β-cyfluthrin and held at 16, 22, 27, 32, or 37 °C, for 0, 4, 8, 12, and 24 weeks after application.

Temperature (°C)	Week	Mean (± SE) percent mortality[a,b]			
		24 h		168 h	
		Liquid combo[c]	β-cyfluthrin[d]	Liquid combo[c]	β-cyfluthrin[d]
16	0	24.0 ± 6.8 Aa	2.0 ± 2.0 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	100.0 ± 0.0 Aa	90.0 ± 4.5 Ab
	8	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	98.0 ± 2.0 Aa	48.7 ± 11.6 Bb
	12	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	42.7 ± 7.1 Ba	16.0 ± 5.1 Cb
	24	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	4.0 ± 2.5 Ca	0.0 ± 0.0 Da
22	0	16.0 ± 5.1 Aa	2.0 ± 2.0 Ab	100.0 ± 0.0 Aa	80.0 ± 7.1 Ab
	4	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	80.0 ± 7.1 Ba	68.0 ± 3.7 Aa
	8	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	14.0 ± 5.1 Ca	6.0 ± 4.0 Ba
	12	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	0.0 ± 0.0 Da	0.0 ± 0.0 Ba
	24	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	0.0 ± 0.0 Da	0.0 ± 0.0 Ba
27	0	4.0 ± 4.0 Aa	6.0 ± 4.0 Aa	100.0 ± 0.0 Aa	84.0 ± 7.5 Ab
	4	0.0 ± 0.0 Aa	5.2 ± 3.4 Aa	88.0 ± 5.8 Ba	68.0 ± 5.8 Ab
	8	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	34.0 ± 5.1 Ca	30.0 ± 7.1 Aa
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	26.6 ± 3.8 Ca	36.0 ± 7.5 Aa
	24	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	0.0 ± 0.0 Da	0.0 ± 0.0 Ba
32	0	14.0 ± 6.8 Aa	0.0 ± 0.0 Aa	100.0 ± 0.0 Aa	92.0 ± 5.8 Aa
	4	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	100.0 ± 0.0 Aa	96.0 ± 2.5 Aa
	8	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	36.9 ± 9.7 Ba	28.0 ± 3.7 Ba
	12	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	15.5 ± 4.7 Ba	8.0 ± 3.7 Ba
	24	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	0.0 ± 0.0 Ca	2.0 ± 2.0 Ba
37	0	24.0 ± 6.8 Aa	2.0 ± 2.0 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	100.0 ± 0.0 Aa	90.0 ± 4.5 Ab
	8	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	98.0 ± 2.0 Aa	48.7 ± 11.6 Bb
	12	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	16.0 ± 7.5 Ba	8.2 ± 3.8 Ca
	24	0.0 ± 0.0 Ba	0.0 ± 0.0 Aa	0.0 ± 0.0 Ca	2.0 ± 2.0 Ca

[a] Within each temperature and column, means followed by different uppercase letters are significantly different among residual weeks (Tukey's HSD, $P < 0.05$).

[b] Within each temperature, residual week, and exposure time (24 h or 168 h), means followed by different lowercase letters are significantly different between insecticides (Tukey's HSD, $P < 0.05$).

[c] Liquid combo = deltamethrin + (*S*)-methoprene + piperonyl butoxide (Gravista™).

[d] β-cyfluthrin = Tempo® SC Ultra.

Table 3.3. Mean (± SE) percent mortality of *Tribolium castaneum* adults exposed for 24 h and 168 h on concrete arenas treated with liquid combo or β-cyfluthrin and held at 16, 22, 27, 32, or 37 °C, for 0, 4, 8, 12, and 24 weeks after application.

Temperature (°C)	Week	Mean (± SE) percent mortality[a,b]			
		24 h[e]		168 h	
		Liquid combo[c]	β-cyfluthrin[d]	Liquid combo[c]	β-cyfluthrin[d]
16	0	0.0 ± 0.0	0.0 ± 0.0	6.0 ± 4.0 Ab	39.3 ± 7.1 Aa
	4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Aa	6.0 ± 4.0 Ba
	8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba
	12	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba
	24	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba
22	0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 2.0 Ab	52.0 ± 4.9 Aa
	4	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 2.0 Ab	32.0 ± 5.8 Ba
	8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Aa	0.0 ± 0.0 Ca
	12	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Aa	0.0 ± 0.0 Ca
	24	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Aa	0.0 ± 0.0 Ca
27	0	0.0 ± 0.0	0.0 ± 0.0	28.0 ± 5.8 Ab	45.0 ± 2.1 Aa
	4	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 3.3 Bb	42.0 ± 3.7 Aa
	8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Bb	14.0 ± 5.1 Ba
	12	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Ba	0.0 ± 0.0 Ca
	24	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Ba	0.0 ± 0.0 Ca
32	0	0.0 ± 0.0	0.0 ± 0.0	68.0 ± 6.6 Ab	92.0 ± 4.9 Aa
	4	0.0 ± 0.0	0.0 ± 0.0	16.4 ± 5.2 Ba	20.0 ± 7.1 Ba
	8	0.0 ± 0.0	0.0 ± 0.0	18.0 ± 8.0 Ba	24.0 ± 5.1 Ba
	12	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Ba	0.0 ± 0.0 Ca
	24	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Ba	0.0 ± 0.0 Ca
37	0	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0	0.0 ± 0.0	84.0 ± 8.1 Ba	100.0 ± 0.0 Aa
	8	0.0 ± 0.0	0.0 ± 0.0	34.0 ± 4.0 Cb	94.0 ± 4.0 Aa
	12	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Da	0.0 ± 0.0 Ba
	24	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 Da	0.0 ± 0.0 Ba

[a] Within each temperature and column, means followed by different uppercase letters are significantly different among residual weeks (Tukey's HSD, $P < 0.05$).

[b] Within each temperature, residual week, and exposure time (24 h or 168 h), means followed by different lowercase letters are significantly different between insecticides (Tukey's HSD, $P < 0.05$).

[c] Liquid combo = deltamethrin + (*S*)-methoprene + piperonyl butoxide (Gravista™).

[d] β-cyfluthrin = Tempo® SC Ultra.

[e] No mortality occurred after 24 h, therefore these data were not analyzed.

3.3.2. *Sitophilus oryzae*

Sitophilus oryzae was highly susceptible after 168 h of exposure on wood and metal, where mortality was ≥86.0% for both insecticides at every temperature and residual week, and it

was 100.0% on metal. On concrete, β-cyfluthrin was generally the more effective insecticide after both 24 h and 168 h. After 24 h, liquid combo was the more effective insecticide on metal, and mortality generally increased with temperature on all three surfaces (Tables 3.4-3.6).

3.3.2.1. Wood Bioassay Arenas

Mortality of *S. oryzae* after 24 h on wood was significantly affected by residual week ($F = 1029.83$; df = 3, 160; $P < 0.001$), temperature ($F = 232.92$; df = 4, 160; $P < 0.001$), the insecticide × residual week interaction ($F = 2.79$; df = 3, 160; $P = 0.042$), the insecticide × temperature interaction ($F = 11.39$; df = 4, 160; $P < 0.001$), the residual week × temperature interaction ($F = 80.28$; df = 12, 160; $P < 0.001$), and the insecticide × residual week × temperature interaction ($F = 14.88$; df = 12, 160; $P < 0.001$), but not by insecticide ($F = 2.12$; df = 1, 160; $P = 0.147$) (Table 3.4). At weeks 0 and 4, mortality after 24 h increased with temperature for both insecticides, from ≤18.0% at 16 °C to 74.0-100.0% at 37 °C, but no mortality occurred at weeks 8 and 12 at any temperature. β-cyfluthrin caused greater mortality than liquid combo at week 4 at 16 and 22 °C and at week 0 at 27 and 37 °C, whereas liquid combo caused greater mortality than β-cyfluthrin at week 0 at 22 °C and at week 4 at 32 °C.

Table 3.4. Mean (± SE) percent mortality of *Sitophilus oryzae* adults exposed for 24 h and 168 h on wood arenas treated with liquid combo or β-cyfluthrin and held at 16, 22, 27, 32, or 37 °C, for 0, 4, 8, and 12 weeks after application.

Temperature (°C)	Week	Mean (± SE) percent mortality[a,b]			
		24 h		168 h	
		Liquid combo[c]	β-cyfluthrin[d]	Liquid combo[c]	β-cyfluthrin[d]
16	0	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba	98.0 ± 2.0 Aa	96.0 ± 2.5 Aa
	4	6.0 ± 4.0 Ab	18.0 ± 3.7 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	8	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba	100.0 ± 0.0 Aa	94.0 ± 4.0 Aa
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba	100.0 ± 0.0 Aa	94.0 ± 4.0 Aa
22	0	26.0 ± 6.0 Aa	6.0 ± 4.0 Bb	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	20.0 ± 3.2 Ab	42.0 ± 3.7 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	8	0.0 ± 0.0 Ba	0.0 ± 0.0 Ba	100.0 ± 0.0 Aa	86.0 ± 6.0 Bb
	12	0.0 ± 0.0 Ba	0.0 ± 0.0 Ba	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
27	0	54.0 ± 6.0 Ab	80.0 ± 7.1 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	66.0 ± 2.5 Aa	72.0 ± 3.7 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	8	0.0 ± 0.0 Ba	0.0 ± 0.0 Ba	100.0 ± 0.0 Aa	92.0 ± 4.9 Aa
	12	0.0 ± 0.0 Ba	0.0 ± 0.0 Ba	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
32	0	52.0 ± 5.8 Ba	56.0 ± 6.8 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	94.0 ± 4.0 Aa	50.0 ± 8.9 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	8	0.0 ± 0.0 Ca	0.0 ± 0.0 Ba	100.0 ± 0.0 Aa	92.0 ± 4.9 Aa
	12	0.0 ± 0.0 Ca	0.0 ± 0.0 Ba	100.0 ± 0.0 Aa	86.0 ± 4.0 Bb
37	0	74.0 ± 5.1 Bb	98.0 ± 2.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	8	0.0 ± 0.0 Ca	0.0 ± 0.0 Ba	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	12	0.0 ± 0.0 Ca	0.0 ± 0.0 Ba	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa

[a] Within each temperature and column, means followed by different uppercase letters are significantly different among residual weeks (Tukey's HSD, $P < 0.05$).

[b] Within each temperature, residual week, and exposure time (24 h or 168 h), means followed by different lowercase letters are significantly different between insecticides (Tukey's HSD, $P < 0.05$).

[c] Liquid combo = deltamethrin + (*S*)-methoprene + piperonyl butoxide (Gravista™).

[d] β-cyfluthrin = Tempo® SC Ultra.

Table 3.5. Mean (± SE) percent mortality of *Sitophilus oryzae* adults exposed for 24 h and 168 h on metal arenas treated with liquid combo or β-cyfluthrin and held at 16, 22, 27, 32, or 37 °C, for 0, 4, 8, and 12 weeks after application.

Temperature (°C)	Week	Mean (± SE) percent mortality[a,b]			
		24 h		168 h[e]	
		Liquid combo[c]	β-cyfluthrin[d]	Liquid combo[c]	β-cyfluthrin[d]
16	0	12.0 ± 5.8 Aa	0.0 ± 0.0 Aa	100.0 ± 0.0	100.0 ± 0.0
	4	8.0 ± 3.7 Ba	0.0 ± 0.0 Ab	100.0 ± 0.0	100.0 ± 0.0
	8	36.0 ± 5.1 Aa	0.0 ± 0.0 Ab	100.0 ± 0.0	100.0 ± 0.0
	12	4.0 ± 4.0 Ba	0.0 ± 0.0 Aa	100.0 ± 0.0	100.0 ± 0.0
22	0	26.0 ± 6.0 Ba	6.0 ± 4.0 Ab	100.0 ± 0.0	100.0 ± 0.0
	4	32.0 ± 3.7 Aa	12.0 ± 7.4 Ab	100.0 ± 0.0	100.0 ± 0.0
	8	58.0 ± 3.7 Aa	18.0 ± 3.7 Ab	100.0 ± 0.0	100.0 ± 0.0
	12	16.0 ± 6.8 Ba	0.0 ± 0.0 Bb	100.0 ± 0.0	100.0 ± 0.0
27	0	66.0 ± 8.1 Aa	38.0 ± 3.7 Ab	100.0 ± 0.0	100.0 ± 0.0
	4	48.0 ± 5.8 Ba	6.0 ± 4.0 Bb	100.0 ± 0.0	100.0 ± 0.0
	8	78.0 ± 3.7 Aa	36.0 ± 5.1 Ab	100.0 ± 0.0	100.0 ± 0.0
	12	40.0 ± 3.2 Ba	6.0 ± 4.0 Bb	100.0 ± 0.0	100.0 ± 0.0
32	0	30.0 ± 4.5 Ca	36.0 ± 5.1 Ba	100.0 ± 0.0	100.0 ± 0.0
	4	100.0 ± 0.0 Aa	76.0 ± 4.0 Ab	100.0 ± 0.0	100.0 ± 0.0
	8	100.0 ± 0.0 Aa	62.0 ± 3.7 Ab	100.0 ± 0.0	100.0 ± 0.0
	12	62.0 ± 3.7 Ba	30.0 ± 7.1 Bb	100.0 ± 0.0	100.0 ± 0.0
37	0	82.0 ± 3.7 Ba	62.0 ± 3.7 Bb	100.0 ± 0.0	100.0 ± 0.0
	4	100.0 ± 0.0 Aa	92.0 ± 3.7 Ab	100.0 ± 0.0	100.0 ± 0.0
	8	100.0 ± 0.0 Aa	78.0 ± 3.7 Ab	100.0 ± 0.0	100.0 ± 0.0
	12	100.0 ± 0.0 Aa	86.0 ± 6.0 Ab	100.0 ± 0.0	100.0 ± 0.0

[a] Within each temperature and column, means followed by different uppercase letters are significantly different among residual weeks (Tukey's HSD, $P < 0.05$).

[b] Within each temperature, residual week, and exposure time (24 h or 168 h), means followed by different lowercase letters are significantly different between insecticides (Tukey's HSD, $P < 0.05$).

[c] Liquid combo = deltamethrin + (*S*)-methoprene + piperonyl butoxide (Gravista™).

[d] β-cyfluthrin = Tempo® SC Ultra.

[e] All adults were dead after 168 h, therefore these data were not analyzed.

Table 3.6. Mean (± SE) percent mortality of *Sitophilus oryzae* adults exposed for 24 h and 168 h on concrete arenas treated with liquid combo or β-cyfluthrin and held at 16, 22, 27, 32, or 37 °C, for 0, 4, 8, and 12 weeks after application.

Temperature (°C)	Week	Mean (± SE) percent mortality[a,b]			
		24 h		168 h	
		Liquid combo[c]	β-cyfluthrin[d]	Liquid combo[c]	β-cyfluthrin[d]
16	0	0.0 ± 0.0 Aa	4.0 ± 2.5 Ba	78.0 ± 5.8 Ab	98.0 ± 2.0 Aa
	4	0.0 ± 0.0 Ab	28.0 ± 3.7 Aa	78.0 ± 3.7 Ab	100.0 ± 0.0 Aa
	8	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba	48.0 ± 3.7 Bb	100.0 ± 0.0 Aa
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba	60.0 ± 3.2 Bb	100.0 ± 0.0 Aa
22	0	0.0 ± 0.0 Ab	18.0 ± 3.7 Aa	70.0 ± 5.5 Ab	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Ab	30.0 ± 3.2 Aa	78.0 ± 7.4 Ab	100.0 ± 0.0 Aa
	8	0.0 ± 0.0 Ab	22.0 ± 3.7 Aa	72.0 ± 5.8 Ab	96.0 ± 2.5 Aa
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba	70.0 ± 10.5 Ab	100.0 ± 0.0 Aa
27	0	0.0 ± 0.0 Ab	48.0 ± 3.7 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Ab	48.0 ± 3.7 Aa	78.0 ± 8.6 Bb	100.0 ± 0.0 Aa
	8	0.0 ± 0.0 Ab	42.0 ± 3.7 Aa	66.0 ± 7.5 Bb	100.0 ± 0.0 Aa
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Ba	68.0 ± 7.4 Bb	100.0 ± 0.0 Aa
32	0	2.0 ± 2.0 Ab	62.0 ± 3.7 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Ab	72.0 ± 3.7 Aa	100.0 ± 0.0 Aa	62.0 ± 7.4 Bb
	8	0.0 ± 0.0 Ab	68.0 ± 3.7 Aa	72.0 ± 8.6 Ba	74.0 ± 5.1 Ba
	12	0.0 ± 0.0 Ab	44.0 ± 7.5 Ba	54.0 ± 7.5 Bb	100.0 ± 0.0 Aa
37	0	8.0 ± 3.7 Ab	78.0 ± 3.7 Ba	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Bb	94.0 ± 4.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	8	0.0 ± 0.0 Bb	82.0 ± 3.7 Aa	84.0 ± 2.5 Ba	88.0 ± 3.7 Ba
	12	0.0 ± 0.0 Bb	78.0 ± 5.8 Ba	68.0 ± 8.6 Bb	100.0 ± 0.0 Aa

[a] Within each temperature and column, means followed by different uppercase letters are significantly different among residual weeks (Tukey's HSD, $P < 0.05$).

[b] Within each temperature, residual week, and exposure time (24 h or 168 h), means followed by different lowercase letters are significantly different between insecticides (Tukey's HSD, $P < 0.05$).

[c] Liquid combo = deltamethrin + (*S*)-methoprene + piperonyl butoxide (Gravista™).

[d] β-cyfluthrin = Tempo® SC Ultra.

