

**RESIDUAL TOXICITIES OF SYNERGIZED PYRETHRINS AND METHOPRENE
APPLIED AS AEROSOL INSECTICIDES**

by

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Abstract

Tribolium spp. are major pests in structures used for the processing and storage of grain-based products (e.g., flourmills, warehouses, retail stores). Consumers and regulators have little tolerance for insect-damaged or contaminated food products. The direction and breadth of pest-control strategies in the food industry have changed significantly over the past few years, creating the need to optimize insecticides through improved integrated pest management (IPM) techniques, specifically through the identification of new control agents that are low in mammalian toxicity, as well as any factors that might affect susceptibility to these agents. There is currently renewed interest in developing reduced-risk, low toxicity chemicals that can be effectively utilized in a setting in which grain and other food commodities are vulnerable to insect infestation, as a means of replacing outdated, and at times, less effective methods of insect control. Over the past decade, developed countries have made significant progress toward alternative insect control strategies by employing a variety of applied insecticides. Two classes of insecticide include natural pyrethrum and insect growth regulators (IGRs), which are substances that mimic insect hormones essential to normal development and reproduction.

Pyrethrin is a highly efficient, broad spectrum, botanical insecticide that causes a rapid knockdown in exposed insects. Synergists are used to extend the economic usage of natural pyrethrins and because pyrethrum is rapidly metabolized, it is often mixed with a synergist. Methoprene, a juvenile hormone analog, is labeled as an aerosol and surface treatment inside mills, warehouses and other food storage facilities. There is little recent research with large-scale aerosol applications in stored-food facilities; furthermore, there are few published references regarding the efficacy of using methoprene in combination with synergized pyrethrin, in aerosol form. Therefore, the purpose of this research was to evaluate the use of aerosol applications of two aerosol concentrations on flour and finished stored-product packaging materials for the control of *Tribolium* spp. Results of this research show that *T. castaneum* are effectively controlled with 1% aerosol application, while the 3% formulation is required to effectively control *T. confusum*. With regards to the various packaging material surfaces, few differences between the surfaces emerged.

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Appendix Table 3.7C. Percentage (mean \pm SE) of larvae morphological characteristics: individuals remaining in the larvae stage, those that died while in the larvae stage and those that were arrested between larvae and pupae development, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated cotton bag surface, for the two exposed *Tribolium* species, at each post-application storage interval.....263

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Literature Review

Biology, Significance and Control of *Tribolium* spp.

Life History Characteristics

Flour beetles of the genus *Tribolium* and related genera of the family Tenebrionidae include the red flour beetle, *Tribolium castaneum* Herbst and the confused flour beetle, *Tribolium confusum* Jacquelin DuVal, two major pests of stored grain products (Champ and Dyte, 1976). These *Tribolium* spp. can be major pests in structures used for the processing and storage of grain-based products (e.g., flourmills, warehouses, retail stores) (Campbell and Runnion, 2003), constituting important primary and secondary pests in many cereal products (Arnaud et al., 2005). *Tribolium* spp. are holometabolous insects, displaying a complete metamorphosis life cycle, in which all four-life stages differ in appearance (Rees, 2004). The white, microscopic eggs are laid amongst the infested commodity, and secrete a substance that provides them with a rather sticky outer membrane, allowing fine particles, such as flour to cling to their surfaces. This property conveniently provides a degree of camouflage, as well as permitting their adherence to surfaces or other packaging materials, throughout the egg life stage (Alabi et al., 2008). Upon hatching, the larvae immediately move in search of food. Their small size readily allows them access to finished products and other packaged materials, which are often major weak links for an impending insect invasion (Conner and Via, 1992). These slender, elateriform, mobile larvae appear a yellow-brown color, living and feeding directly within the infested commodity. Each individual can display as many as 5-11 larval instars; however, 7-8 are most common. This instar variation often tends to be a result of the environment, food (availability and quality), temperature, humidity, or due to other pressures exerted on the individual insect (Howe, 1956). The pupae are whitish, immobile, unprotected and non-feeding (Weston and Rattlingourd, 2000), and therefore cause less overall product damage.

Tribolium spp. adults are reddish-brown, ranging from 3 to 4 mm long and live and feed in the infested commodity (Walter, 1990). The adult *T. castaneum* and *T. confusum* have subtle morphological differences. The antennae of *T. castaneum* end in a three-segmented club, whereas *T. confusum* antennae end in a 4-segmented club that gradually enlarges toward the top,

but does not form a distinctive club (Bousquet, 1990; Walter, 1990). The head of *T. castaneum* does not have a visible beak and the thorax that has slightly curved sides, which conceals the joint between the thorax and the abdomen. In contrast, *T. confusum* has a visible beak, and a less rounded thorax, which makes their bodies appear more parallel, and allowing for the joint between the thorax and the abdomen to be visible and distinct (Ryan et al., 1970). Tenebrionids are adapted to arid environments, with features for increased water retention (Duncan, 2003). The relationships among species of *Tribolium* were loosely considered by Hinton (1948), based on their geographic distributions and morphological characters such as body size, the number of enlarged antennal segments forming the club, and the form of margins on the vertex and pronotum. The dates of lineage splits separating *T. castaneum* from its relatives remain obscure. It was Hinton's supposition that *Tribolium* species groups were very old due to their wide geographic distributions. Angelini and Jockusch (2008) assessed phylogenetic relationships within *Tribolium*, and review several lines of evidence supporting monophyly of *T. castaneum* species group. In their study, the relationships of *Tribolium* species are elucidated with DNA sequence data from two mitochondrial and three nuclear markers using several phylogenetic inference methods, including parsimony, likelihood, and Bayesian inference using various partitioning strategies. Within the limits of taxon sampling, monophyly of *T. castaneum* and *T. confusum* species groups was strongly supported, and the combined analyses strongly support a sister group relationship between these lineages.

Mixed populations of *T. castaneum* and *T. confusum* do not occur often, as the competition between the two species has been reported to be intense. However, when these mixed populations do occur, they tend to do so only at low densities, because as population densities increase, the level of competition also increases, often concluding with one of species dominating and out-competing the other (Ryan et al., 1970). Cannibalistic behavior has been observed in *Tribolium* populations, whereby eggs, early larvae, and pupae are consumed by the feeding adults (Via, 1999). Previous studies have reported nutritional benefits to the larvae from egg consumption, such that upon emergence into the adult stage, a significant increase in fecundity was observed (Ho and Dawson, 1966; Sokoloff et al., 1966ab; Mertz and Robertson, 1970; Via, 1999). Cannibalism as observed within mixed-species *Tribolium* spp. populations is a

potent force in the regulation that mediates the interspecific competition between *T. castaneum* and *T. confusum* (Ho, 1966; Via, 1999).

Tribolium spp. can complete development within a wide range of temperatures and relative humidity (r.h.) conditions. Howe (1956) demonstrated that when beetles were reared between 20° and 37.5° C, and at an r.h. greater than 70%, adult development could be achieved in as little as 19-20 days. Accordingly, *T. confusum* can develop in environments with r.h. as low as 10%, a level that is prohibitive for the development of most other stored product insect pests. However, developmental rates of the two *Tribolium* species will also vary depending upon rearing temperature conditions. When *T. castaneum* populations were reared at 30° and 34° C, the development times for eggs, larvae, and pupae were observed as: 3 d and 2 d; 20 d and 15 d; and, 4 d and 3 d, respectively. The adult reproductive maturation time of *T. castaneum* and *T. confusum* was shown to be 5 d and 4 d, respectively, while the total development time from egg to adult was observed as 32 d and 24 d, respectively (Howe, 1956; Fedina and Lewis, 2007). Both, *T. castaneum* and *T. confusum* adult beetles are long-lived, often with life spans of more than three years (Walter, 1990). In isolation, male *T. castaneum* live for as long as 2.5 years, and male *T. confusum* for up to 3.5 years. However, in groups or populations, the life expectancy of adult *T. castaneum* is reduced to six months, while that of *T. confusum* is still somewhat longer (Skoloff, 1972).

Because *Tribolium* feed primarily externally on stored grains (i.e., ‘secondary colonizers’), they will often have a greater reproductive capacity than primary feeders, which increases their potential for both causing losses and damaging grain products (Weston and Rattlingourd, 2000). *Tribolium* spp. males become sexually mature two days post-eclosion, whereas, females become sexually mature within a few days after emergence. The adult females reach their maximum egg-laying capacity between five to ten days old, and can successfully mate as early as 3 hours post eclosion, however o not begin laying eggs until almost four days later (Dawson, 1965; Fedina and Lewis, 2007). With the stimulus of a mate, females can lay on average 10 eggs per day over a period of 3-6 months, after which time the oviposition rates begin to slowly decline. Previous fecundity studies have shown that female *T. castaneum* can maintain high rates of oviposition for over 100 days at 25° C, sometimes laying eggs for over 300 days (Good, 1936; Howe, 1962). The female *Tribolium* adults are quite prolific, lying between 500-

1500 eggs (Sokoloff, 1974), more or less continuously during their life spans (Fedina and Lewis, 2007). Their atypically long adult lifespan, combined with the prolonged amount of time in which they are reproductively active, is suggested as a coping strategy of sorts, enabling them to effectively contend with fluctuating conditions during characteristic colonization-overexploitation cycles (Fedina and Lewis, 2007), as would commonly be experienced in mills, food warehouses and other stored product facilities.

Many of *Tribolium* spp. life-history traits show considerable phenotypic plasticity in response to environmental variation. Varying temperature, r.h., or food quality has been shown to greatly influence flour beetle development and behavior (Sokoloff, 1974). *Tribolium* spp. populations exhibit density fluctuations that affect both their biotic and abiotic environments. As efficient colonizers, *Tribolium* spp. can often achieve a relatively high intrinsic population growth rate, fast maturation, rapid larval development, as well as rapid dispersal activity (Fedina and Lewis, 2008). The rate of oviposition can be influenced by a range of both internal and external factors, including environmental conditions (i.e., temperature or r.h.) (Good, 1936; Park and Frank, 1948; Howe, 1962), the type and quality of available food material (Good, 1936), as well as conditions of over crowding (Birch et al., 1951). Nutritional quality of the flour medium is of the utmost importance to *Tribolium* spp. population dynamics, such that any slight change to the immediate environment can cause a significant decrease in the oviposition rate (Fedina and Lewis, 2007).