Mortality of *S. oryzae* after 168 h on wood was significantly affected by insecticide (F = 24.47; df = 1, 160; $P < 0.001$), residual week (F = 6.65; df = 3, 160; $P < 0.001$), temperature (F = 3.12; df = 4, 160; $P = 0.017$), the insecticide × residual week interaction (F = 7.67; df = 3, 160; $P < 0.001$), the insecticide × temperature interaction (F = 2.51; df = 4, 160; $P = 0.044$), the residual week × temperature interaction (F = 2.50; df = 12, 160; $P = 0.005$), and the insecticide × residual week × temperature interaction (F = 2.15; df = 12, 160; $P = 0.017$) (Table 3.4). Mortality after

168 h was ≥86.0% for both insecticides at every temperature and residual week. Liquid combo caused 98.0-100.0% mortality and β-cyfluthrin caused 86.0-100.0%. Liquid combo caused greater mortality than β-cyfluthrin at week 8 at 22 °C and at week 12 at 32 °C, when β-cyfluthrin mortality was 86.0%.

3.3.2.2. Metal Bioassay Arenas

Mortality of *S. oryzae* after 24 h on metal was significantly affected by insecticide (F = 306.23; df = 1, 160; $P < 0.001$), residual week (F = 51.90; df = 3, 160; $P < 0.001$), temperature (F = 384.89; df = 4, 160; $P < 0.001$), the insecticide × residual week interaction (F = 9.79; df = 3, 160; $P < 0.001$), the residual week × temperature interaction (F = 17.96; df = 12, 160; $P < 0.001$), and the insecticide × residual week × temperature interaction (F = 2.87; df = 12, 160; P = 0.001), but not by the insecticide × temperature interaction (F = 2.05; df = 4, 160; P = 0.090) (Table 3.5). Mortality after 24 h increased with temperature for both insecticides. At 16 °C, liquid combo caused 4.0-36.0% mortality and β-cyfluthrin caused none, whereas at 37 °C liquid combo caused 82.0-100.0% and β-cyfluthrin 62.0-92.0%. Liquid combo caused greater mortality than β-cyfluthrin in 17 of the 20 temperature × residual week combinations and did not cause lower mortality in any combination.

All *S. oryzae* adults exposed on metal were dead after 168 h for both insecticides at every temperature and residual week (100.0% mortality, Table 3.5).

3.3.2.3. Concrete Bioassay Arenas

Mortality of *S. oryzae* after 24 h on concrete was significantly affected by insecticide (F = 2521.42; df = 1, 160; $P < 0.001$), residual week (F = 72.14; df = 3, 160; $P < 0.001$), temperature (F = 238.62; df = 4, 160; $P < 0.001$), the insecticide × residual week interaction (F = 68.93; df = 3, 160; $P < 0.001$), the insecticide × temperature interaction (F = 193.87; df = 4, 160;

$P < 0.001$), the residual week × temperature interaction ($F = 7.38$; df = 12, 160; $P < 0.001$), and the insecticide × residual week × temperature interaction ($F = 10.45$; df = 12, 160; $P < 0.001$) (Table 3.6). Liquid combo caused ≤8.0% mortality after 24 h at every temperature and residual week, whereas β-cyfluthrin caused 0-94.0%. β-cyfluthrin caused greater mortality than liquid combo in 15 of the 20 temperature × residual week combinations. In the remaining five (weeks 0, 8, and 12 at 16 °C and week 12 at 22 and 27 °C), neither insecticide caused more than 4.0% mortality. β-cyfluthrin mortality increased with temperature, reaching 44.0-94.0% at 32-37 °C, and declined to 0% by week 12 at 16-27 °C.

Mortality of *S. oryzae* after 168 h on concrete was significantly affected by insecticide ($F = 353.89$; df = 1, 160; $P < 0.001$), residual week ($F = 46.41$; df = 3, 160; $P < 0.001$), temperature ($F = 10.96$; df = 4, 160; $P < 0.001$), the insecticide × residual week interaction ($F = 38.38$; df = 3, 160; $P < 0.001$), the insecticide × temperature interaction ($F = 34.23$; df = 4, 160; $P < 0.001$), the residual week × temperature interaction ($F = 6.06$; df = 12, 160; $P < 0.001$), and the insecticide × residual week × temperature interaction ($F = 10.70$; df = 12, 160; $P < 0.001$) (Table 3.6). β-cyfluthrin caused 96.0-100.0% mortality after 168 h at every temperature and residual week, except at weeks 4 (62.0%) and 8 (74.0%) at 32 °C and week 8 (88.0%) at 37 °C. Liquid combo caused 48.0-78.0% mortality at 16 and 22 °C at every residual week. At 27-37 °C, liquid combo caused 100.0% mortality at week 0, which declined to 54.0-84.0% at weeks 8 and 12. β-cyfluthrin caused greater mortality than liquid combo at every residual week at 16 and 22 °C, at weeks 4-12 at 27 °C, and at week 12 at 32 and 37 °C, whereas liquid combo caused greater mortality than β-cyfluthrin at week 4 at 32 °C.

3.3.3. *Rhyzopertha dominica*

Rhyzopertha dominica was highly susceptible after 168 h of exposure on metal, where mortality was 98.0-100.0% for both insecticides at every temperature and residual week. On wood, both insecticides caused 100.0% mortality through week 12 at 32 and 37 °C, but mortality declined with residual week at 16-27 °C. Mortality was generally lowest on concrete, particularly at 16 °C. Liquid combo was the more effective insecticide after 24 h on metal, whereas β-cyfluthrin was the more effective insecticide on concrete and, at 16-27 °C, on wood (Tables 3.7-3.9).

3.3.3.1. Wood Bioassay Arenas

Mortality of *R. dominica* after 24 h on wood was significantly affected by insecticide ($F = 4.83$; df = 1, 160; $P = 0.029$), temperature ($F = 10.56$; df = 4, 160; $P < 0.001$), the insecticide × residual week interaction ($F = 9.41$; df = 3, 160; $P < 0.001$), the insecticide × temperature interaction ($F = 3.56$; df = 4, 160; $P = 0.008$), and the insecticide × residual week × temperature interaction ($F = 2.91$; df = 12, 160; $P = 0.001$), but not by residual week ($F = 0.27$; df = 3, 160; $P = 0.844$) and the residual week × temperature interaction ($F = 0.99$; df = 12, 160; $P = 0.463$) (Table 3.7). Mortality after 24 h was ≤16.0% for both insecticides, and no mortality occurred at 16 or 22 °C. At 37 °C, liquid combo caused greater mortality than β-cyfluthrin at week 0 (14.0% vs. 0%), whereas β-cyfluthrin caused greater mortality than liquid combo at weeks 4 and 8 (14.0-16.0% vs. 0%).

Mortality of *R. dominica* after 168 h on wood was significantly affected by insecticide ($F = 22.73$; df = 1, 160; $P < 0.001$), residual week ($F = 75.52$; df = 3, 160; $P < 0.001$), temperature ($F = 77.22$; df = 4, 160; $P < 0.001$), the insecticide × residual week interaction ($F = 4.59$; df = 3, 160; $P = 0.004$), the insecticide × temperature interaction ($F = 4.58$; df = 4, 160; $P = 0.002$), the

residual week × temperature interaction (F = 15.50; df = 12, 160; P < 0.001), and the insecticide × residual week × temperature interaction (F = 2.19; df = 12, 160; P = 0.014) (Table 3.7). Both insecticides caused 100.0% mortality after 168 h at week 0 at every temperature, and at 32 and 37 °C mortality remained 100.0% through week 12. At 16-27 °C, mortality declined with residual age, to 54.0-70.0% for liquid combo and 70.0-90.0% for β-cyfluthrin at weeks 8 and 12. β-cyfluthrin caused greater mortality than liquid combo at week 4 at 16 and 27 °C and at week 8 at 22 and 27 °C. Liquid combo did not cause greater mortality than β-cyfluthrin at any temperature or residual week.

3.3.3.2. Metal Bioassay Arenas

Mortality of *R. dominica* after 24 h on metal was significantly affected by insecticide (F = 1356.72; df = 1, 160; P < 0.001), residual week (F = 57.90; df = 3, 160; P < 0.001), temperature (F = 190.76; df = 4, 160; P < 0.001), the insecticide × temperature interaction (F = 20.25; df = 4, 160; P < 0.001), the residual week × temperature interaction (F = 7.59; df = 12, 160; P < 0.001), and the insecticide × residual week × temperature interaction (F = 6.24; df = 12, 160; P < 0.001), but not by the insecticide × residual week interaction (F = 2.10; df = 3, 160; P = 0.103) (Table 3.8). Liquid combo caused 42.0-100.0% mortality after 24 h, compared with 0-98.0% for β-cyfluthrin. β-cyfluthrin caused no mortality at 16 °C and ≤36.0% at 22-32 °C, but 42.0-98.0% at 37 °C. Liquid combo caused greater mortality than β-cyfluthrin at every residual week at 16-32 °C and at weeks 8 and 12 at 37 °C. At 22 and 27 °C, liquid combo mortality declined from 98.0-100.0% at week 0 to 42.0-46.0% at week 12.

Table 3.7. Mean (± SE) percent mortality of *Rhyzopertha dominica* adults exposed for 24 h and 168 h on wood arenas treated with liquid combo or β-cyfluthrin and held at 16, 22, 27, 32, or 37 °C, for 0, 4, 8, and 12 weeks after application.

Temperature (°C)	Week	Mean (± SE) percent mortality[a,b]			
		24 h		168 h	
		Liquid combo[c]	β-cyfluthrin[d]	Liquid combo[c]	β-cyfluthrin[d]
16	0	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	76.0 ± 2.5 Bb	90.0 ± 4.5 Aa
	8	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	68.0 ± 3.7 Ba	78.0 ± 6.6 Aa
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	70.0 ± 8.9 Ba	82.0 ± 3.7 Aa
22	0	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	96.0 ± 4.0 Aa	94.0 ± 4.0 Aa
	8	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	70.0 ± 4.5 Bb	90.0 ± 4.5 Aa
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	56.0 ± 8.1 Ba	70.0 ± 4.5 Ba
27	0	6.0 ± 4.0 Aa	0.0 ± 0.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	5.3 ± 3.4 Aa	6.0 ± 4.0 Aa	86.0 ± 5.1 Bb	100.0 ± 0.0 Aa
	8	0.0 ± 0.0 Aa	2.0 ± 2.0 Aa	54.0 ± 5.1 Cb	84.0 ± 7.5 Ba
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	66.0 ± 7.5 Ca	70.0 ± 3.2 Ba
32	0	6.0 ± 4.0 Aa	0.0 ± 0.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	8	0.0 ± 0.0 Aa	10.0 ± 6.3 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	12	0.0 ± 0.0 Aa	6.0 ± 4.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
37	0	14.0 ± 6.0 Aa	0.0 ± 0.0 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Bb	16.0 ± 5.1 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	8	0.0 ± 0.0 Bb	14.0 ± 6.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	12	0.0 ± 0.0 Ba	14.0 ± 7.5 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa

[a] Within each temperature and column, means followed by different uppercase letters are significantly different among residual weeks (Tukey's HSD, $P < 0.05$).

[b] Within each temperature, residual week, and exposure time (24 h or 168 h), means followed by different lowercase letters are significantly different between insecticides (Tukey's HSD, $P < 0.05$).

[c] Liquid combo = deltamethrin + (*S*)-methoprene + piperonyl butoxide (Gravista™).

[d] β-cyfluthrin = Tempo® SC Ultra.

Table 3.8. Mean (± SE) percent mortality of *Rhyzopertha dominica* adults exposed for 24 h and 168 h on metal arenas treated with liquid combo or β-cyfluthrin and held at 16, 22, 27, 32, or 37 °C, for 0, 4, 8, and 12 weeks after application.

Temperature (°C)	Week	Mean (± SE) percent mortality[a,b]			
		24 h		168 h	
		Liquid combo[c]	β-cyfluthrin[d]	Liquid combo[c]	β-cyfluthrin[d]
16	0	54.0 ± 4.0 Aa	0.0 ± 0.0 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	52.0 ± 5.8 Aa	0.0 ± 0.0 Ab	100.0 ± 0.0 Aa	98.0 ± 2.0 Aa
	8	48.0 ± 3.7 Aa	0.0 ± 0.0 Ab	100.0 ± 0.0 Aa	98.0 ± 2.0 Aa
	12	42.0 ± 5.8 Aa	0.0 ± 0.0 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
22	0	98.0 ± 2.0 Aa	12.0 ± 7.4 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	84.0 ± 5.1 Ba	2.5 ± 2.5 Ab	100.0 ± 0.0 Aa	98.0 ± 2.0 Aa
	8	68.0 ± 3.7 Ba	0.0 ± 0.0 Ab	100.0 ± 0.0 Aa	98.2 ± 1.8 Aa
	12	42.0 ± 3.7 Ca	0.0 ± 0.0 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
27	0	100.0 ± 0.0 Aa	16.0 ± 5.1 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	98.0 ± 2.0 Aa	16.0 ± 6.8 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	8	86.0 ± 6.0 Aa	10.0 ± 4.5 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	12	46.0 ± 6.8 Ba	0.0 ± 0.0 Ab	100.0 ± 0.0 Aa	98.0 ± 2.0 Aa
32	0	98.0 ± 2.0 Aa	36.0 ± 2.5 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	96.0 ± 2.5 Aa	26.0 ± 5.1 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	8	54.0 ± 5.1 Aa	10.0 ± 4.5 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	12	76.0 ± 8.1 Aa	26.0 ± 6.8 Ab	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
37	0	100.0 ± 0.0 Aa	98.0 ± 2.0 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	94.0 ± 6.0 Aa	76.4 ± 6.9 Ba	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	8	100.0 ± 0.0 Aa	58.0 ± 3.7 Bb	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	12	100.0 ± 0.0 Aa	42.0 ± 3.7 Bb	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa

[a] Within each temperature and column, means followed by different uppercase letters are significantly different among residual weeks (Tukey's HSD, $P < 0.05$).

[b] Within each temperature, residual week, and exposure time (24 h or 168 h), means followed by different lowercase letters are significantly different between insecticides (Tukey's HSD, $P < 0.05$).

[c] Liquid combo = deltamethrin + (*S*)-methoprene + piperonyl butoxide (Gravista™).

[d] β-cyfluthrin = Tempo® SC Ultra.

Table 3.9. Mean (± SE) percent mortality of *Rhyzopertha dominica* adults exposed for 24 h and 168 h on concrete arenas treated with liquid combo or β-cyfluthrin and held at 16, 22, 27, 32, or 37 °C, for 0, 4, 8, and 12 weeks after application.

Temperature (°C)	Week	Mean (± SE) percent mortality[a,b]			
		24 h		168 h	
		Liquid combo[c]	β-cyfluthrin[d]	Liquid combo[c]	β-cyfluthrin[d]
16	0	0.0 ± 0.0 Aa	6.0 ± 4.0 Aa	68.0 ± 5.8 Aa	72.0 ± 3.7 Aa
	4	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	68.0 ± 3.7 Aa	78.0 ± 6.6 Aa
	8	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	54.0 ± 6.0 Aa	56.0 ± 5.1 Aa
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	30.0 ± 4.5 Ba	42.0 ± 3.7 Aa
22	0	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	70.0 ± 4.5 Ab	88.0 ± 5.8 Aa
	4	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	42.0 ± 8.0 Bb	76.0 ± 6.8 Aa
	8	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	46.0 ± 5.1 Bb	100.0 ± 0.0 Aa
	12	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	34.0 ± 5.1 Bb	100.0 ± 0.0 Aa
27	0	2.0 ± 2.0 Ab	34.0 ± 5.1 Aa	74.0 ± 2.5 Bb	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Ab	22.0 ± 3.7 Aa	32.0 ± 5.8 Cb	100.0 ± 0.0 Aa
	8	0.0 ± 0.0 Ab	10.0 ± 4.5 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	12	0.0 ± 0.0 Ab	32.0 ± 8.6 Aa	32.0 ± 5.8 Cb	100.0 ± 0.0 Aa
32	0	4.0 ± 2.5 Ab	46.0 ± 5.1 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Ab	74.0 ± 4.0 Aa	100.0 ± 0.0 Aa	94.0 ± 4.0 Aa
	8	0.0 ± 0.0 Ab	52.0 ± 5.8 Aa	96.0 ± 4.0 Aa	100.0 ± 0.0 Aa
	12	0.0 ± 0.0 Aa	10.0 ± 6.3 Ba	98.0 ± 2.0 Aa	100.0 ± 0.0 Aa
37	0	4.0 ± 2.5 Aa	24.0 ± 8.1 Aa	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
	4	0.0 ± 0.0 Ab	42.0 ± 18.3 Aa	86.0 ± 14.0 Aa	96.0 ± 4.0 Aa
	8	0.0 ± 0.0 Ab	36.0 ± 10.3 Aa	64.0 ± 17.2 Aa	82.0 ± 18.0 Aa
	12	0.0 ± 0.0 Ab	24.0 ± 6.8 Aa	100.0 ± 0.0 Aa	80.0 ± 20.0 Aa

[a] Within each temperature and column, means followed by different uppercase letters are significantly different among residual weeks (Tukey's HSD, $P < 0.05$).

[b] Within each temperature, residual week, and exposure time (24 h or 168 h), means followed by different lowercase letters are significantly different between insecticides (Tukey's HSD, $P < 0.05$).

[c] Liquid combo = deltamethrin + (*S*)-methoprene + piperonyl butoxide (Gravista™).

[d] β-cyfluthrin = Tempo® SC Ultra.