Unlike many insects, the flour beetles subsist in an infested food resource that is shared by both the adult and the juvenile stages (Weston and Rattlingourd, 2000; Fedina and Lewis, 2007). *Tribolium* adults can survive, develop and reproduce under conditions that are not suitable for the development of other stored product insect pests, allowing high population densities to be easily built up (Champ and Dyte, 1976; Hill, 1990). As the population density increases, the shared flour medium that *Tribolium* spp. stages occupy begins to lose its nutritional quality, often accumulating waste products and other ethyl- and methylbenzoquinones, produced by adults and released as defensive compounds (Sokoloff, 1974). This density dependence has also been shown to cause extended larval developmental times (Nakakita, 1990; Kotaki, 1995), increased preadult mortality due to cannibalism by the active adult and larvae stages on the inactive egg

and pupae stages (Ho and Dawson, 1966; Sokoloff, 1974; Via, 1999; Alabi et al., 2008), and further induced dispersal (Ziegler, 1977). *Tribolium* spp. has been known to cause significant damage in a number of stored commodities, within many stored product industry environments. The ease of their adaptability to survive harsh environments as well as developmental conditions, combined with their high reproductive capability, allows these insect pests the potential to cause significant product damage in and around food storage facilities.

Significance: Damage Caused by *Tribolium* spp.

Tribolium spp. has been associated with stored food for more than 4000 years (Levinson and Levinson, 1985). Considered serious cosmopolitan pests of stored grains worldwide (Fedina and Lewis, 2007), they attack stored grain products such as flour, cereals, meal, crackers, beans, spices, pasta, cake mix, dried pet food, dried flours, chocolate, nuts, and seeds (Via, 1999; Weston and Rattlingourd, 2000; Mahroof et al., 2003). *Tribolium* spp. are worldwide insect pests of mills, food warehouses, retail stores, and urban homes (Rees, 2004), and are often disseminated worldwide through transported grains (Ryan et al., 1970). *T. castaneum* generally resides in both grain stores and mills, whereas *T. confusum* is more often found only in milling settings (Rees, 2004). *Tribolium* spp. are often more difficult to kill than other stored product beetles, though the order of toxicity will often vary depending on the particular insecticide utilized (Arthur, 2008a). Today, *Tribolium* spp. continues to rank as a serious pest, in many parts of the world, both developed and undeveloped (Bugchio and Wilkins, 2004).

Stored product pests are economically important and are responsible for millions of dollars of loss every year, causing both quantitative and qualitative losses. In many developing countries, the overall post-harvest losses of cereals and legumes averages at rates of nearly 10–15% (Neethirajana et al., 2007), or about \$1.4 to \$2.8 billion per year (USDA, 2006). Grain losses resulting from insect infestations of all postharvest product losses have a major economic impact on the food industry due to the costs associated with the treatment and monitoring, rejection and return of contaminated products, loss of consumer goodwill, and failure to pass inspection or meet regulations (Campbell and Arbogast, 2004). The methods currently in use to control *Tribolium* populations have proven to be necessary in preventing a contamination of food materials, primarily used for human consumption. These methods specifically address the issues

of stored, handled or processed food materials, and aid in preventing revenue losses (Daglish, 2005).

Wheat milling is a particular major food industry with a very low tolerance of insect infestation. Flour mills contain complex networks of storage bins, processing equipment, and machinery for moving grain and milled material (Campbell and Arbogast, 2004). Within a milling setting, grain spillages and other residues in machinery and empty silos can support these residual infestations of stored-product insects (Sinclair and White, 1980). Infestations can arise from within the supply chain, resulting in contaminated food products being shipped through the supply chain to the product manufacturer (Daglish, 2005; Faustini, 2006). However, once all precautions have been taken to ensure the processing and packaging of insect-free food, the food processor has little control over subsequent post-harvest shipping and storage. In developed countries, total food costs are increased with infestations, because of the amount of food that is lost after incurring all costs of growing, harvesting, processing, packaging, shipping, warehousing and retailing (Highland, 1977a). The situation is significantly magnified by the presence of insects in finished and packaged goods, which directly affects consumer confidence (Collins, 1998).

Both *T. castaneum* and *T. confusum* may be present in large numbers in damaged grain, but neither species is a primary feeder of sound (i.e., undamaged) grain. Flour beetles are often attracted to grain that has high moisture content and it is this preference in conjunction with their secretion of benzoquinones, which lead to persistent odors that often encourages mold growth in the infested commodities/products (Assié et al., 2007; Walter, 1990). *T. castaneum* in particular is known to utilize many different flour types (Sokoloff et al., 1966a, b). Via and Connor (1995) showed that this polyphagy results from local adaptation to the most common flour resource, as opposed to an individually generalized resource use. In granaries or flour mills, *Tribolium* spp. may often be exposed to only one or two grain types; however on occasion, mills may contain many types of grain, which then provides the opportunity for increased selection in a more spatially heterogeneous environment, perhaps leading to the evolution of a more generalized *Tribolium* species (Via and Conner, 1995). Because residual infestations can be the primary source of insects infesting stored grain, industry places a heavy emphasis on managing these

populations through the adoption and use of good hygiene practices, which call for an elimination of food sources that allow grain insects to survive and reproduce (Hagstrum and Flinn, 1996; Dargatzis, 2005). Product contamination by whole insects, eggs, insect fragments, frass, and cast skins often occurs in *Tribolium* infested processing plants and warehouses (Baur, 1984). Federal laws strictly regulate the presence of insects in processing facilities, as well as the amount of insect fragments in processed goods sold to consumers, insect management remains an important tool used to uphold consumer confidence (Neethirajana et al., 2007). Therefore, either the need exists to improve upon current IPM techniques, by refining methods previously utilized, or through the investigation and development of new techniques that prove to be safer and more environmentally friendly, than those currently being utilized for general insect pest management purposes (Bell, 2000).