Mortality of *R. dominica* after 168 h on metal was significantly affected by insecticide ($F = 5.00$; df = 1, 160; $P = 0.027$), but not by residual week ($F = 0.73$; df = 3, 160; $P = 0.537$), temperature ($F = 1.00$; df = 4, 160; $P = 0.412$), the insecticide × residual week interaction ($F = 0.73$; df = 3, 160; $P = 0.537$), the insecticide × temperature interaction ($F = 1.00$; df = 4, 160; $P = 0.412$), the residual week × temperature interaction ($F = 0.74$; df = 12, 160; $P = 0.714$), and the insecticide × residual week × temperature interaction ($F = 0.74$; df = 12, 160; $P = 0.714$) (Table

3.8). Liquid combo caused 100.0% mortality after 168 h at every temperature and residual week, and β-cyfluthrin caused 98.0-100.0%. The two insecticides did not differ within any temperature × residual week combination.

3.3.3.3. Concrete Bioassay Arenas

Mortality of *R. dominica* after 24 h on concrete was significantly affected by insecticide ($F = 211.23$; df = 1, 160; $P < 0.001$), residual week ($F = 4.59$; df = 3, 160; $P = 0.004$), temperature ($F = 40.95$; df = 4, 160; $P < 0.001$), the insecticide × temperature interaction ($F = 33.63$; df = 4, 160; $P < 0.001$), the residual week × temperature interaction ($F = 3.03$; df = 12, 160; $P < 0.001$), and the insecticide × residual week × temperature interaction ($F = 3.33$; df = 12, 160; $P < 0.001$), but not by the insecticide × residual week interaction ($F = 2.64$; df = 3, 160; $P = 0.051$) (Table 3.9). Liquid combo caused ≤4.0% mortality after 24 h at every temperature and residual week, whereas β-cyfluthrin caused 0-6.0% at 16-22 °C and 10.0-74.0% at 27-37 °C. β-cyfluthrin caused greater mortality than liquid combo at every residual week at 27 °C, at weeks 0-8 at 32 °C, and at weeks 4-12 at 37 °C.

Mortality of *R. dominica* after 168 h on concrete was significantly affected by insecticide ($F = 79.19$; df = 1, 160; $P < 0.001$), residual week ($F = 5.26$; df = 3, 160; $P = 0.002$), temperature ($F = 54.62$; df = 4, 160; $P < 0.001$), the insecticide × temperature interaction ($F = 21.15$; df = 4, 160; $P < 0.001$), the residual week × temperature interaction ($F = 5.86$; df = 12, 160; $P < 0.001$), and the insecticide × residual week × temperature interaction ($F = 5.04$; df = 12, 160; $P < 0.001$), but not by the insecticide × residual week interaction ($F = 1.57$; df = 3, 160; $P = 0.198$) (Table 3.9). Mortality after 168 h was lowest at 16 °C, where it declined from 68.0-72.0% at week 0 to 30.0-42.0% at week 12 for both insecticides. At 22 and 27 °C, β-cyfluthrin caused 76.0-100.0% mortality, whereas liquid combo caused 32.0-74.0% at every residual week except week 8 at 27

°C (100.0%). β-cyfluthrin caused greater mortality than liquid combo at every residual week at 22 °C and at weeks 0, 4, and 12 at 27 °C. At 32 °C, both insecticides caused 94.0-100.0% mortality, and at 37 °C mortality ranged from 64.0 to 100.0% with no difference between insecticides.

3.4. Discussion

Surface type, temperature, insect species, and insecticide formulation interacted to influence the initial and residual efficacy of two formulations of insecticide, β-cyfluthrin and liquid combo. Among these factors, surface type exerted the most consistent influence, resulting in significantly higher mortality on metal surfaces against *S. oryzae* and *R. dominica* adults up to 12 weeks post application over the 16-37 °C range. However, *S. oryzae* and *R. dominica* mortality significantly decreased on concrete surfaces over the 16-37 °C range. Contrary to the expectation that elevated temperature would accelerate the loss of activity on porous substrates, mortality at 32-37 °C was generally comparable to or greater than mortalities observed at 16-22 °C for all three species, except for *T. castaneum* exposed to liquid combo on metal. The relative performance of the two formulations was surface-dependent, with liquid combo more effective on metal and β-cyfluthrin more effective on concrete.

The greater and more persistent mortality on metal is consistent with previous evaluations of contact insecticides on structural substrates. Hydroprene applied at 19 mg a.i./m² was least persistent on concrete and generally most persistent on metal over 56 d (Arthur et al., 2009), and deltamethrin, cyfluthrin, methoprene, and pyriproxyfen were most persistent on metal against *T. granarium* larvae over 3 months (Arthur et al., 2018). Comparable advantages of non-porous substrates have been reported for organophosphates against liposcelid psocids, which remained effective considerably longer on galvanized steel than on concrete (Collins et al., 2000), and for

malathion and pirimiphos-methyl, which remained 100% effective against *S. oryzae* on metal for 180 d (Jankov et al., 2013). A similar ranking was reported for novaluron, which limited adult emergence to ≤18.3% on metal, ≤32.5% on concrete, and ≤45.0% on wood 12 weeks after application at 0.209 mg/m² (Yasir et al., 2021). Non-porous surfaces retain the deposit at the surface, where it remains available for tarsal contact, whereas porous substrates absorb part of the active ingredient into the matrix (Chadwick, 1985). Concrete is also alkaline, which contributes to the degradation of insecticides (Vassilakos et al., 2014; Lampiri et al., 2023), and sealing concrete with a commercial waterproofing sealant before application markedly extended the residual activity of cyfluthrin (Arthur, 1994). Even freshly applied deltamethrin was considerably less effective on concrete than on metal against *S. oryzae* and *O. surinamensis* (Lampiri et al., 2023). These processes are consistent with the rapid loss of efficacy against *T. castaneum* on concrete, on which no mortality was recorded from week 12 onward at any temperature.

Temperature appeared to influence efficacy mainly through the response of the insects rather than through a faster loss of residual efficacy. Mortality at week 0 generally increased with temperature, and higher temperatures prolonged rather than shortened residual activity on several surfaces. *Rhyzopertha dominica* mortality on wood remained at 100% through week 12 at 32 and 37 °C but declined to 54-70% with liquid combo at 16-27 °C. β-cyfluthrin remained highly effective against *T. castaneum* on concrete through week 8 at 37 °C, whereas no mortality occurred by week 8 at 16 and 22 °C. Studies in which residues were aged across a range of temperatures support the conclusion that temperature has a smaller influence on residual persistence than surface type. Methoprene applied to varnished wood prevented adult emergence of *T. castaneum* for 24 weeks at 20, 30, and 35 °C, and surface type was more important than

temperature in determining residual efficacy (Wijayaratne et al., 2012). Likewise, temperatures of 25-40 °C had no overall effect on the residual activity of a pyrethrin + methoprene aerosol against two dermestid species over 6 weeks (Lanka et al., 2019). Although insecticide residues were not measured in the present study, the mortality data suggest that greater locomotor activity at warm temperatures outweighed any increase in the rate of residue degradation. In contrast, Arthur (2018) observed slower knockdown of *T. castaneum* on deltamethrin-treated concrete aged in a grain bin that accumulated more hours above 32.2 °C, although those bioassays were conducted under common laboratory conditions after aging. The response of *T. castaneum* to liquid combo on metal was the principal exception in the present study, with high mortality maintained through week 8 at 16 °C but not at 22-32 °C, a pattern consistent with slower loss of residual efficacy at low temperature.

The positive association between temperature and mortality contrasts with the negative temperature coefficient of toxicity frequently reported for pyrethroids. The toxicity of several pyrethroids to three lepidopteran species decreased as temperature increased (Sparks et al., 1982), and deltamethrin and cypermethrin were 2.4- and 2.2-fold less toxic, respectively, to *Musca domestica* L. (Diptera: Muscidae), house fly, at 34 °C than at 20 °C, whereas chlorpyrifos, profenofos, emamectin benzoate, and fipronil showed positive temperature coefficients (Khan and Akram, 2014). Among stored-product insects, brief exposure to −5 °C increased the toxicity of deltamethrin to field and selected populations of *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), granary weevil, more than that of the other contact insecticides tested (Kljajić et al., 2014). Studies of short exposures on treated surfaces are consistent with this pattern. *Tribolium castaneum* exposed for 0.5-2 h to cyfluthrin wettable powder on concrete recovered progressively at 25-35 °C as residues aged over 8 weeks, reaching at least 90%

survival by week 6, whereas few beetles survived at 20 °C (Arthur, 1999). Similarly, *T. castaneum* exposed for 3 h to deltamethrin on concrete recovered to a greater extent at 27 and 32 °C than at 18 and 22 °C (Gerken et al., 2025).

The opposite trend observed in the present study is likely explained by the length of exposure. Adults remained on the treated surface for 24 h or 7 d, and continuous contact probably prevented the recovery that occurs when insects are removed from the residue after a short exposure. Under these conditions, the greater locomotor activity of insects at warm temperatures (Stejskal et al., 2019) increases contact with the treated surface and the amount of insecticide acquired, and this appears to outweigh any intrinsic increase in pyrethroid toxicity at low temperature. Conversely, reduced activity at 16-22 °C probably limited contact with the deposit and accounts for the low mortality after 24 h at these temperatures. Positive effects of temperature have also been reported for other insecticide classes against the species tested here. Spinosad dust applied to wheat was more effective against *S. oryzae* and *R. dominica* at 30 °C than at 20 °C (Athanassiou et al., 2008), and abamectin applied to wheat and maize was more effective against *R. dominica*, *S. oryzae*, and *T. confusum* at 31 °C than at 25 °C (Kavallieratos et al., 2009). The toxicity of spinosad, emamectin benzoate, lufenuron, and thiamethoxam to *S. oryzae* on wheat increased 1.6- to 3.0-fold between 20 and 30 °C (Malik et al., 2018), and survival and progeny production of *T. castaneum* exposed to chlorfenapyr on concrete were generally lower at 32 °C than at 27 °C (Arthur, 2013). Even for a single class of product, the direction of the temperature effect can differ among species. Mortality of *Tribolium* spp. exposed to diatomaceous earth increased between 22 and 32 °C (Arthur, 2000b; Vayias and Athanassiou, 2004), whereas most diatomaceous earths tested by Fields and Korunic (2000) were more effective against *T. castaneum* at lower temperatures but less effective against *Cryptolestes*

ferrugineus (Stephens) (Coleoptera: Laemophloeidae), rusty grain beetle. Together, these studies indicate that the effect of temperature on insecticide efficacy depends on both the mode of action and the duration of exposure, and that results from short-exposure bioassays should not be extrapolated directly to insects that remain in contact with treated surfaces for extended periods. They also indicate that surface comparisons made at a single temperature, such as the 26 °C used by Lampiri et al. (2023), may not predict efficacy across the range of temperatures that occurs in storage structures.

Species differed markedly in susceptibility, and the low susceptibility of *Tribolium* spp. relative to other stored-product beetles is well documented. *Tribolium confusum* was the least susceptible of three species to α-cypermethrin, deltamethrin, and spinosad on four surfaces (Lampiri et al., 2023), the least susceptible of eight species to spinosad (Toews et al., 2003), and the least susceptible species on spinetoram-treated concrete (Vassilakos et al., 2014). *Rhyzopertha dominica* and *S. oryzae* were highly susceptible after 168 h of exposure on wood and metal, whereas efficacy against *T. castaneum* declined substantially within 4 weeks on wood and concrete at most temperatures. A comparable difference was reported by Sehgal et al. (2014), in which β-cyfluthrin applied to concrete at labeled rates controlled *R. dominica* but not field strains of *T. castaneum*, even at four times the highest labeled rate. The presence of food in the arenas may have contributed to the lower efficacy against *T. castaneum*, which can recover from exposure to pyrethroid residues when food is available (Arthur, 2000a). Gerken et al. (2025) reported that a light dusting of flour allowed more than 90% of *T. castaneum* to recover after exposure to deltamethrin, and flour can absorb β-cyfluthrin from treated concrete within 24 h (Arthur et al., 2015).

The surface-dependent performance of the two formulations is likely related to differences in their composition. The greater 24 h mortality caused by liquid combo on metal may reflect synergism of deltamethrin by PBO, which inhibits the cytochrome P450 monooxygenases involved in pyrethroid detoxification. Exposure to a deltamethrin + methoprene + PBO formulation on wheat for 1-72 h progressively reduced the movement of *R. dominica*, which had virtually ceased at 48 and 72 h (Arthur et al., 2020). On concrete, formulation type can strongly influence efficacy. An emulsifiable concentrate of deltamethrin applied at 0.02-0.5 g/m² caused no mortality of *S. oryzae* on concrete, whereas a suspension concentrate applied at 0.1 g/m² caused complete mortality (Quinn et al., 2018). Likewise, an emulsifiable concentrate of deltamethrin caused no more than 16.7% mortality of *O. surinamensis* on concrete after 14 d of exposure (Lampiri et al., 2023). Tempo® SC Ultra is a suspension concentrate and was previously shown to be at least as effective on concrete as a formulation combining β-cyfluthrin with imidacloprid (Athanassiou et al., 2013). Because (*S*)-methoprene acts primarily on immature stages, its contribution to the efficacy of liquid combo was not captured by the adult mortality evaluated in this study.

These findings have direct implications for residual treatment programs in warehouses, mills, and shipping containers. Treatments applied to metal surfaces are likely to remain effective against *S. oryzae* and *R. dominica* for at least 12 weeks under a wide range of temperatures, whereas treatments on concrete and wood, particularly where *T. castaneum* is the target species, may require re-application within 4 to 8 weeks. Low mortality after 24 h at 16-22 °C indicates that insects contacting treated surfaces only briefly during cool periods may survive. The present study was conducted at constant temperatures and a single application rate, and treated arenas were aged at the same temperature at which insects were exposed. Under field

conditions, such as those in the shipping containers evaluated in Chapter 2, fluctuating temperatures, dust accumulation, light, and residual food are likely to modify efficacy. For example, exposure to a 12:12 h light:dark cycle reduced the residual efficacy of spinetoram on concrete (Vassilakos and Athanassiou, 2015). Surface treatments should therefore be integrated with rigorous sanitation (Gerken et al., 2025).

3.5. Conclusions

This study showed that the residual efficacy of β-cyfluthrin and liquid combo applied to structural surfaces is determined jointly by surface type, temperature, target species, and formulation, rather than by any single factor. Metal surfaces provided the most persistent control, resulting in 98-100% mortality of *S. oryzae* and *R. dominica* after 168 h for at least 12 weeks across 16-37 °C, whereas concrete provided the least residual control. *Tribolium castaneum* was the most difficult species to control on porous surfaces. Initial mortality generally increased with temperature, and efficacy at 32-37 °C persisted as long as or longer than at cooler temperatures, so insects crossing treated surfaces briefly during cool periods are the least likely to be controlled. Liquid combo was generally more effective on metal and β-cyfluthrin on concrete, indicating that no single product performed best on every surface. For pest management in warehouses, mills, and shipping containers, these results indicate that metal surfaces may need re-treatment less often, whereas concrete and wood surfaces, particularly where *T. castaneum* is the target, may require re-treatment within 4-8 weeks. Selection of a residual insecticide and its re-treatment interval should therefore be based on the predominant surface in a structure, the expected temperature range, and the target pest species, and surface treatments should be combined with thorough sanitation to limit insect recovery. Future work should evaluate these

formulations under fluctuating temperatures, assess progeny production to capture the effect of methoprene, and extend residual evaluations beyond 12 weeks for *S. oryzae* and *R. dominica*.

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Chapter 4 - Evaluation of Methoprene-Incorporated Packaging to Suppress Stored-Product Insect Populations and Inhibit Infestation of Bagged Rice Under Simulated Warehouse Conditions

Abstract

Methoprene-incorporated packaging reduces the development of stored-product insects in laboratory studies, but its performance on pallets of bagged commodities under realistic storage conditions has not been evaluated. This study evaluated methoprene-incorporated woven polypropylene packaging (ProvisionGard™) for protecting bagged milled rice in two field-scale, climate-controlled simulated warehouses over 182 d. Each simulated warehouse served as one replicate and contained one pallet of 25 rice bags (~11.3 kg each) in methoprene-incorporated packaging and one pallet in untreated packaging. Each simulated warehouse was infested with 200 each of *Tribolium castaneum* (Herbst), red flour beetle, *Oryzaephilus surinamensis* (L.), sawtoothed grain beetle, and *Rhyzopertha dominica* (F.), lesser grain borer, adults and *Trogoderma variabile* Ballion, warehouse beetle, and *Plodia interpunctella* (Hübner), Indian meal moth, larvae, with insects reintroduced every 25-30 d. Five bags per pallet were sampled every 30 d, insect activity was monitored with beetle and sticky traps, and all bags were opened and sieved at 182 d. Pallet temperatures followed the outdoor seasonal pattern and increased from a monthly mean of 22.8 °C in January to 30.4 °C in June. Live insects were not detected in sampled bags until 91 d, and no insect-damaged kernels were found in any subsample. From 91 to 150 d, fewer sampled bags contained live insects in methoprene-incorporated packaging than in untreated packaging (23% vs. 57%, $P = 0.004$). At 182 d, methoprene-incorporated pallets held 57% fewer insects than untreated pallets (122.5 ± 28.5 vs. 287.0 ± 130.0 insects per pallet),

and the numbers of *T. castaneum*, *O. surinamensis*, and *R. dominica* were 49-64% lower, but these differences were not significant with two replicates ($P \geq 0.318$). The number of infested bags was similar between packaging types (12 of 50 vs. 14 of 50 bags), and *T. variabile* was recovered only from untreated bags. About 81% of the insects recovered at 182 d were in the bottom layer of the pallets, closest to the floor where insects were released. *Tribolium castaneum* (64%) and *O. surinamensis* (32%) dominated beetle trap captures, and no *R. dominica* were captured. These results indicate that methoprene-incorporated packaging can reduce insect numbers within bags but does not prevent insects from infesting bags. It should therefore be used together with improved bag closures, sanitation, residual insecticides, and fumigation as part of an integrated pest management program for food-processing facilities, storage warehouses, and shipping containers.