Management Options for *Tribolium* spp.

For more than 40 years, *T. castaneum* has shown its ability to develop resistance to insecticides, thus permitting resistant strains to spread geographically (Assié, 2007). A stored product integrated pest management (IPM) program usually emphasizes the prevention of established pest populations through the use of quick, targeted responses, used to suppress pests when they do become established (Campbell et al., 2002). Comprehensive IPM programs designed for commercial food processing facilities typically rely on an effective monitoring system to obtain reliable information about insect populations (Burkholder, 1990; Hagstrum and Flinn, 1996), often utilizing pheromone-baited traps for monitoring purposes (Campbell et al., 2002). Successful and comprehensive IPM programs designed for commercial food processing facilities also typically rely on controlled application of residual insecticides. Other recommended components include sanitation, such as cleaning the inside of mills and warehouses and by eliminating food sources that can otherwise support infestations (Hedges and Lacey, 1996).

An effective stored product IPM program emphasizes the prevention of pest populations from becoming established, and allows for a quick targeted response to suppress pests when they do become established (Faustini, 2006). In the past, control of insects had resulted in resistance to a number of common insecticides (Bugchio and Wilkins, 2004), and resistance has been reported from many countries since the 1970s (Champ and Dyte, 1976). Resistance to the most

commonly used insecticides has become widespread in many insect species, and the continued use of these conventional insecticides intensifies this selection for resistance, further aggravating the problem (Assié, 2007). Both *T. castaneum* and *T. confusum* have evolved an ability to interact with a diverse chemical environment, as has been evidenced by large expansions in odorant and gustatory receptors, as well as p450 and other detoxification enzymes (Rees, 2004). Among the suggested causes of the spread of these *Tribolium* resistant populations is a significant increase in the worldwide grain trade (Assié, 2007). Flour beetles feed and survive on small amounts of grain and flour; hence, sanitation plays a crucial role in controlling these pests by eliminating food resources necessary for larval growth and development (Mahroof et al., 2005). In a mill setting or other food processing, storage and warehouse facilities, early intervention is required to manage potential surges of insect resistance (Assié, 2007), often requiring that any long-term control program needs to consider the potential occurrence of insecticide resistance (Bughio and Wilkins, 2004). Any employed strategy must limit the impact and spread of resistant strains of insects, by preventing the full genetic expression of resistance within a population (Uyenoyama, 1986). Many sectors of the food industry still rely on calendar-based pesticide applications that are often applied to a whole structure. However, with the loss and further restricted-use of certain residual insecticides, interest is being renewed in improving current IPM techniques. Ultimately, the development of pest management programs for the food industry that are targeted both in time and space will increase the effectiveness of pest suppression to acceptable levels and reduce the risk of any related, negative non-target effects (Campbell and Arbrogast, 2004).

Chemical Control

Fumigations and Methyl Bromide Usage

Fumigation is described as a last choice for control when all other control methods have failed to work, and should be performed only when an established, long-term monitoring program definitively indicates that an economically important pest cannot be controlled under any method other than that of fumigation (Walter, 2006). Fumigations are used on stored products against stored product pests in structures such as flourmills or food factories, and are carried out in chambers, warehouses, silos, food stores, containers, railway boxcars, barges and ships (Bell, 2000). Only a limited number of fumigants are still available, some of which are

under consideration of being removed from use through regulatory action (Walter, 2006). Of the three most widespread fumigants, methyl bromide production and distribution is currently being phased out, and phosphine is undergoing regulatory review in several developed countries, while sulphuryl fluoride is available for use only in some countries and only for treatment of structures, not food materials (Bell, 2000).

One of the biggest challenges to the processing, milling and food plant industries has been the loss of the fumigant methyl bromide (Arthur and Phillips, 2002). Fumigation with methyl bromide as well as other related fumigants has remained the method of choice for a large percentage of post-harvest insect management needs. Methyl bromide, first used in 1932 as a broad spectrum, whole-structure, gaseous fumigant, has since been readily utilized for managing stored product insects in food-processing facilities (Mahroof et al., 2005). Used as a disinfestation, or quarantine measure against stored product insects, in structures such as flourmills, warehouses, silos and food stores, methyl bromide is widely employed for its ability to rapidly kill insects, mites, microflora, and nematodes (Bell, 2000). Due to its desirable physical and chemical properties, i.e., non-flammable, nonexplosive and noncorrosive, as well as possessing little odor or taste at concentrations commonly utilized during commodity-based fumigations, it quickly became the required fumigant for insect control purposes (Fields et al., 2002; Walter, 2006). Currently, there are few fumigants registered that can be used in place of methyl bromide, and none of these registered fumigants have shown methyl bromides' propensity for rapid disinfestation of infested products (Arthur and Phillips, 2002; Small, 2007).