Keywords: insect growth regulator, methoprene, insecticide-incorporated packaging, stored-product insects, bagged rice, warehouse

4.1. Introduction

Stored-product insects are cosmopolitan pests that infest raw and processed commodities throughout the post-harvest supply chain, including farm storage, mills, processing plants, warehouses, retail outlets, and the railcars, ships, and shipping containers (Campbell et al., 2004; Hagstrum and Subramanyam, 2006; Hagstrum and Phillips, 2017). Globally, nearly one-third of the food produced for human consumption is lost or wasted each year, and a substantial portion of this loss occurs post-harvest during handling, storage, and transportation (FAO, 2011). In the United States alone, stored-product insects cause more than $500 million in damage annually (Hagstrum and Subramanyam, 2006). Beyond direct consumption of the product, infestation reduces grain quality and market grade and contaminates food with insect fragments and exuviae

(Hagstrum and Subramanyam, 2006). Because consumer and regulatory tolerance for live insects or insect-damaged products is extremely low, even modest infestations can result in rejected shipments, product recalls, and loss of customer confidence.

Rice is a staple food for more than half of the world's population (Muthayya et al., 2014). Milled rice is commonly packaged in woven polypropylene bags, stacked and stored on pallets, and held in warehouses or loaded into shipping containers for domestic distribution, export, and humanitarian food-aid programs (USDA-AMS, 2022). Rice mills and storage facilities support a diverse community of stored-product insects whose activity fluctuates seasonally (McKay et al., 2017). This community includes internal feeders such as *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), lesser grain borer, whose feeding reduces both measured and perceived rice quality and therefore its economic value (Su et al., 2019), and external feeders such as *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), red flour beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), sawtoothed grain beetle, *Trogoderma variabile* Ballion (Coleoptera: Dermestidae), warehouse beetle, and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), Indian meal moth (Hagstrum and Subramanyam, 2006; Mohandass et al., 2007; McKay et al., 2017). Many of these species are highly mobile and are captured both inside and outside food facilities, allowing them to repeatedly colonize stored products from surrounding landscapes and adjacent structures (Campbell and Mullen, 2004; Scheff et al., 2022).

Historically, fumigation with methyl bromide or phosphine has been the primary method for disinfesting grain and grain-based commodities. The production and use of methyl bromide for structural and commodity treatments have been phased out under the Montreal Protocol, with remaining uses largely restricted to quarantine and pre-shipment applications (Fields and White, 2002). Phosphine remains widely used, but it requires warm temperatures, long exposure

periods, and well-sealed enclosures to achieve complete mortality of all life stages, and fumigation provides no residual protection. Poor fumigation practices and lack of fumigation monitoring have contributed to the development of phosphine resistance in key species, including *T. castaneum* and *R. dominica* (Cato et al., 2017). Long-term monitoring in flour mills has shown that *T. castaneum* populations rebound within weeks to months after fumigation through survival of insects in refugia and immigration from outside the treated structure (Campbell and Arbogast, 2004; Campbell et al., 2010). These limitations have shifted stored-product pest management toward integrated pest management (IPM) programs that emphasize prevention, monitoring, sanitation, and reduced-risk insecticides that provide sustained protection (Phillips and Throne, 2010; Arthur, 2012; Hagstrum and Phillips, 2017).

Packaging is the final barrier between a finished product, and the insect populations present in a storage or transportation environment. However, protecting packaged products from infestation remains a persistent challenge for the food industry (Marsh and Bugusu, 2007). Stored-product insects are generally classified as either penetrators, which can chew directly through packaging materials, or invaders, which enter through existing openings such as seams, closures, and punctures created by other insects or by handling (Highland, 1991; Mullen et al., 2012). Larvae of *P. interpunctella* and *T. variabile* and adults of *R. dominica* are capable of penetrating a range of packaging films, whereas species such as *T. castaneum* and *O. surinamensis* readily exploit small gaps and imperfect seals to gain access to package contents (Highland, 1991; Riudavets et al., 2007; Scheff et al., 2018b). Woven polypropylene bags with sewn closures, as are commonly used for bagged rice, are particularly vulnerable because the weave and stitching create numerous potential points of entry. Consequently, considerable effort has been directed at developing insect-resistant packaging that combines physical barriers with

chemical protection (Mullen et al., 2012). Insecticide-incorporated packaging materials and long-lasting insecticide-incorporated netting containing pyrethroids such as deltamethrin have been shown to reduce survival, mobility, and reproductive output of several stored-product beetles (Kavallieratos et al., 2017; Paudyal et al., 2017a; Morrison et al., 2018; Scheff et al., 2020, 2021).

Methoprene is a juvenile hormone analog insect growth regulator (IGR) that disrupts normal development and metamorphosis, preventing immature stages from successfully developing into reproductive adults (Oberlander et al., 1997). Because of its low mammalian toxicity, methoprene is exempt from the requirement of a tolerance in or on all food commodities in the United States when used to control insect larvae (U.S. EPA, 2003). Methoprene has a long history of use as a grain protectant and is particularly effective against *R. dominica*, providing long-term protection of stored wheat, brown rice, rough rice, and corn (Arthur, 2004, 2016a; Athanassiou et al., 2011a). More recently, methoprene has been incorporated directly into the polymer matrix of commercial food packaging (ProvisionGard™ technology), from which the active ingredient is gradually released to the package surface, providing continuous exposure to insects that contact the packaging (Arthur, 2016b; Scheff et al., 2016).

Laboratory studies have consistently demonstrated that methoprene-incorporated packaging can disrupt the development of stored-product insects. Exposure to methoprene-treated polymer and woven packaging reduced egg-to-adult emergence of *T. castaneum* and *T. variabile* and adversely affected larvae exposed to the treated material (Scheff et al., 2016, 2017). Methoprene-treated foil packaging has been evaluated for its effects on larval penetration and invasion by *P. interpunctella* and *T. variabile* (Scheff et al., 2018a), and methoprene-treated packaging suppressed larval development of the quarantine pest *Trogoderma granarium* Everts,

khapra beetle, and the related *Trogoderma inclusum* LeConte, larger cabinet beetle (Scheff et al., 2019). Standardized methodologies for evaluating methoprene-treated packaging films have also been developed (Arthur, 2016b). Collectively, these studies indicate that continuous exposure to methoprene-treated packaging can substantially reduce the ability of immature insects to complete development, although the magnitude of the effect depends on species, life stage, packaging type, and exposure duration (Arthur, 2016b; Scheff et al., 2016, 2017, 2018a, 2019).

Despite this progress, nearly all evaluations of methoprene-incorporated packaging have been conducted in small laboratory bioassay arenas, typically with a single species, a single life stage, and exposure periods spanning less than one generation (Arthur, 2016b; Scheff et al., 2016, 2017, 2018a, 2019). These studies were designed primarily to determine whether the treated packaging prevents the growth and development among common stored product insect species associated with food processing and storage areas. In commercial practice, however, packaged products are stored for months in pallet stacks within warehouses and shipping containers, where a mixed-species pest community has access to alternative food in the form of spillage and residues, and where populations are continually supplemented by immigration from the surrounding environment (Campbell and Mullen, 2004; Campbell et al., 2010; McKay et al., 2017). Under such conditions, widespread use of methoprene-treated packaging could potentially have effects that extend beyond the individual package, by reducing the number of insects that successfully develop and reproduce within a storage space and thereby suppressing the resident population over multiple generations. Conversely, insects developing in untreated spillage or in neighboring untreated products may sustain populations that continually challenge the treated packaging potentially leading to cross-contamination between storage areas. To date, the impact of insecticide-treated packaging on resident insect populations and on long-term prevention of

infestation in semi-field applications has not been thoroughly determined. This information is critical for its broader adoption within IPM programs for stored and transported commodities domestically and internationally.

Pilot-scale or semi-field warehouse studies provide an important link between laboratory bioassays and commercial facilities because they allow replicated, controlled releases of insects into realistic storage environments while monitoring populations with the same tools used in industry. Field-scale climate-controlled sheds at the USDA-ARS Center for Grain and Animal Health Research in Manhattan, Kansas, have previously been used to evaluate residual insecticides, aerosols, pheromone-based monitoring of stored-product insects, and long-lasting insecticide-incorporated netting (Toews et al., 2005; Kharel et al., 2014; Wilkins et al., 2021). Previous long-lasting insecticide-incorporated netting was deployed in the field-scale climate-controlled sheds to demonstrate its effect on preventing insects from reaching a commodity (Wilkins et al., 2021). Results demonstrated that the number of insects reaching stored commodities was reduced by 89-93% and progeny production in those commodities by 98-100% despite repeated insect releases (Wilkins et al., 2021).

The preceding chapters of this thesis evaluated contact insecticide treatments applied to the wood and metal surfaces of shipping containers before loading as one component of an IPM program for bagged commodities. Additionally, the effect of surface material and temperature was evaluated on the residual efficacy of two insecticides utilized as surface treatments in processing and storage warehouses as another component of an IPM program for bagged commodities. The present study addresses a third component, namely the protection by the packaging itself through the use of insecticide-incorporated packaging. The overall objective of this study was to determine the ability of methoprene-incorporated woven polypropylene

packaging to reduce infestation of bagged rice under simulated warehouse storage conditions. Specifically, pallets of bagged long-grain milled rice in methoprene-incorporated or untreated packaging were held in field-scale simulated warehouses, infested with a mixed community of *T. castaneum*, *T. variabile*, *O. surinamensis*, *R. dominica*, and *P. interpunctella*, and re-infested at regular intervals to simulate immigration over a 182-d storage period spanning multiple insect generations. The specific aims were to (1) compare insect-infested bags and insect-damaged kernels in bagged rice held in methoprene-incorporated and untreated packaging over time, (2) assess changes in rice quality, including moisture content and test weight, (3) document packaging integrity and evidence of insect penetration or invasion, and (4) describe insect activity in the storage environment over time, as measured by pheromone- and food-baited traps. We hypothesized that methoprene-incorporated packaging would reduce infestation and damage of bagged rice relative to untreated packaging by limiting successful development of insects within the packaged product.

4.2. Materials and Methods

4.2.1. Experimental Sheds

The study was conducted in two identical field-scale, climate-controlled sheds (6.0 × 2.9 × 2.6 m; approximately 45 m^3) designed to simulate warehouse storage conditions (hereafter, simulated warehouses), located at the USDA-ARS Center for Grain and Animal Health Research (CGAHR), Manhattan, Kansas (Kharel et al., 2014). Each simulated warehouse was set to maintain a temperature of approximately 26-30 °C, a range favorable for development of all test species. Each simulated warehouse contained one pallet of rice in methoprene-incorporated single-ply woven polypropylene packaging and one pallet of rice in untreated packaging (single-ply woven polypropylene without methoprene). Each simulated warehouse was treated as one

replicate (block), giving two replicates of each packaging type in two separate warehouse environments. The experiment was conducted over a 182-d storage period from 29 December 2025 (day 0) to 29 June-2 July 2026 (day 182), which allowed several generations of each test species to develop.

4.2.2. Packaging and Rice

Methoprene-incorporated packaging consisted of single-ply woven polypropylene bags containing methoprene at an active level of 1 μg/cm^2 (ProvisionGard™ Technology, LLC, Greensboro, NC), and the methoprene loading rate of the treated packaging was verified by an independent laboratory. Untreated control packaging consisted of identical single-ply woven polypropylene bags without methoprene. All bags were manufactured by JohnPac, LLC (Crowley, LA). Before bagging, the rice was frozen to disinfest it. Each bag was filled with ~11.3 kg of extra-long-grain milled white rice (Riviana Foods Inc., Houston, TX) and closed with a single line of stitching (single-stitched seam) across the top using a handheld bag-sewing machine, leaving 2.5-5.0 cm of overhang above the seam. Each sewn bag was inspected to confirm a uniform stitch across the closure. To prevent cross-contamination with methoprene, all control bags were filled and sewn before the treated bags.

4.2.3. Pallet Configuration and Environmental Monitoring

Bags were stacked on new heat-treated wooden export pallets (101 × 122 cm, model H-1260, Uline, Pleasant Prairie, WI). Each pallet held 25 bags arranged five bags per layer in five layers, using an alternating (interlocking) stacking pattern on a single slip sheet to stabilize the load and prevent the bottom layer of bags from bursting. Each simulated warehouse therefore contained 50 bags (25 treated and 25 untreated), for a total of 100 bags across both replicates. Bags were numbered from the bottom of the stack upward, so that layer 1 (bags 1-5) was the

bottom layer in contact with the slip sheet and pallet and layer 5 (bags 21-25) was the top layer. Temperature and relative humidity (RH) were recorded hourly with HOBO® data loggers (Onset Computer Corporation, Bourne, MA). One logger was placed in the open shed to record the ambient environment, and three loggers were placed within each pallet stack in the bottom, middle, and top layers. The arrangement of the untreated and methoprene-incorporated pallets within a simulated warehouse is shown in Fig. 4.1.



Figure 4.1. Arrangement of pallets in a simulated warehouse (6.0 × 2.9 × 2.6 m). Each simulated warehouse contained one pallet of untreated woven polypropylene bags (control; bags labeled C, foreground) and one pallet of methoprene-incorporated woven polypropylene bags (methoprene-treated; bags labeled T, background). Each pallet held 25 bags of milled rice stacked in five interlocking layers on a slip sheet over a heat-treated wooden pallet (101 × 122 cm).

4.2.4. Grain Spillage and Insect Traps

A food patch simulating grain spillage was placed in each simulated warehouse. Each food patch consisted of a 150 × 25 mm plastic Petri dish containing 10 g of rice mixed with 1% (w/w) flour, sufficient to cover the bottom of the dish. Four 10-mm holes were made around the

side wall of each dish, and the inner floor of the dish was roughened with sandpaper so that insects could freely enter and exit. The food patch was placed along one wall of the simulated warehouse, equidistant from the two pallets, and remained in place without replacement for the entire trial.

Insect activity within each simulated warehouse was monitored with one beetle trap (Storgard® Dome™ trap) baited with a Quick-Change multi-species lure and one hanging diamond sticky trap baited with an Indian meal moth pheromone lure (Trécé Inc., Adair, OK). Traps and lures were replaced at each sampling timepoint. Examples of insects captured in the beetle and sticky traps are shown in Fig. 4.2.

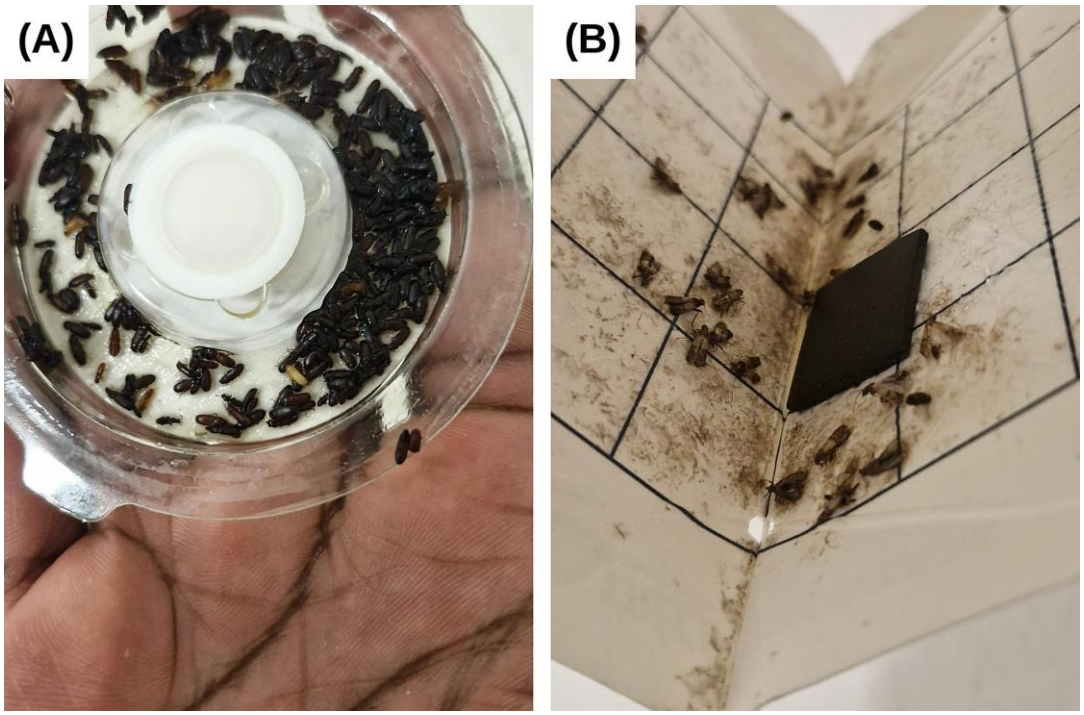


Figure 4.2. Traps used to monitor insect activity in the simulated warehouses. (A) Beetle trap baited with a Quick-Change multi-species lure, showing captured adult beetles. (B) Diamond sticky trap baited with an Indian meal moth pheromone lure, showing captured *Plodia interpunctella* (Hübner) adults. Traps and lures were replaced at each sampling timepoint.

4.2.5. Test Insects and Infestation

Five stored-product insect species were used: *T. castaneum*, *T. variabile*, *O. surinamensis*, *R. dominica*, and *P. interpunctella*. All insects were obtained from pesticide susceptible laboratory colonies maintained at the USDA-ARS CGAHR, Manhattan, KS, in 0.95-L glass jars fitted with fine-mesh screen lids and held in environmental chambers at 60-65% RH in complete darkness. Colonies of *T. castaneum*, *O. surinamensis*, *R. dominica*, and *P. interpunctella* were held at 27 ± 1 °C, and the *T. variabile* colony was held at 30 ± 1 °C. *Tribolium castaneum* was reared on organic whole-wheat flour with 5% (w/w) brewer's yeast, and *R. dominica* was reared on organic whole hard red winter wheat kernels. *Oryzaephilus surinamensis* was reared on crimped wheat mixed with rolled oats and brewer's yeast. *Trogoderma variabile* was reared on ground dog food with rolled oats. *Plodia interpunctella* was reared on a diet of cracked wheat, wheat shorts, wheat germ, brewer's yeast, honey, glycerin, and water.

On day 0, each simulated warehouse was infested with 200 unsexed 1- to 2-week-old adult *T. castaneum*, 200 medium-sized *T. variabile* larvae, 200 unsexed 1- to 2-week-old adult *O. surinamensis*, 200 unsexed 1- to 2-week-old adult *R. dominica*, and 200 third- to fourth-instar *P. interpunctella* larvae. Insects were released on the floor between the treated and untreated pallets, opposite the food patch, so that neither packaging type was favored by proximity to the release point. To simulate continued immigration of insects into the storage environment, the same numbers and life stages of each species were reintroduced into each simulated warehouse every 25-30 d, with the timing of each release determined by the availability of *P. interpunctella* larvae of the required size, giving five additional releases during the trial.

4.2.6. Monthly Bag Sampling

Bags were sampled after 30, 60, 91, 120, and 150 d of storage in the warehouses. At each sampling timepoint, five bags per pallet (treated and control) were selected at random in each warehouse, using a random number table. Thus, 10 bags per replicate (five treated and five control) were sampled. Each bag was sampled only once during the trial, so that by 150 d all 25 bags on each pallet had been sampled. Bags remained in place on the pallet during and after sampling. Each selected bag was pierced once on the side with a grain trier, and approximately 500 g of rice was withdrawn from the center of the bag by inserting the trier at several angles through the same opening. Immediately after sampling, the sampling hole was resealed with food-grade woven polypropylene tape (Filmloc, Flowery Branch, GA). The bag sampling procedure and a rice sample are shown in Fig. 4.3.

In the laboratory, the moisture content and test weight of each sample were determined using a Mini GAC® 2500 grain analysis computer (DICKEY-john Corp., Auburn, IL) with the long-grain milled rice calibration. Each sample was then sieved, and all insects recovered were identified to species level and recorded as live or dead. After insects were removed, the sample was divided with a sample divider to obtain a subsample of approximately 7-10 g, in which the number of insect-damaged kernels (IDK) and undamaged kernels were counted following the USDA Federal Grain Inspection Service rice inspection guidelines (USDA-AMS-FGIS, 2020). The percentage of IDK was calculated as [number of damaged kernels / (number of damaged kernels + number of undamaged kernels)] × 100. The remaining rice was placed in a 0.95-L plastic jar fitted with a screened lid and held at 27 °C and 65% RH in complete darkness for 8 weeks to allow emergence of any adult progeny.

4.2.7. Pallet Inspection and Trap Monitoring

At each sampling timepoint, each pallet was visually inspected, and changes in appearance were documented, including off-color, mold growth, floury residue, odor, and insects moving into or out of the packaging. Grain quality and packaging integrity of each pallet were scored on a 1-4 scale (1 = worst, 4 = no visible change) using the criteria in Table 4.1 and the reference photographs in Figs. 4.4 and 4.5, and tears, holes, seam failures, and chewing damage were recorded. At the same time, beetle and sticky traps were collected, and captured insects were counted by species.

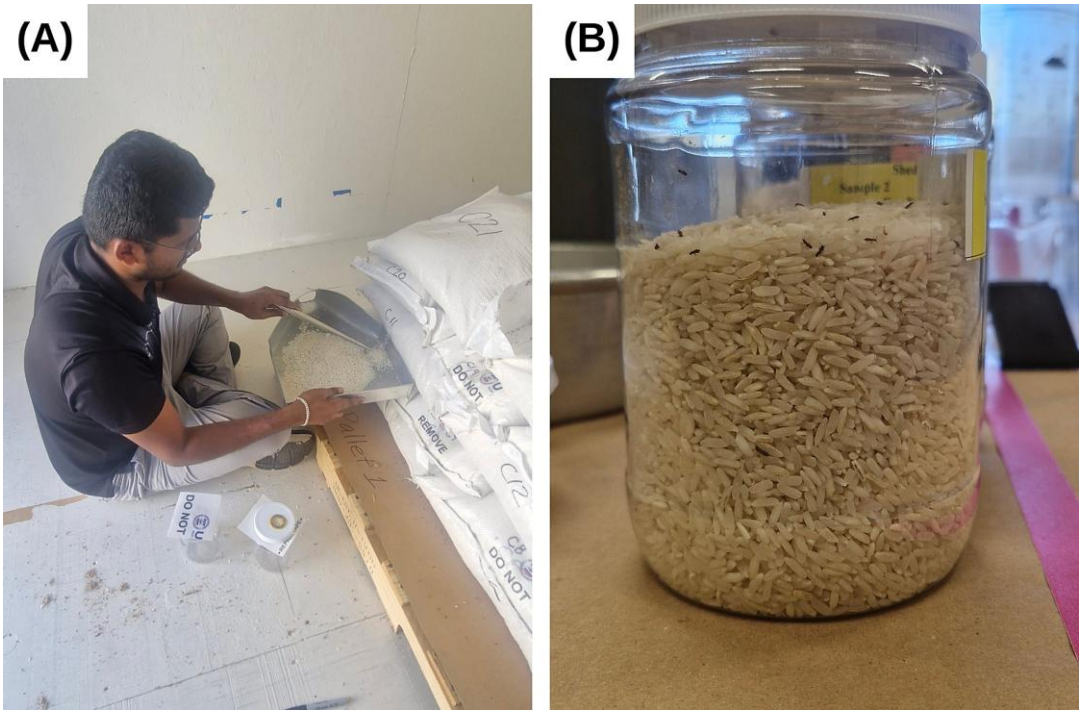


Figure 4.3. Monthly sampling of bagged rice. (A) Withdrawal of an approximately 500-g sample from the center of a bag with a grain trier inserted through a single side opening, which was resealed with woven polypropylene tape immediately after sampling. (B) Rice sample in a 0.95-L plastic jar with a screened lid, and adult insects are visible on the grain surface.

Table 4.1. Visual scoring criteria (1 = worst, 4 = no change) used to assess grain quality and packaging integrity of pallets at each monthly inspection and of individual bags at the final bag opening (182 d).

Score	Grain quality	Packaging integrity
1	Bags completely discolored, extreme rotten or musty odor, grain wet or damp, live insects present when sampled, broken kernels and floury fine material, grain and bag moldy	Bag falls apart or seams fail, grain spilling out of the bag, floury residue when gently probed, obvious infestation, holes or signs of insects chewing through or entering the bag
2	Bags darkened or discolored, signs of mold or fungal growth, off odor when sampled, bag floury or actively infested when probed, insects detected, grain may feel damp, rice discolored	Live insects crawling into or out of the bag, grain spilling around the bag, pallet, or layers, seams or weave showing signs of failure
3	Slight discoloration of the bag (possibly dust), no off odor, grain in normal condition and not discolored, no insects, broken kernels, or signs of infestation when sampled	Weave beginning to separate, with some grain spilling, seams opening (signs of stress), signs of infestation or chewing on the bag, webbing, frass, or cast skins present
4	Bags as on day 0, no discoloration, off odor, musty smell, mold, or signs of infestation	Bags in the same condition as at the start of the trial, seams and weave intact, no tears, damage, or signs of infestation

Pallets were scored at each 30-d inspection (30-150 d), and all 25 bags on each pallet were scored individually at the final bag opening at day 182.

4.2.8. Final Bag Opening

At 182 d (29 June-2 July 2026), all traps were collected from each simulated warehouse, and all 50 bags in each simulated warehouse (2 pallets × 25 bags) were removed from the pallets. Each bag was inspected and scored for grain quality and packaging integrity on the same 1-4 scale, and its position in the pallet stack and any visible damage were recorded. The entire contents of each bag were emptied and sieved to recover all insects, which were counted and identified to species. Any larvae and pupae collected were held until adult emergence to confirm species identity. The moisture content and test weight of each bag were determined as described above, and broken kernels and fine dust/frass recovered by sieving were weighed. Insect-damaged kernels were counted in three 100-kernel subsamples from each bag.



Figure 4.4. Reference scale used to score grain quality of pallets and individual bags (1 = worst, 4 = no change). (A) Score 1: extensive discoloration and mold on the bags, with damp, musty grain. (B) Score 2: darkened or discolored bags with off odor or signs of active infestation. (C) Score 3: slight discoloration of the bag surface, with grain in normal condition and no insects. (D) Score 4: bags and grain unchanged from the start of storage. Full criteria are given in Table 4.1.

4.2.9. Data Analysis

Each simulated warehouse contained one pallet of each packaging type, so the pallet was the experimental unit and each simulated warehouse served as one replicate (block), giving two replicates of each packaging type. Data from the two replicates were pooled and are presented as means ± standard error (SE) of the two pallets of each packaging type. In tables and figures, untreated packaging is labeled control and methoprene-incorporated packaging is labeled methoprene-treated. Analyses of variance (ANOVA) were fitted in R version 4.3.3 (R Core Team, 2024) and tested with Type III sums of squares and sum-to-zero contrasts using the car

package (Fox and Weisberg, 2019). Means were separated with Tukey's honestly significant difference (HSD) test using the emmeans package (Lenth, 2023) at $\alpha = 0.05$. Insect counts were log(x + 1)-transformed before analysis to stabilize variances, and untransformed means are presented. When an interaction was not significant ($P > 0.05$), it was removed, and main effects were tested in the additive model.

For the final bag opening, the numbers of *T. castaneum*, *O. surinamensis*, *R. dominica*, and *T. variabile* and the total number of insects per pallet, the number of insects per bag, the number of infested bags, and the number of insects per infested bag were analyzed with a randomized complete block ANOVA with packaging type as the fixed effect and replicate as the block. Pallet means of moisture content, test weight, broken kernels, fine dust/frass, and grain quality and packaging integrity scores were analyzed in the same way. The number of infested bags is also presented as the total for both replicates (out of 50 bags per packaging type). The percentage reduction in insects in methoprene-incorporated packaging was calculated from the pooled means as [(control − methoprene-treated) / control] × 100. To describe the vertical distribution of infestation, the number of insects per bag in each pallet layer was compared among the five layers within each packaging type, with replicate as the block. Moisture content, test weight, broken kernels, and fine dust/frass in each layer were analyzed with packaging type, layer, and their interaction as fixed effects and replicate as the block.



Figure 4.5. Reference scale used to score packaging integrity of pallets and individual bags (1 = worst, 4 = no change). (A) Score 1: bag failure, with seams split and grain spilling from the bag. (B) Score 2: seams and weave failing, with grain spilling around the bags and layers. (C) Score 3: seams beginning to open under stress, with insects and frass along the seam. (D) Score 4: seams and weave intact, with no tears or signs of infestation. Full criteria are given in Table 4.1.

For the monthly bag samples, moisture content and test weight were analyzed with packaging type, sampling timepoint, and their interaction as fixed effects and replicate as the block (30, 60, 91, and 150 d, because moisture content and test weight were not recorded at 120 d). The number of live insects recovered from the five sampled bags of each pallet was analyzed in the same way for all five sampling timepoints. The presence or absence of live insects in each sampled bag from 91 to 150 d was analyzed with a binomial generalized linear model with packaging type, sampling timepoint, and replicate as fixed effects, and effects were tested with Type III likelihood-ratio χ^2 tests using the car package. Because bags on the same pallet are

subsamples of one experimental unit, this bag-level analysis was used only to support the pallet-level comparisons.

Because the traps in each simulated warehouse monitored insects from both pallets, trap captures were analyzed for sampling timepoint only. The number of insects captured per beetle trap and the number of *P. interpunctella* captured per sticky trap at each sampling timepoint were compared among sampling timepoints by one-way ANOVA on log(x + 1)-transformed counts, with the two replicates as observations, followed by Tukey's HSD test. The species composition of beetle trap captures was expressed as the percentage of all insects captured in both beetle traps at each sampling timepoint represented by each species. Temperature and RH were summarized for each pallet logger as the overall mean and range of daily means calculated from hourly readings, and the overall means for each pallet layer and packaging type are presented as means ± SE of the two replicates.

4.3. Results

4.3.1. Environmental Conditions

Temperature and relative humidity (RH) within the top, middle, and bottom layers of control and methoprene-treated pallets are summarized in Table 4.2, and daily mean temperature and RH in the three layers are shown in Fig. 4.6. Mean temperatures within the pallets ranged from 25.48 ± 0.51 °C in the bottom layer of control pallets to 27.20 ± 0.76 °C in the top layer of methoprene-treated pallets. Within every pallet, the top layer was the warmest and the bottom layer the coolest, and mean temperatures in the top layer exceeded those in the bottom layer by 1.1-1.7 °C. Mean temperatures differed by less than 0.6 °C between control and methoprene-treated pallets in the same layer (Table 4.2).

Table 4.2. Overall mean (± SE) temperature and relative humidity within the top, middle, and bottom layers of control and methoprene-treated pallets of bagged rice during the 182-d storage trial. Values in parentheses are the lowest and highest daily means recorded in either replicate.

	Temperature (°C)[a]		Relative humidity (%)[a]	
Pallet layer	Control pallet	Methoprene-treated pallet	Control pallet	Methoprene-treated pallet
Top layer	26.93 ± 0.29 (18.6-31.8)	27.20 ± 0.76 (19.2-32.1)	35.80 ± 1.73 (26.9-62.1)	33.81 ± 0.91 (25.8-47.7)
Middle layer	25.97 ± 0.52 (17.2-31.8)	26.52 ± 0.77 (18.1-32.0)	37.09 ± 1.21 (25.7-61.4)	34.36 ± 1.15 (25.2-48.3)
Bottom layer	25.48 ± 0.51 (16.6-31.5)	25.88 ± 0.58 (17.7-31.5)	38.80 ± 1.32 (31.4-61.9)	36.97 ± 1.07 (29.6-48.3)

[a] Values are overall means ± SE of the two replicates (n = 2) for the 182-d trial, calculated from daily means of hourly HOBO® logger readings (28 December 2025 to 30 June 2026).

Although the simulated warehouses were climate-controlled, pallet temperatures followed the outdoor seasonal pattern (Fig. 4.6). Temperatures were lowest in January and early February and increased steadily from March to the end of the trial. The monthly mean temperatures across all pallet loggers rose from 22.8 °C in January to 30.4 °C in June. The lowest daily mean temperatures were recorded on 26-27 January 2026, during an arctic outbreak in Manhattan, KS, when the outdoor minimum temperature fell to −22 °C (Extreme Weather Watch, 2026). During this period, the daily mean temperatures within the pallets fell to 16.6-19.2 °C in one replicate, whereas they remained at or above 22.0 °C in the other (Table 4.2). Mean RH within the pallets ranged from 33.81 ± 0.91% to 38.80 ± 1.32% (Table 4.2). In contrast to temperature, RH was highest in the bottom layer and lowest in the top layer of every pallet. RH declined from the start of the trial through the winter, remained lowest from February to April (monthly means of 30.9-34.4%), and increased during May and June to a monthly mean of 43.2% in June (Fig. 4.6).