Methyl bromide has been used in a large capacity within the sphere of agriculture as a principal product in the fumigation of soil before planting crops, as well as in post-harvest storage and facility fumigation, and for government-required quarantine treatments. Often used as a disinfestation, or quarantine measure against stored product insects, it is also effective in controlling many soil insects, diseases, nematodes, and weeds, as well as insects and other organisms present in stored or shipped commodities, as well as storage, shipping and processing facilities (Osteen, 2003). Methyl bromide is now categorized as a restricted use pesticide, and registered use is currently required under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (Morrissey, 1996). It is currently undergoing a mandated phaseout due to its extensive

ozone-depleting characteristic. Ozone is a rare form of oxygen that is highly reactive. Most ozone is found in the lower, two layers of the earth's atmosphere, the troposphere and the stratosphere. The ozone that is present in the troposphere is normally found at concentrations of 10 to 30 parts per billion (ppb). However, over 90% of the earth's ozone is present in the stratosphere, which contains ozone concentrations upwards of 10,000 ppb. This stratospheric region extends from 10 to 100 miles above the earth's surface, and comprises what we know as the "ozone layer", specifically, the area of the stratosphere in which the ozone concentrations are greatest (Ristaino and Thomas, 1997). This stratospheric ozone layer is essential for life on Earth and provides a protective layer for the earth's surface, by playing a key role in moderating the climate of the earth by absorbing ultraviolet radiation from the sun (UV-B) such that it essentially acts as a sunscreen for the planet (Robock, 1996). A strong correlation exists between decreased stratospheric ozone and the increased UV-B at the earth's surface, resulting in increases in sunburn, skin cancer, eye danger, crop damage, and other negative environmental impacts (Ristaino and Thomas, 1997).

Halogenated aliphatic hydrocarbons, such as chlorofluoromethanes (CFCs), and other compounds such as methyl bromide have been detected throughout the troposphere in greatly increasing amounts since the early 1970s. Methyl bromide is the major carrier of bromine into the stratosphere, and upon breakdown into bromine atoms, it participates in a series of ozone-depleting chemical reactions (Cox et al., 1995). Bromine is up to 50 times more reactive than chlorine in depleting ozone, because it reacts with reservoir chlorine species, able to react with additional ozone molecules (Ristaino and Thomas, 1997). Unlike chlorine, which is present in the stratosphere mostly from human activities, the presence of bromine compounds in the atmosphere can result from both natural and anthropogenic sources, with the largest anthropogenic source resulting from agricultural usage (Ristaino and Thomas, 1997). The United Nations Environment Program (UNEP) calculated the average atmospheric lifetime of methyl bromide to be 0.7 years, and assessed it as having an ozone depleting potential (ODP) between 0.4 and 0.6 (UNEP, 2000), which corresponds to a value between 40 and 60% of CFC-11's ODP value (Mellouki et al., 1992; Solomon et al., 1992; USDA, 2004). A gaseous chemical with an ODP relative value that is greater than 0.2 is listed as a Class I ozone-depleting substance (Morrissey, 1996; Ristaino and Thomas, 1997). Chemicals within this Class I list are known to

cause significant damage to the ozone layer. The ODP of methyl bromide is dependent upon the atmospheric abundance of chlorine, such that the higher the abundance of atmospheric chlorine, the higher the ODP of methyl bromide. In 1992, methyl bromide was placed on the accelerated phaseout, and as of 1997, the EPA, acting under The Clean Air Act scheduled the domestic U.S. phaseout date for production and importation of methyl bromide for January 1, 2001 (UNEP, 2000).

The episodic loss of ozone that occurs each spring over the Antarctic continent was first reported in 1985. Due to the lower temperatures that occur between midwinter and spring, the Antarctic stratosphere is most sensitive during this time to the growth of inorganic chlorine, resulting in further thinning of the ozone concentrations (Farman et al., 2003). Mapping of this reoccurring and increasing ozone depletion event has been monitored since 1985, and during the first four-year monitoring period, the loss of ozone was equivalent to the size of the entire Antarctic continent, equating to roughly a 70% reduction (Stolarski et al., 1992). As of 2007, destruction of the ozone layer has caused the ozone hole to increase to over 10.6 million square miles, making the ozone hole the deepest it has ever been (EPA, 2008). Concern over this ozone depletion led to negotiations among countries that ultimately resulted in the 1987 drafting of the Montreal Protocol on Substances That Deplete the Ozone Layer, an international treaty designed to protect the ozone layer by the reduction and elimination of chemicals thought responsible for this depletion. The treaty governs the production and trade of ozone-depleting substances, requiring an eventual elimination of the production of the substances deemed most ozone depleting (Ristaino and Thomas, 1997; Osteen, 2003). Opened for signature on Sept 16, 1987, the treaty was entered into force on Jan 1, 1989. Based on a 1992 ruling, which classified methyl bromide as an ozone depleting substance, all developed countries were scheduled to begin eliminating the production and usage of methyl bromide. Since this initial draft, this protocol has undergone seven revisions, with the latest taking place in Beijing in 1999. It is believed that if this international agreement is followed, the ozone layer is expected to recover by the year 2050 (Subramanyam, 2006). This ambitious environmental treaty set the standards for reductions of ozone-depleting substances worldwide and has currently been signed by more than 150 countries, including the United States (UNEP, 2000).

In December 1995, representative parties to the Montreal Protocol met, and developing countries agreed to eliminate production of methyl bromide by 2015, following a 20% reduction in 2005 and a 100% reduction in 2015. Developing countries, which at the time accounted for only 18% of the global consumption of methyl bromide, agreed to freeze their use of the compound in 2002, which will be based on the average of the 1995 to 1998 consumption levels (Morrissey, 1996). Under the Federal Clean Air Act, the phaseout of methyl bromide mandated a freeze in the domestic production and importation of methyl bromide for developed nations, such that it was capped at the 1991 level, thereafter referred to as the 'baseline'. At this time the U.S. consumption, defined as the annual production plus imports, minus exports, was estimated to be about 25,500 metric tons (~28, 109 tons). The Clean Air Act mandated that interim supply reductions of methyl bromide, for the U.S. as well as other developed countries, follow a 25% reduction from baseline levels between 1999 and 2000, preceded by a 50% reduction from the baseline levels between 2001 to 2002, and 70% reduction from baseline levels between 2003 to 2004, with a projected 100% phaseout by the end of 2005. In addition, the treaty enacted a cap of the use of methyl bromide for quarantine and pre-shipment purposes at the 1996-1998 average level, with a provision to further restrict this use in future, as alternatives are introduced. These provisions or allowable exceptions are otherwise known as critical use exemptions (CUEs) (Ristaino and Thomas, 1997). Due to its overall effectiveness, the availability of methyl bromide has been limited only to quarantine applications, which are performed to prevent the introduction, spread or establishment of quarantine pests, for pre-shipment and certain other approved critical uses, all of which were exempted from the previous international methyl bromide ban. However, not exempt are preventative treatments of stored commodities or facilities not related to quarantine or pre-shipment requirements, and thus requiring a CUE (Osteen, 2003).

Under the Montreal Protocol “a use of methyl bromide should qualify as ‘critical’ only if the nominating Party determines that: (i) The specific use is critical because the lack of availability of methyl bromide for that use would result in a significant market disruption; and (ii) there are no technically and economically feasible alternatives or substitutes available to the user that are acceptable from the standpoint of environment and public health and are suitable to the crops and circumstances of the nomination” (UNEP, 2004). Entities regulated by this

proposed action include: producers, importers and exporters of methyl bromide; applicators and distributors of methyl bromide; users of methyl bromide, e.g. farmers of vegetable crops, fruits and seedlings; and owners of stored food commodities and structures such as grain mills and processors, government and non-government researchers (UNEP, 2004). After 2004, CUEs were exempted on a yearly basis in developed countries (Osteen, 2003). Beyond the scheduled 100% planned phaseout, these CUEs allow a country to legally use upwards of 20 metric tons of methyl bromide, per year, for emergency uses. These exemptions further permit an application for approval can be submitted after this emergency usage has been deemed necessary and applied (Osteen, 2003). In accordance with the Federal Clean Air Act and the Montreal Protocol, the EPA authorized 4,813 metric tons of methyl bromide for approved critical uses, in 2008, with 3,083 metric tons supplied for new production or import (EPA, 2008).

Because these fumigants are often required for international commerce, exemptions will continue to be permitted until a time in which suitable alternatives can be found, tested and accepted by all necessary parties (Walter, 2006). For many uses, no single alternative is available that is as effective and economical as methyl bromide, causing overall industry concern that this mandatory phaseout could effect short-term losses at all levels of agriculture by lowering yields, increasing costs and reducing import availabilities of crops, until more cost-effective alternatives are developed and made available. The effects of these losses could in turn be passed onto the U.S. consumers in the form of higher commodity prices and perhaps reduced supplies of stored grains and other commodities (Osteen, 2003). The National Pesticide Impact Assessment Program (NAPIAP) estimated annual economic losses upwards of \$1.3 to 1.5 billion, should the usage of methyl bromide be banned in the United States. Most of the estimated losses were due to loss of soil fumigation (\$800 to 900 million), with \$450 million due to loss of quarantine fumigation used for imports. However, these loss estimates assumed that few or no alternatives would be available or readily utilized (UNEP, 2000). When the EPA conducted a cost-benefit analysis of the elimination of methyl bromide, they estimated \$1.2 to 2.3 billion in losses could occur if the methyl bromide were not phased-out, due to the health costs related to the continued use of methyl bromide. It was estimated that between \$244 and 952 billion in benefits would result primarily from a reduction in 2,800-skin cancer deaths over the period from 1994 to 2010, assuming the phaseout of methyl bromide proceeds as scheduled (Slaper et al., 1996).

Regulatory constraints have reduced the availability and have significantly increased the cost of using methyl bromide, making this fumigant a much less attractive choice (Walter, 2006). From 1996 to 2003, the price of methyl bromide increased over 146%. These rising costs have and will ultimately reduce users' net revenues and lower the material cost effectiveness, which should further discourage its continued use, and further encourage use of available alternatives (Osteen, 2003). The loss of methyl bromide presents a unique opportunity to develop smaller-scale insect control strategies within a food production or storage facilities that are vulnerable to insect pest infestations. Ultimately, this can be done in a way that improves IPM, through selective applications of safer control agents and methods, which will ultimately benefit food manufacturers and handlers by decreasing the levels of chemicals currently needed to protect the vulnerable food supply from insect pests, all while leading to a wholesome, more dependable food supply for consumers (Faustini, 2006). Continuing research and development, as well as registration of new pesticides may improve the effectiveness of new alternatives that could potentially reduce both the established and the perceived negative economic effects of the methyl bromide phaseout plan. With the de-commercialization, restrictions and repealed use of methyl bromide, aerosolized treatments are being touted as a potential replacement alternative, as they are a more cost-effective means of controlling insect populations in a variety of food storage/processing facilities (Waddell et al., 2000).