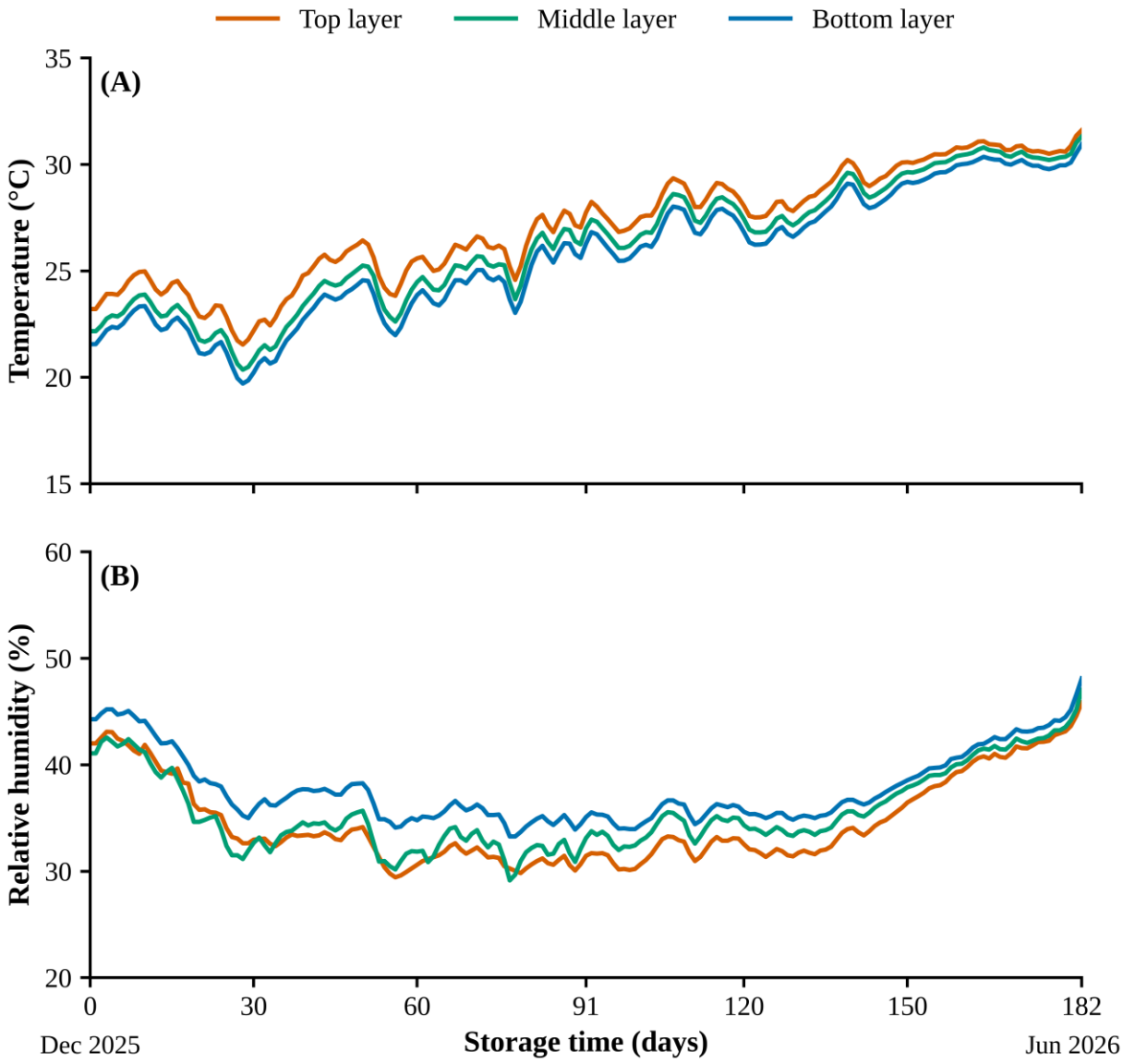


Figure 4.6. Daily mean temperature (A) and relative humidity (B) in the top, middle, and bottom layers of pallets of bagged milled rice during the 182-d storage trial (28 December 2025 to 30 June 2026). Each line is the mean of the four pallets (control and methoprene-treated pallets in two replicates), calculated from hourly HOBO® data logger readings.

4.3.2. Trap Captures

Insects were captured in beetle traps at every sampling timepoint, and 1,563 insects were captured in beetle traps over the entire trial (Figs. 4.7 and 4.8). The number of insects per beetle trap differed among sampling timepoints ($F = 14.72$; df = 5, 6; $P = 0.003$). Captures were

greatest at 91 and 150 d (195.5 and 194.5 insects per trap, respectively) and lowest at 30, 60, and 182 d (81.0-98.0 insects per trap) (Fig. 4.7). *Tribolium castaneum* made up 64% and *O. surinamensis* 32% of all beetle trap captures throughout the study. *Trogoderma variabile* and *P. interpunctella* together accounted for the remaining 4% (Fig. 4.8). The proportion of *T. castaneum* ranged from 41% at 120 d to 78% at 60 d, while the proportion of *O. surinamensis* showed the inverse pattern (22-55%). *Trogoderma variabile* were captured in beetle traps from 91 d onward, and *P. interpunctella* were captured in beetle traps mainly at 91 d.

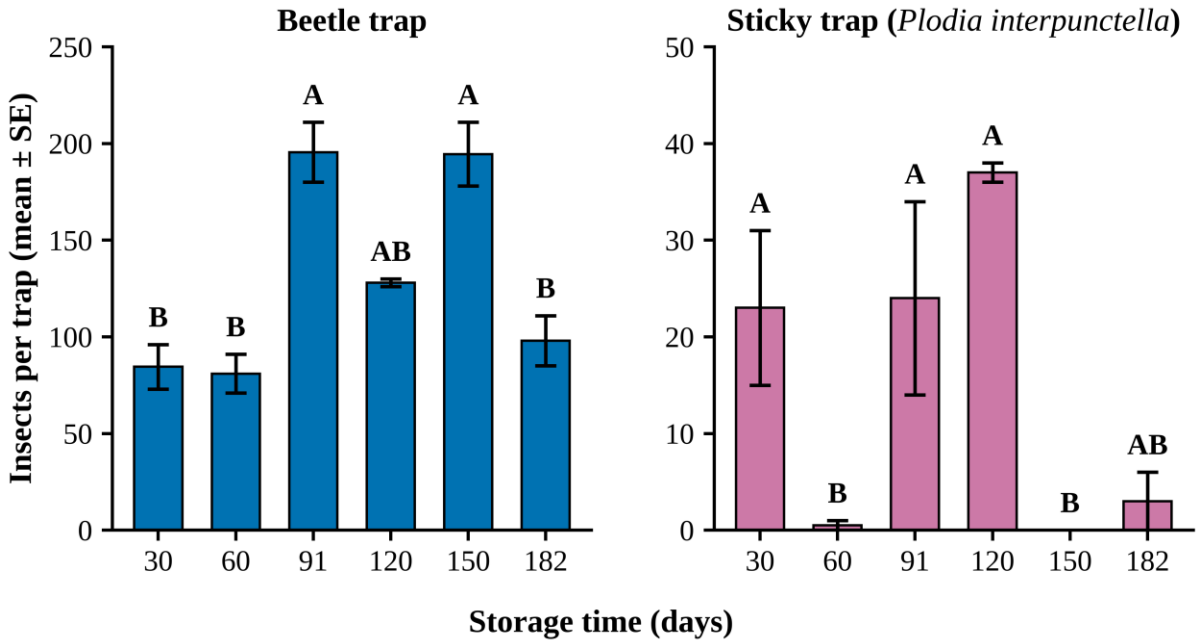


Figure 4.7. Mean (± SE) number of insects captured per beetle trap and per sticky trap (*Plodia interpunctella* only) at each sampling timepoint (n = 2 replicates, one trap of each type per replicate). Within each trap type, bars with the same letter do not differ among sampling timepoints (one-way ANOVA on log(x + 1)-transformed counts and Tukey's HSD test, $P > 0.05$).

Plodia interpunctella captures on sticky traps also differed among sampling timepoints ($F = 11.32$; df = 5, 6; $P = 0.005$). *Plodia interpunctella* were captured at 30, 91, and 120 d, whereas few or none were captured at 60, 150, and 182 d, and captures at 30, 91, and 120 d were greater than those at 60 and 150 d (Fig. 4.7). No *R. dominica* were captured in either trap type

during the trial, even though *R. dominica* were recovered from bags on every pallet at the final opening (Table 4.4).

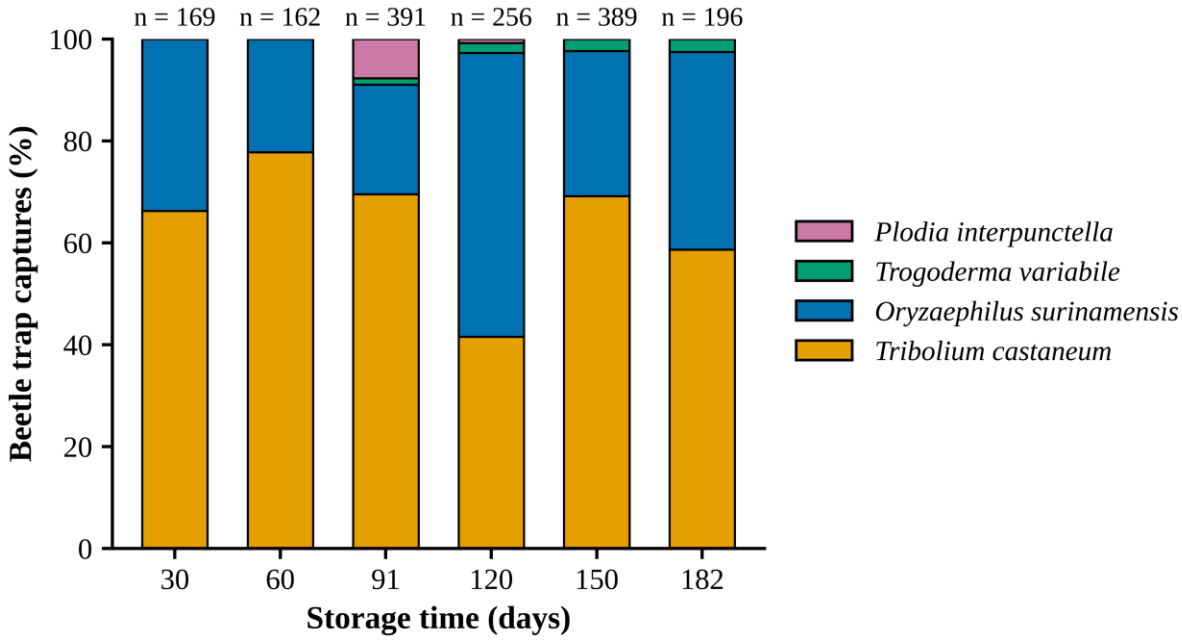


Figure 4.8. Species composition (%) of insects captured in beetle traps at each sampling timepoint, with both replicates combined. n = total number of insects captured in both beetle traps at each sampling timepoint. No *Rhyzopertha dominica* were captured.

4.3.3. Monthly Bag Samples

Moisture content, test weight, and insect infestation of the bags sampled every ~30 d are presented in Table 4.3. Moisture content was not affected by packaging type ($F = 0.21$; df = 1, 10; $P = 0.657$) or by the packaging type × sampling timepoint interaction ($F = 1.23$; df = 3, 7; $P = 0.368$), but it differed among sampling timepoints ($F = 11.13$; df = 3, 10; $P = 0.002$). Moisture content was greatest at 30 d (10.27%) and moisture content decreased on subsequent storage timepoints. Test weight was not affected by packaging type ($F = 0.22$; df = 1, 10; $P = 0.649$), sampling timepoint ($F = 0.67$; df = 3, 10; $P = 0.587$), or their interaction ($F = 0.97$; df = 3, 7; $P = 0.458$) and remained between 59.12 and 59.90 lb/bu. No insect-damaged kernels (IDK) were found in any monthly sample from either packaging type (0 of 43,032 kernels examined).

No live insects were recovered from any sampled bags, control or methoprene-treated, after 30 d or at 60 d of storage. However, there were three dead insects found in the control bags at 60 d. Live insects were first detected at 91 d of storage. After 91 d, all 10 control bags sampled contained live insects (11 insects), compared with 1 of 10 methoprene-treated bags (2 insects) (Table 4.3, Fig. 4.9). At 120 d, live insects were found in 1 of 10 control bags (2 insects) and 2 of 10 methoprene-treated bags (9 insects). At 150 d, live insects were found in 6 of 10 control bags (56 insects) and 4 of 10 methoprene-treated bags (26 insects), and the insects recovered were mainly *T. castaneum* and *T. variabile*, including larvae. From 91 to 150 d, live insects were found in 17 of 30 control bags (57%) and 7 of 30 methoprene-treated bags (23%). The probability that a sampled bag contained live insects was lower in methoprene-incorporated packaging ($\chi^2 = 8.36$; df = 1; $P = 0.004$) and differed among sampling timepoints ($\chi^2 = 9.86$; df = 2; $P = 0.007$). In total, 69 live insects were recovered from control bags and 37 from methoprene-treated bags, and the number of live insects per pallet was not affected by packaging type ($F = 0.60$; df = 1, 13; $P = 0.453$) or by the packaging type × sampling timepoint interaction ($F = 2.22$; df = 4, 9; $P = 0.147$). The number of live insects per pallet differed among sampling timepoints ($F = 9.67$; df = 4, 13; $P < 0.001$) and was greater at 150 d than at 30, 60, and 120 d, with 91 d intermediate (Fig. 4.9). No adult progeny emerged from any rice sample held for 8 weeks at 27 °C and 65% RH after sampling.

Table 4.3. Moisture content, test weight, and insect infestation of bagged rice in control and methoprene-treated packaging sampled at 30-d intervals.

	Control				Methoprene-treated			
Day	Moisture (%)[a]	Test weight (lb/bu)[a]	Bags with live insects[b]	Live insects (n)[c]	Moisture (%)[a]	Test weight (lb/bu)[a]	Bags with live insects[b]	Live insects (n)[c]
30	9.95 ± 0.61	59.28 ± 0.68	0/10	0	10.59 ± 0.17	59.46 ± 0.22	0/10	0
60	9.03 ± 0.41	59.87 ± 0.37	0/10	0	8.77 ± 0.49	59.12 ± 0.24	0/10	0
91	9.07 ± 0.29	59.61 ± 0.13	10/10	11	8.62 ± 0.46	59.57 ± 0.29	1/10	2
120[d]	NR	NR	1/10	2	NR	NR	2/10	9
150	8.67 ± 0.31	59.71 ± 0.27	6/10	56	8.31 ± 0.13	59.90 ± 0.34	4/10	26
Total			17/50	69			7/50	37

[a] Moisture content and test weight are mean ± SE (n = 2 replicates, each value the mean of five bags per pallet).

[b] Bags with live insects = number of sampled bags containing ≥ 1 live insect out of 10 bags (five per pallet in each replicate).

[c] Live insects (n) = total live insects (adults and larvae) recovered from the 10 bags.

[d] NR = not recorded due to equipment error.

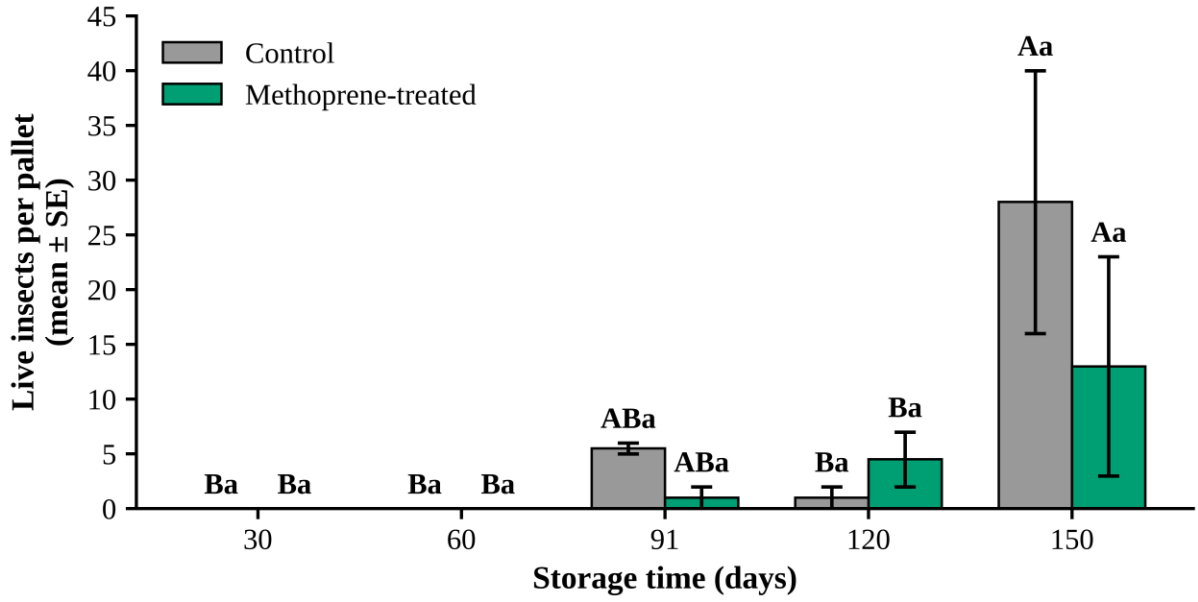


Figure 4.9. Mean (± SE) number of live insects recovered from the five bags sampled from each control and methoprene-treated pallet at each sampling timepoint (n = 2 replicates). Different uppercase letters indicate differences among sampling timepoints, and the same lowercase letter indicates no difference between packaging types within a sampling timepoint (randomized complete block ANOVA on log(x + 1)-transformed counts and Tukey's HSD test, $P < 0.05$).

4.3.4. Pallet Observations and Inspection Scores

At the start of the trial (day 0), all four pallets consisted of 25 newly filled and sewn bags stacked in five layers, with no tears, open seams, or other visible damage and no insects present on or around the pallets. At every monthly inspection from 30 to 150 d, all four pallets received the highest score (4) for both grain quality and packaging integrity, indicating that no visible damage to the grain or packaging was detected on the outside of the pallets. The few changes recorded during this period were on control pallets and included one bag with a slightly opened seam at 60 and 91 d, a few *T. variabile* and *P. interpunctella* adults on the pallet at 91 d, insects on the pallet floor at 120 d, and discoloration around the sampling hole of a previously sampled bag at 150 d. Pallet-level scores were not recorded at 182 d. Instead, all bags were scored individually at the final bag opening (Section 4.3.5).

At the end of the trial (182 d), visible signs of insect activity were present on both packaging types, and all were recorded on bags in the bottom three layers of the pallets (layers 1-3). These included insects on the outside of bags, insect cast skins and dead insects on bags, live insects inside bags when opened, discoloration around sampling holes, and one bag with a loosened weave.

4.3.5. Final Bag Opening

At the final bag opening (182 d), all 100 bags were emptied and sieved, and 819 insects were recovered (Table 4.4). Methoprene-treated pallets held 122.5 ± 28.5 insects, compared with 287.0 ± 130.0 insects on control pallets, a reduction of 57.3%, but this difference was not significant ($F = 1.11$; df = 1, 1; $P = 0.483$). The reduction differed between the two replicates (3.8% and 77.5%). Insects per bag averaged 4.90 ± 1.14 in methoprene-treated packaging and 11.48 ± 5.20 in control packaging ($P = 0.484$). Insects were recovered from 12 of 50

methoprene-treated bags (24%) and 14 of 50 control bags (28%), and the number of infested bags per pallet did not differ between packaging types ($P = 0.500$). Infested bags held 20.4 ± 4.8 insects in methoprene-treated packaging and 39.1 ± 13.0 insects in control packaging ($P = 0.483$). The highest number recovered from a single bag was 89, from a control bag.