Aerosol Insecticide Applications

Consumers and regulators have little tolerance for insect-damaged or contaminated food products, which pose serious consequences and challenges for pest management professionals (Arthur and Peckman, 2006). Therefore, the direction and breadth of pest-control strategies in the food industry have changed significantly over the past few years, creating the need to optimize insecticides through improved IPM techniques, specifically through the identification of new control agents that are low in mammalian toxicity, as well as identifying any factors that might affect susceptibility to these agents. These changes include more-restrictive regulatory positions on the reduction of residual insecticide chemicals, and the utilization of more sophisticated methods for measuring insecticidal residues, as well as a reduced reliance on insecticides in conjunction with food-processing and food-storage. These regulatory positions stress the importance of implementation and integration of policies and procedures to better manage pest populations, such as actively maintaining of an acceptable-insecticide list, improving upon

current pest management techniques, and through the further advancement of insect-monitoring approaches as a means of further directing insect control practices (Faustini, 2006). Adopted in response to the growing level of government and consumer concern over insecticide residues in food, these regulations have led to reduced availability of traditional chemical insecticides, placing increased importance on the continued development of alternative IPM-based methods (Arthur and Peckman, 2006).

Residual insecticides can be successfully used as a part of an integrated approach to target insect control purposes. First defined for pesticide applications in 1973, residual insecticides are often grouped and classified according to the chemical structure of their active ingredient (a.i.), often indicating their common modes of action, or entry pathways, including inorganics, organophosphates, insect growth regulators, carbamates, and both natural pyrethrins as well as synthetic pyrethroids (Arthur and Peckman, 2006). Pest management professionals often utilize aerosolized liquid applications otherwise known as fogging, space sprays, cold aerosols, ultra low dosage fogging or ultra-low volume (ULV), to manage stored product insects. Residual contact insecticides are commonly applied on a recurring basis, often at regular treatment intervals of 2-3 weeks (Toews et al., 2005b), and can be used to both control insects inside food processing facilities, flourmills, warehouses, indoor storage facilities and grocery stores. Aerosols have been highly regarded for IPM purposes because they provide good coverage of all exposed surfaces, as well as providing a level of residual control, depending on the substrate(s) that they are applied to. Aerosols can also target specific areas, and also prolong the time interval between structural fumigations or heat treatments, all of which allow aerosols to be more readily utilized, especially as the food, shipping and other agricultural industries are searching for effective fumigant replacement strategies (Toews et al., 2005a, 2006b).

Research today includes not only the identification of potential new insecticides that can be used on stored products, but also a thorough examination of the factors that can affect efficacy of these residual insecticides. Since their introduction in the 1940s, the bioefficacy of such 'fogging' devices has been greatly improved through an understanding of the influence of the physical properties of insecticidal sprays (Whitmore et al., 2001). There are a number of factors that affect the final insecticidal efficacy, and thus the performance of applied residual

insecticides, including: the specific insecticidal class and formulation that is being used as well as the dispersion of the aerosol within a target area, surface and substrate effects, sanitation, specifically the presence of food material within the treated facility; the differences among the targeted insect species and their associated responses to the applied aerosol insecticide, the level of exposure required to kill exposed insects, pertinent environmental effects such as temperature and r.h., and, the economics involved in determining effective application rates (White et al., 1992; Arthur and Phillips, 2002; Arthur and Peckman, 2006; Toews et al., 2006b).

During the fogging process, the insecticide is mixed with compressed air and moved through a nozzle, a process known as “atomization”, which creates a dense fog, which then spreads throughout a facility (Schick, 1997; Peckman and Arthur, 2006). Pressurized aerosol/fogging insecticides consist of particles ranging from 0.1 to 50 microns (μm), the size of which can be controlled by the nozzle. Droplet parameters within a required aerosol range are specified for each insecticide that is labeled for ULV dispersal (Brown et al., 1999). Air currents move the applied insecticidal fog around and under otherwise easily accessible equipment, which greatly increases the opportunity for exposed insects to come in contact with the applied insecticide (Peckman and Arthur, 2006), greatly increasing the effectiveness of aerosol insecticides in the control of stored product insect pests, when used in facilities that are heavily laden with equipment and other barriers that insects use as refuge. Methods employed before the introduction of ULV space sprays required large dispersal rates of diluted pesticides, often in excess of 60 fluid ounces per minute. Thus, with the advent of ULV applications, the effectiveness of ULV dispersal is now equal to or greater than other high volume application methods (Brown et al., 1999). When compared to high volume methods, the amount of aerosol dispersed to achieve control is thus greatly reduced (Peckman and Arthur, 2006).