Oryzaephilus surinamensis and *R. dominica* were the most abundant species in the bags and accounted for 42.2% and 35.3% of all insects recovered, followed by *T. castaneum* (20.9%) and *T. variabile* (1.5%). Methoprene-incorporated packaging reduced the mean numbers of *T. castaneum* by 52.6%, *O. surinamensis* by 63.8%, and *R. dominica* by 48.7% relative to untreated packaging, but none of these differences was significant ($P = 0.318$-0.649, Table 4.4). *Trogoderma variabile* were recovered only from control bags (6.0 ± 4.0 per pallet) and were absent from all methoprene-treated bags ($P = 0.226$). No *P. interpunctella* were recovered from any bag, and one *Sitophilus oryzae* (L.), rice weevil, was recovered from a control bag.

Table 4.4. Number of stored-product insects, by species, and infestation of bagged rice in control and methoprene-treated packaging at the final bag opening (182 d).

Variable	Control[a]	Methoprene-treated[a]	Reduction (%)[d]	*P*[e]
Insects per pallet (n)				
T. castaneum	58.0 ± 38.0	27.5 ± 0.5	52.6	0.649
O. surinamensis	127.0 ± 43.0	46.0 ± 8.0	63.8	0.318
R. dominica	95.5 ± 45.5	49.0 ± 21.0	48.7	0.631
T. variabile	6.0 ± 4.0	0.0 ± 0.0	100.0	0.226
Total insects[b]	287.0 ± 130.0	122.5 ± 28.5	57.3	0.483
Infestation of bags				
Insects per bag	11.48 ± 5.20	4.90 ± 1.14	57.3	0.484
Infested bags (of 50)[c]	14 (28%)	12 (24%)	14.3	0.500
Insects per infested bag	39.1 ± 13.0	20.4 ± 4.8	47.8	0.483

[a] Values are mean ± SE per pallet (n = 2 replicates).

[b] Total insects includes one *Sitophilus oryzae* (L.), rice weevil, recovered from a control bag. No *P. interpunctella* were recovered from any bag.

[c] Infested bags are the total number of bags containing ≥ 1 insect out of 50 bags per packaging type (25 bags per pallet × 2 replicates), with the percentage in parentheses.

[d] Reduction (%) = [(control − methoprene-treated) / control] × 100.

[e] *P* values are for packaging type in a randomized complete block ANOVA with replicate as the block (df = 1, 1), on log(x + 1)-transformed counts, except infested bags per pallet and insects per infested bag, which were not transformed.

Infestation at the final bag opening was concentrated in the bottom of the pallets (Table 4.5, Fig. 4.10). The bottom layer (layer 1) contained 81.4% of all insects recovered, and layers 1 and 2 together contained 99.8%. One bag in layer 3 of a control pallet contained two insects, and no insects were recovered from layers 4 and 5 of any pallet. All five bottom-layer bags were infested on three of the four pallets, and four of five were infested on the remaining methoprene-treated pallet. The number of insects per bag differed among layers in both control ($F = 43.94$; df = 4, 4; $P = 0.002$) and methoprene-treated packaging ($F = 17.10$; df = 4, 4; $P = 0.009$). In control packaging, layers 1 and 2 (44.5 ± 18.7 and 12.7 ± 7.1 insects per bag) held more insects than layers 3-5 (≤ 0.2 insects per bag), whereas in methoprene-treated packaging only layer 1 (22.2 ± 7.4 insects per bag) differed from the other layers (≤ 2.3 insects per bag).

Moisture content, broken kernels, and fine dust/frass at 182 d were not affected by packaging type ($P \geq 0.166$) or by the packaging type × layer interaction ($P \geq 0.659$), but all three differed among layers (Table 4.5). Fine dust/frass decreased from 3.63 g in layer 1 to 0.44 g in layer 5 ($F = 43.15$; df = 4, 13; $P < 0.001$), broken kernels decreased from 35.95 g in layer 1 to 20.47 g in layer 5 ($F = 3.70$; df = 4, 13; $P = 0.032$), and moisture content decreased from 10.20% in layer 1 to 9.46% in layer 5 ($F = 3.93$; df = 4, 13; $P = 0.026$). Test weight did not differ among layers ($P = 0.635$). When averaged across whole pallets, moisture content (9.78-10.00%), test weight (59.26-59.31 lb/bu), broken kernels (25.68-27.69 g), and fine dust/frass (1.39-1.41 g) did

not differ between packaging types ($P \geq 0.217$). No IDK were found in any bag from either packaging type (0 of 30,000 kernels examined in three 100-kernel subsamples per bag).

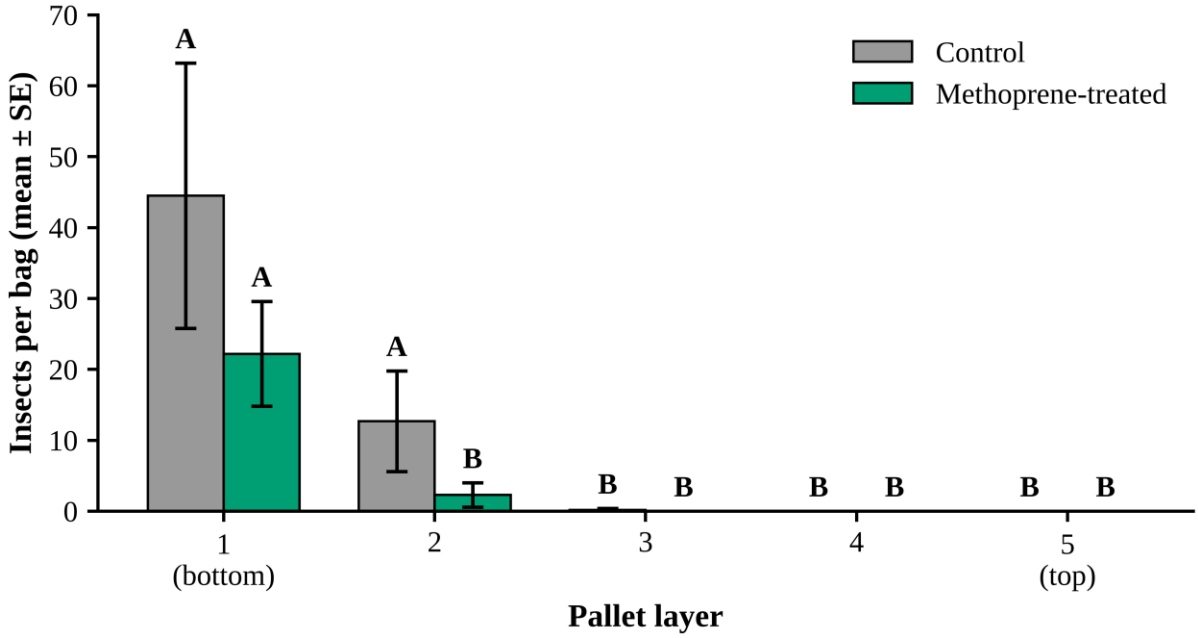


Figure 4.10. Mean (± SE) number of insects per bag in each pallet layer at the final bag opening (182 d) in control and methoprene-treated packaging. Within each packaging type, bars with the same letter do not differ among layers (randomized complete block ANOVA on log(x + 1)-transformed counts and Tukey's HSD test, $P > 0.05$). Layer 1 = bottom bags resting on the pallet, layer 5 = top bags.

Table 4.5. Insect infestation, moisture content, test weight, broken kernels, and fine dust/frass of bagged rice by pallet layer at the final bag opening (182 d).

	Insects per bag[a]		Both packaging types[b]			
Layer[c]	Control	Methoprene-treated	Moisture content (%)	Test weight (lb/bu)	Broken kernels (g)	Fine dust/frass (g)
1	44.5 ± 18.7 A	22.2 ± 7.4 A	10.20 ± 0.36 A	59.22 ± 0.29	35.95 ± 4.01 A	3.63 ± 0.38 A
2	12.7 ± 7.1 A	2.3 ± 1.7 B	10.16 ± 0.25 AB	59.17 ± 0.15	27.97 ± 0.84 AB	1.53 ± 0.14 B
3	0.2 ± 0.2 B	0.0 ± 0.0 B	10.00 ± 0.17 AB	59.52 ± 0.20	26.23 ± 1.90 AB	0.87 ± 0.15 BC
4	0.0 ± 0.0 B	0.0 ± 0.0 B	9.64 ± 0.20 AB	59.15 ± 0.13	22.80 ± 2.91 AB	0.53 ± 0.11 C
5	0.0 ± 0.0 B	0.0 ± 0.0 B	9.46 ± 0.36 B	59.36 ± 0.14	20.47 ± 4.01 B	0.44 ± 0.01 C

[a] Insects per bag are mean ± SE (n = 2 replicates) for each packaging type, and within each packaging type, means followed by the same letter do not differ among layers (Tukey's HSD, $P > 0.05$).

[b] Packaging type did not affect moisture content, test weight, broken kernels, or fine dust/frass ($P \geq 0.166$), so these are mean ± SE of the four pallets (n = 4), and means in a column followed by the same letter do not differ among layers (Tukey's HSD, $P > 0.05$). Test weight did not differ among layers.

[c] Layers are numbered from the bottom of the stack: layer 1 (bottom) = bags 1-5, layer 5 (top) = bags 21-25.

Grain quality and packaging integrity scores at the final opening were high on all pallets. Mean grain quality scores were 3.90 ± 0.02 on control pallets and 3.80 ± 0.00 on methoprene-treated pallets ($P = 0.126$), and mean packaging integrity scores were 3.80 ± 0.00 and 3.78 ± 0.10, respectively ($P = 0.874$). Bags scoring below 4 were found only in layers 1-3, and most were in the bottom layer, which also had the greatest infestation. Grain quality scores below 4 were recorded for 4 of 50 control bags and 8 of 50 methoprene-treated bags, and packaging integrity scores below 4 for 9 and 10 of 50 bags, respectively. Mean scores in the bottom layer were 3.25 for grain quality and 3.20 for packaging integrity, compared with 3.80-4.00 in layers 2-5.

Inspection of the opened bags at 182 d showed small gaps between the stitches along the single line of stitching that closed each bag.

4.4. Discussion

The objective of this study was to determine whether methoprene-incorporated packaging reduces infestation of bagged rice and influences stored-product insect activity under simulated warehouse conditions. To our knowledge, this is the first evaluation of methoprene-incorporated packaging on full pallets of a bagged commodity in simulated warehouses, with a mixed community of five stored-product insect species and repeated introductions over 182 d. Previous evaluations of methoprene-incorporated packaging have been conducted in small laboratory arenas over periods shorter than one generation (Scheff et al., 2016, 2017, 2018a, 2019; Arthur, 2016b). Three main findings emerged from this pallet-scale study. First, infestation of the bagged

rice developed slowly, was not detected until 91 d, and remained low enough that no insect-damaged kernels were found at any time. Second, insects that entered the bags were concentrated in the bottom layer of the pallets, nearest the floor where insects were released. Third, methoprene-incorporated packaging reduced the number of insects in bags at 182 d by 57% and reduced the proportion of sampled bags that contained live insects, but the reduction in insect numbers was not significant with two replicates, and the packaging did not reduce the number of bags that became infested.

Environmental conditions in the simulated warehouses strongly influenced the course of the trial. Although the simulated warehouses were climate-controlled, temperatures within the pallets followed the outdoor seasonal pattern and rose from a monthly mean of 22.8 °C in January to 30.4 °C in June. For most stored-product insects, 25-33 °C is optimal for growth and reproduction, whereas at 13-25 °C insects can complete development and produce offspring, but more slowly (Fields, 1992). Pallet temperatures were at or below the lower limit of this optimal range during the first 60 d of storage and fell to 16.6-19.2 °C in one replicate during an arctic outbreak in late January 2026. Larvae of *T. castaneum*, for example, fail to develop into normal adults at 20 °C, and larval development is slower and mortality higher at low humidity (Howe, 1956). A similar seasonal pattern has been observed in commercial facilities, where few beetles were captured inside a food storage warehouse between November and June, most likely because of lower indoor temperatures (Arthur et al., 2014), and where trap captures of stored-product insects at a rice mill increased with temperature (McKay et al., 2017). The cool conditions during the first 60 d, combined with the time required for the released insects to locate the bags, enter them, and produce offspring, probably explain why no live insects were found in the sampled

bags at 30 and 60 d and why infestation was first detected at 91 d, after pallet temperatures exceeded 25 °C.

Relative humidity within the pallets was low throughout the trial (mean RH of 34-39%), particularly from February to April, when cold outdoor air was heated to maintain shed temperatures, and it increased only in May and June as outdoor air became warmer and more humid. The low RH dried the rice during storage, and moisture content declined from 10.27% at 30 d to 8.49-8.90% from 60 to 150 d in both packaging types. Low grain moisture and dry air are unfavorable for the population growth of many stored-product insects (Hagstrum and Subramanyam, 2006), and these conditions may have contributed to the slow development of infestation and the absence of measurable kernel damage. Within the pallets, the bottom layer was consistently the coolest and most humid position, which is consistent with its proximity to the floor and the reduced air movement at the base of the stack. Temperature and RH were similar in control and methoprene-treated pallets, so differences between packaging types cannot be attributed to differences in microclimate.

Trap captures reflected the activity of insects within each simulated warehouse as a whole, including insects from the repeated releases, rather than the response to a particular packaging type, because both pallets were held in the same simulated warehouse. Similar whole-warehouse monitoring approaches have been used to follow insect populations in pilot-scale warehouses (Toews et al., 2005; Wilkins et al., 2021), and in untreated pilot-scale warehouses, captures in pheromone-baited pitfall traps followed the population trends determined by direct sampling of food patches (Toews et al., 2009). *Tribolium castaneum* and *O. surinamensis* together made up 96% of beetle trap captures, which is consistent with the high mobility of walking beetles such as *T. castaneum* and their response to pitfall traps baited with pheromone

and food attractants (Campbell, 2012). Beetle trap captures were greatest at 91 and 150 d, after pallet temperatures exceeded 25 °C, which agrees with the positive relationship between temperature and trap captures at a rice mill (McKay et al., 2017) and with the increase in flight initiation of stored-product insects as temperatures rise (Cox et al., 2007). Captures declined again at 182 d, even though temperatures were highest at that time, which suggests that trap captures also depended on factors other than temperature, such as the timing of releases and the movement of insects into the rice. *Plodia interpunctella* captures on the pheromone-baited sticky traps were greatest at 30, 91, and 120 d. Because the sticky trap lure is a female sex pheromone, these captures represent males and indicate periods of adult emergence and mating activity of the released larval cohorts (Mohandass et al., 2007). The reason that no *P. interpunctella* were captured at 150 d, despite continued releases, is not clear and may reflect the timing of adult emergence relative to the trap replacement interval.

No *R. dominica* were captured in either trap type, although *R. dominica* made up 35.3% of the insects recovered from bags at 182 d. *Rhyzopertha dominica* is a primary, internal feeder whose immature stages develop inside kernels (Edde, 2012). The multi-species lure in the beetle trap attracts more than 25 stored-product insect species, which would include *R. dominica* (Trécé Inc., n.d.). Captures of *R. dominica* in northeast Kansas vary with season, landscape, and the attractant used (Scheff et al., 2022), and adults that remained within the grain may have had little opportunity to encounter the beetle trap on the floor. Trap captures alone would therefore have underestimated the presence of *R. dominica* in the stored product, and they did not show which insects were developing inside the bags. This result shows that monitoring of bagged commodities should combine traps with direct inspection or sampling of the product.

Infestation of packaged products can result from insects that are already present in the product before packaging or from insects that enter after packaging, either through existing openings or through openings that they create (Athanassiou et al., 2011b). In the present study, the rice was frozen before bagging, so it was not infested at the start of storage. No live insects were recovered from bags sampled at 30 and 60 d, and no progeny emerged from any of the subsamples, which indicates that the rice was most likely not infested within the first 60 d of storage. The distribution of these insects within the pallets was one of the clearest results of this study. At the final opening, the bottom layer contained 81.4% of all insects recovered, the second layer contained nearly all of the remainder, and no insects were recovered from the two upper layers of any pallet. The insects were released on the floor of the simulated warehouses, and most of the species used, including *T. castaneum*, *O. surinamensis*, and *R. dominica* adults and *T. variabile* and *P. interpunctella* larvae, readily walk and fly in search of food. The bags in the bottom layer were the first that these insects encountered as they moved from the floor onto the pallet, and the wooden pallet and slip sheet also provided crevices and sheltered spaces close to these bags. Movement of insects from the floor, residues, and the surrounding environment into stored products is a well-documented source of infestation in food facilities (Campbell and Mullen, 2004; Campbell et al., 2010). The cooler, more humid conditions in the bottom layer may also have made these bags more suitable for some species. Fine dust/frass, broken kernels, and moisture content also decreased from the bottom to the top of the stack. The greater amount of fine dust/frass in the bottom layer is consistent with the greater insect activity there. The larger amount of broken kernels in the bottom bags may also reflect compression from the weight of the four layers of bags above them, and the higher moisture content reflects the more humid conditions at the base of the stack.

The packaging used in this study was single-ply woven polypropylene closed with a single line of stitching, which is typical of commercial bags for rice and other grain products. Woven fabrics and sewn seams contain small openings, such as gaps between the stitches, needle holes, and spaces in the weave, that can be exploited by insects. Stored-product insects are commonly classified as penetrators, which chew through packaging materials, and invaders, which enter through existing openings (Highland, 1991; Mullen et al., 2012), and most common stored-product insects enter packaged food through existing openings rather than by penetrating intact packaging (Athanassiou et al., 2011b). *Tribolium castaneum* and *O. surinamensis* are typical invaders that enter packages through small gaps and seams, whereas *R. dominica* adults and the larvae of *T. variabile* and *P. interpunctella* can penetrate many packaging materials (Highland, 1991; Riudavets et al., 2007; Scheff et al., 2018b). Insects on the outside of bags, a loosened weave structure of the packaging material, and a partly opened seam were observed during the trial. These observations indicate that insects may have entered both packaging types through the seams and other openings in the package. Inspection of the opened bags showed small gaps between the stitches along the single line of stitching. Gaps of this size would allow small insects such as *O. surinamensis* and *T. castaneum* to enter the bags without chewing through the fabric, so the closure, rather than the methoprene-incorporated fabric, was probably the weakest point of the package. In addition, each bag was pierced once with a grain trier during monthly sampling. Although every sampling hole was resealed with polypropylene tape immediately after sampling, discoloration and insect activity were observed near sampling holes, and these resealed holes may have provided an additional point of entry. Because every bag in both packaging types was sampled once, this potential entry route was the same for both packaging types. Over time, as the insect populations in the simulated warehouses increased and

spread, insects searching for food and shelter could locate and exploit these openings in the package.

Methoprene is a juvenile hormone analog that disrupts development and metamorphosis of immature stages but has little direct toxicity to adults (Oberlander et al., 1997). Packaging-incorporated methoprene acts on insects that contact the treated packaging surface, and laboratory studies have shown that exposure to methoprene-incorporated polymer, woven, and foil packaging reduces larval development and egg-to-adult emergence of *T. castaneum*, *T. variabile*, and other *Trogoderma* spp. (Scheff et al., 2016, 2017, 2018a, 2019). Continual exposure to methoprene-treated woven packaging, the same type of material used in the present study, completely suppressed adult emergence of *T. castaneum* and reduced emergence of *T. variabile*, and it reduced the fecundity of *T. castaneum* adults but not that of *T. variabile* adults (Scheff et al., 2017). The stage of the insect that contacts the treated surface is also important. Wandering-phase larvae of *P. interpunctella* exposed briefly to methoprene on packaging materials produced fewer adults, whereas eggs were able to compensate for exposure to methoprene residues (Jenson et al., 2009), and late-stage larvae have been recommended as the most suitable life stage for evaluating methoprene-treated packaging films (Arthur, 2016b).

In the present study, methoprene-incorporated pallets held 57% fewer insects at 182 d than untreated pallets, and the mean numbers of *T. castaneum*, *O. surinamensis*, and *R. dominica* were 49-64% lower. Fewer sampled bags contained live insects in methoprene-incorporated packaging from 91 to 150 d, and *T. variabile* was recovered only from untreated bags. These results agree in direction with the laboratory studies. The absence of *T. variabile* from methoprene-incorporated bags is consistent with the susceptibility of *T. variabile* larvae to methoprene-treated packaging (Scheff et al., 2016, 2017, 2018a). In contrast, the number of

infested bags was similar between packaging types, which shows that methoprene-incorporated packaging did not prevent insects from infesting the bags. Adult insects are not killed by methoprene and can enter through seams and openings or lay eggs through these openings, and their offspring can develop within the rice, away from the treated packaging surface. This limitation is likely to be greatest for internal feeders such as *R. dominica*, whose larvae develop inside kernels (Hagstrum and Subramanyam, 2006; Edde, 2012). When methoprene is applied directly to the grain, it is highly effective against *R. dominica* and has suppressed progeny production on stored wheat and brown rice for up to 24 months (Arthur, 2016a). Packaging-incorporated methoprene, in contrast, is not distributed through the grain mass, so larvae developing in the center of a bag are unlikely to contact it. In addition, the insects counted at 182 d may have included adults that entered the bags as well as progeny from eggs laid through openings by adults outside the bags (Highland, 1991; Mullen et al., 2012). Methoprene would not be expected to affect these adults, and they would have reduced the measured difference between packaging types. Methoprene is stable across the range of temperatures found in indoor food storage, and its residual persistence depends more on the treated substrate than on temperature (Wijayaratne et al., 2012). The seasonal temperature changes in the present study were therefore unlikely to have reduced methoprene activity.

Packaging that incorporates an adulticide such as deltamethrin acts differently from methoprene-incorporated packaging. Fabric from deltamethrin-incorporated woven polypropylene bags knocked down 99% of *S. oryzae* and *T. castaneum* adults in less than 3 h, and none of the insects tested chewed through miniature bags made from this fabric (Paudyal et al., 2017a). Deltamethrin-incorporated bags have also controlled several stored-product beetles and *T. granarium* in laboratory tests (Kavallieratos et al., 2017; Scheff et al., 2020). In a field

study with bagged maize, however, deltamethrin-incorporated bags kept insect-damaged kernels below 5% for only 4 months, after which damage increased, possibly because the maize was already infested when it was bagged (Paudyal et al., 2017b). Deltamethrin-incorporated packaging therefore targets the adults that enter bags, whereas methoprene-incorporated packaging targets the development of their offspring. The present results suggest that the two modes of action are complementary, and combining methoprene with an adulticide in the packaging, or combining methoprene-incorporated packaging with long-lasting insecticide-incorporated netting, deserves evaluation.

Although the reduction in insect numbers was consistent in direction for every species, it was not statistically significant. Each simulated warehouse contained only one pallet of each packaging type, so the comparison was based on two replicates with one error degree of freedom, and the reduction differed greatly between the two replicates (3.8% and 77.5%). With low and patchy infestation, the number of insects recovered from a pallet depended heavily on a few bags in the bottom layer, and a single untreated bag contained 89 insects. The outcome on each pallet may therefore have been influenced by the chance entry of a small number of reproducing adults into individual bags. The analysis of sampled bags, in which individual bags were the observations, detected a lower proportion of infested bags in methoprene-incorporated packaging. However, bags on the same pallet are subsamples of one experimental unit rather than independent replicates, and treating them as replicates would overstate the evidence for a treatment effect (Hurlbert, 1984). The lack of a significant difference at the pallet level should therefore not be taken as evidence that methoprene-incorporated packaging had no effect. Rather, a 57% reduction with two replicates indicates that a study with more replicated pallets and storage units is needed to determine the size of the effect with confidence. Nevertheless, the

consistent reductions across species and the absence of *T. variabile* from methoprene-incorporated bags indicate that methoprene-incorporated packaging has the potential to reduce insect populations developing within bagged commodities under simulated warehouse conditions, as suggested by laboratory studies (Scheff et al., 2016, 2017).

Grain quality was largely maintained in both packaging types throughout the trial. No insect-damaged kernels were detected in any of the more than 73,000 kernels examined, moisture content, test weight, broken kernels, and fine dust/frass did not differ between packaging types, and grain quality and packaging integrity scores were reduced only in a small number of bags, mostly in the bottom layer. Infestation of milled rice by *R. dominica* and other species can reduce its quality and market value (Su et al., 2019). In a field study of bagged maize, insect-damaged kernels in untreated bags reached 32% after 6 months of storage (Paudyal et al., 2017b). The absence of kernel damage in the present study probably reflects the uninfested milled rice at the start of storage, the relatively low insect densities reached in the bags, and the cool and dry conditions during the first half of the trial. Longer storage periods, warmer conditions, or higher insect pressure, such as those encountered during long-distance shipment or summer storage, may produce greater differences between packaging types.

From a practical perspective, these results indicate that methoprene-incorporated packaging should be viewed as one component of an integrated pest management program rather than a stand-alone barrier (Phillips and Throne, 2010; Mullen et al., 2012). Its effectiveness is likely to be improved by bag closures that reduce openings at the seam, such as double stitching or taped or heat-sealed closures, and by minimizing damage to bags during handling and sampling. Because infestations were concentrated in the bottom layer of each pallet, inspections should focus on bottom-layer bags, and sanitation of floors and pallets, removal of spillage, and

the use of clean pallets or physical separation between the floor and the bags may further reduce infestation of stored bags. Combining treated packaging with residual insecticide treatments of storage and transport surfaces, such as those evaluated in Chapters 2 and 3, may also limit the number of insects that reach the bags.

This study had several limitations. The trial was conducted once, in two simulated warehouses with one pallet of each packaging type in each, so each comparison between packaging types had only two replicates and one error degree of freedom, which limited the power to detect differences. The 182-d storage period included an unusually cold winter that affected one replicate more than the other. In addition, the simulated warehouses (approximately 45 m^3) were much smaller than commercial warehouses, and the repeated releases of 1,000 insects every 25-30 d created concentrated insect pressure close to the pallets, so infestation patterns in larger commercial storage may differ. Future studies should use more replicated pallets in separate storage units and repeat the trial across multiple storage seasons, evaluate different bag closures, and test methoprene-incorporated packaging in combination with adulticidal packaging or netting.

4.5. Conclusion

Methoprene-incorporated woven polypropylene packaging was evaluated on pallets of bagged milled rice in two replicate simulated warehouses over 182 d with a mixed community of five stored-product insect species. Infestation developed slowly under the cool, dry winter conditions, was first detected at 91 d, and was concentrated in the bottom layer of the pallets, closest to the floor where insects were released. Insects infested both packaging types, most likely through the single-stitched seams, the openings of the woven fabric, and/or resealed sampling holes. Methoprene-incorporated packaging reduced the number of insects in bags at

182 d by 57%, reduced the proportion of sampled bags containing live insects, and excluded *T. variabile* from treated bags, but the reduction in insect numbers was not significant with two replicates, and the number of infested bags was similar between packaging types (12 of 50 vs. 14 of 50). No insect-damaged kernels were detected, and moisture content, test weight, and grain quality were similar between packaging types. Methoprene-incorporated packaging can therefore reduce the development of insect populations within bagged commodities, but it does not prevent insects from infesting bags. It is best used together with improved bag closures, sanitation of floors and pallets, and regular monitoring as part of an integrated pest management program for stored and transported bagged commodities, and its effect should be confirmed in a larger, replicated study.

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Chapter 5 - Conclusions and Future Directions

5.1. Overview

The overall goal of this thesis project was to evaluate non-fumigant tools that could reduce the reliance on fumigation for protecting bagged commodities during storage and transport. The three research chapters addressed this goal at three successive points in the supply chain. Research presented in Chapter 2 evaluated residual contact insecticides applied to the wood and metal surfaces of shipping containers before loading, under seasonal field conditions in Arkansas and Kansas. Research presented in Chapter 3 examined, under controlled laboratory conditions, how surface type and temperature determine the efficacy and persistence of the same insecticides against three major stored-product beetles. Research described in Chapter 4 moved from the structure to the commodity itself and evaluated methoprene-incorporated packaging on pallets of bagged rice in simulated warehouses. Together, these studies describe two complementary layers of protection, the treated structure and the treated package, and identify the conditions under which each layer is most and least effective.

5.2. Summary of Major Findings

Residual surface treatments in shipping containers (Chapter 2). β-cyfluthrin and liquid combo applied as pre-loading treatments inside shipping containers reduced the survival of *Tribolium castaneum* and *Sitophilus oryzae* relative to the untreated control at nearly every residual interval over 16 weeks, across four seasonal trials with widely fluctuating temperatures. Efficacy on metal was stable throughout the evaluation, and liquid combo caused complete mortality of both species on metal after 168 h of exposure. On plywood, short-term (24 h) mortality of *T. castaneum* declined to control levels by week 8, but mortality after 168 h remained greater than in the control through week 16, showing that biologically active residues

persisted on wood longer than short exposures indicated. Progeny production on treated surfaces was nearly eliminated for both species, even where adult mortality was incomplete.

Effects of surface type and temperature (Chapter 3). Under controlled conditions, residual efficacy was determined jointly by surface type, temperature, insect species, and formulation rather than by any single factor. Metal provided the most persistent control, with 98-100% mortality of *S. oryzae* and *Rhyzopertha dominica* after 168 h at every temperature from 16 to 37 °C, whereas concrete provided the least, and no *T. castaneum* mortality occurred on concrete from week 12 onward. *Tribolium castaneum* was the most difficult species to control on porous surfaces, with mortality falling to 44% or less within 4 weeks on wood and concrete at most temperatures. Initial mortality generally increased with temperature, liquid combo was generally more effective on metal, and β-cyfluthrin was generally more effective on concrete.

Methoprene-incorporated packaging in simulated warehouses (Chapter 4). On pallets of bagged milled rice held for 182 d with repeated releases of five stored-product species, infestation developed slowly under cool, dry winter conditions, was first detected at 91 d, and was concentrated in the bottom layer of the pallets, closest to the floor where insects were released. No insect-damaged kernels were detected. Methoprene-incorporated packaging reduced the number of insects in bags at 182 d by 57% and reduced the proportion of sampled bags containing live insects from 91 to 150 d, and *Trogoderma variabile* was recovered only from untreated bags. However, the reduction in insect numbers was not significant with two replicates, and the number of infested bags was similar between packaging types (12 of 50 vs. 14 of 50). Insects infested both packaging types, most likely through the single-stitched seams, the openings of the woven fabric, and/or resealed sampling holes.

5.3. Integrated Implications for Pest Management

Several themes were common to the three studies. First, no single tactic provided complete protection. Residual insecticides lost efficacy on porous surfaces within weeks, and methoprene-incorporated packaging reduced insect numbers within bags but did not prevent insects from infesting them. Each tool is therefore best used as one layer of an integrated program that also includes sanitation, exclusion, and monitoring. Second, the products containing methoprene, liquid combo on treated surfaces and methoprene-incorporated packaging, both acted strongly on the next generation: progeny production on liquid combo-treated surfaces was nearly eliminated in Chapter 2, and fewer insects, and no *T. variabile*, were recovered from treated bags in Chapter 4. The main value of insect growth regulators in these systems is therefore to limit population build-up over time rather than to provide rapid knockdown of adults, which should be considered when evaluating their performance with short-term mortality assessments.

Third, *T. castaneum* was consistently the most challenging species. It was the species least affected by residues on wood and concrete in Chapters 2 and 3, and it dominated trap captures in the simulated warehouses in Chapter 4. Because this species is highly mobile and readily survives brief contact with aged residues, structures where it is the main pest are likely to need more frequent re-treatment and closer attention to sanitation. Fourth, the environment strongly influenced each tool. Temperature changed the initial efficacy of the residual insecticides, and the cold winter conditions in Chapter 4 delayed insect activity and infestation. Pest management decisions, including the timing of treatments and re-treatment intervals, should therefore account for the surface type, the expected temperature range, and the season of storage or shipment.

For practical application, the results support a layered approach for bagged commodities in containers and warehouses. First, apply a residual insecticide to the container or storage surfaces close to loading, selecting the product according to the predominant surface, and re-treat porous surfaces more frequently than metal. Second, use methoprene-incorporated packaging with improved closures, such as double-stitched, taped, or heat-sealed seams. Third, keep floors, pallets, and slip sheets clean and free of spillage, because infestation began in the bags nearest the floor. Finally, monitor insect activity with traps suited to the target species.

5.4. Limitations

These conclusions should be interpreted within the limitations of each study. The field trials in Chapter 2 were conducted in empty containers with bioassay arenas rather than in containers loaded with commodities, and they used laboratory strains that were susceptible to insecticides. The laboratory study in Chapter 3 used constant temperatures, whereas temperatures in containers and warehouses fluctuate daily and seasonally. The packaging study in Chapter 4 was conducted once, in two simulated warehouses with a single pallet of each packaging type, so each comparison between packaging types had only two replicates and limited power to detect differences, and the simulated warehouses were much smaller than commercial storage facilities. Residues of the active ingredients on the treated surfaces were not measured in Chapters 2 and 3.

5.5. Future Directions

The findings of this thesis identify several priorities for future research:

1. *Combined tactics in commodity-loaded containers.* Pre-loading surface treatments, methoprene-incorporated packaging, and fumigation should be evaluated together in shipping containers loaded with bagged commodities during simulated or actual transport, to determine whether the layers of protection are additive.

2. *Fluctuating temperatures and longer residual periods.* Residual efficacy should be evaluated under fluctuating temperature regimes that reproduce container and warehouse conditions, and over residual periods longer than 12-16 weeks that match long-distance shipment and storage.

3. *Resistant field populations.* The efficacy of β-cyfluthrin, liquid combo, and methoprene-incorporated packaging should be determined for field-collected populations with documented pyrethroid or phosphine resistance.

4. *Replicated packaging trials and improved closures.* Methoprene-incorporated packaging should be evaluated with replicated pallets in separate storage units, with different bag closures, and with measurement of methoprene residues on the packaging over time.

5. *Monitoring and spatial distribution.* Trap monitoring should be combined with direct sampling of bags in the bottom layers of pallets to better describe insect movement from floors and pallets into stored bags.

6. *Economic evaluation and outreach.* The costs and benefits of combined non-fumigant programs relative to repeated fumigation should be evaluated, and the results should be communicated to the food industry, shippers, and humanitarian food-aid programs that rely on bagged commodities.

5.6. Concluding Remarks

This thesis provides field, laboratory, and simulated warehouse evidence that residual contact insecticides and methoprene-incorporated packaging can contribute to the protection of bagged commodities without relying on fumigation. Their effectiveness, however, depends on the surface treated, the temperature, the insect species, and how well the package is closed. Used together, and combined with sanitation and monitoring, these tools can form a practical

integrated approach for protecting stored and transported commodities and for reducing the reliance on methyl bromide and phosphine in the postharvest supply chain.