The efficacy of some agricultural insecticidal sprays has been improved by electrostatically charging the spray droplets, effectively helping to enhance the control of stored product pests (Whitmore et al., 2001). Because viscosity and dynamic surface tension have long been recognized as dominant factors controlling the atomization of a liquid, facilities utilizing these application systems must be aware of the physical characteristics of these applied residual insecticides. Physical changes to the spray-solution, often through dilution, the use of synergists

or changes to solvents can directly affect properties of the insecticidal spray, most importantly the droplet-size distribution (Tsuda et al., 1988; White et al., 1992; Whitmore et al., 2001; Hoffman, 2007). High voltage power supplies are used to impart charge to the droplets (Matthews 1989). Whenever a liquid is atomized, the droplets then carry an electrostatic charge, due to a natural charge exchange process. ULVs or space spray aerosol insecticides often carry a charge-to-mass ratio 2.66×10^{-5} to 2.16×10^{-4} C/kg. Devices used to create aerosol droplets of a specific size with a preferred trajectory that are able to be effectively dispersed throughout a large room, must create particles with a minimum charge-to-mass ratio about 1×10^{-4} C/kg⁻¹ (Gaunt et al., 2003). This level of charge can be achieved using insecticidal formulations that can be optimized using actuators, placed on the pressurized nozzles, and promote shearing of the electrical double layer. These space charge forces within the charged aerosol cloud will result in the outward movement of droplets away from the center of the spray cloud, improving its distribution and overall bioefficacy (Whitmore et al., 2001).

The selection of insecticides for treatment to food commodities is based on toxicological data, which often indicate both the effectiveness and the persistence of the selected insecticide, under certain storage conditions. There are strict guidelines, implemented by both the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), are used to determine whether an insecticide can be used in, on or around grain products intended for human consumption. These include, but are not limited to the requirements that the chosen insecticide must be effective at economic rates of use, it must be effective against a wide variety of insect pests, a legal maximum residue limit must be established and followed, it must present no hazards to consumers of grain and grain products, as well as users and/or applicators, it must be acceptable to health authorities, it must not give rise to unacceptable residues, it must be free of side effects such that when applied, it will not affect the quality, flavor, smell or handling of the grain, it must be acceptable in the international grain trade, it must not be flammable, explosive or corrosive; and, that its method of application or use proves compatible with previously established grain handling procedures (FAO, 2008). Testing of the insecticide, along with integrating the proposed control strategies into an actual industrial environment, is important for the incorporation into a pest management program. A thorough investigation of specific aerosol insecticide delivery systems, as well as any proposed insecticidal carriers, can help predict the

effects that these mixtures will have on targeted insects, allowing a better, more thorough understand of the rate at which these insecticidal mixtures break down in a stored product environment. Ineffective aerosol insecticide management programs (i.e., facilities are being fogged too often, or too little), can lead to four likely consequences: money being wasted, complaints and law suits by consumers due to contaminated food products, increases in use of insecticides, targeted insect pests developing resistance to active ingredients (Nansen, 2007).

Sanitation as it relates to IPM purposes refers to cleaning and eliminating extraneous food materials within food related facilities. The presence of food materials often compromises the residual efficacy of insecticidal treatments (Arthur 1998, 2000; Arthur and Campbell, 2008), by providing nutrition to the targeted insect pests, which can mitigate the effect of the applied residual insecticides, as well as diminishing the likelihood for the targeted insects to encounter and be successfully exposed to insecticides (Arthur and Peckman, 2006). Although aerosols do show potential for replacing whole-plant fumigations, they may be less effective when used to control insect pest populations/infestations if sanitation is not practiced (UNEP, 2004). And lastly, as several stored product insect pests have become resistant to other residual insecticides that have been overused on a continual basis within stored cereals, such as traditional organophosphorous residual insecticides, a major issue arises regarding the need to control stored product pests, in the food industry (Zettler, 1991; Arthur, 1996; Zettler and Arthur, 1997; Atkinson et al., 2004). It is the differential evolutionary adaptation of insects to their chemical environment that eliminates genetically susceptible individuals, resulting in an increased proportion of resistant individuals, in any given population (Rousch and McKenzie, 1987).

There is currently renewed interest in developing reduced-risk, low toxicity chemicals that can be effectively utilized in a setting in which grain and other food commodities are vulnerable to insect infestation, as a means of replacing outdated, and at times, less effective methods of insect control. Over the past decade, developed countries have made significant progress toward alternative insect control strategies by employing a variety of applied insecticides (Collins, 1998). One such insecticide is pyrethrum, which has been readily used as an insecticide for the better part of this century and has become widespread in both the developed and in the developing world. Through resurgence in popularity, it is necessary to

evaluate the use of pyrethrins as potential grain protectants with respect to the reduction of the application rates, since less restrictive regulations have been imposed, regarding permissible residue levels in foodstuffs (Atkinson et al., 2004). In addition, use of insect growth regulators may also increase, specifically for stored product insect control, as they can be applied directly to stored grains (Toews et al., 2005b).

Chemical Control with Synergized Pyrethrins

Pyrethrins

Pyrethrum is a highly efficient, broad spectrum, toxic agent effective against a wide range of insect pests, with a century long history of safe use. First recognized as having insecticidal properties around 1800 (Katsuda, 1999), pyrethrum acts as a repellent to insects when used at low concentrations (Casida, 1980). The main structural features of pyrethrins were elucidated around 1914, but not reported until 1924. Pest control until the 1940's was moderately successful with the use of compounds that were derived primarily from natural sources; however, by the beginning of the 1940's most of these treatments were displaced by synthetic organic, (i.e., "second generation insecticides"), which tended to provide a more complete control, at a greatly reduced cost, due to their higher potency/persistence (Casida, 1980). Following World War II, pyrethrin-based insecticides were largely replaced by the more stable and less expensive organochlorine (OC), organophosphorous (OP), and carbamate insecticides (Valentine, 1990). In the 1970's, however, concerns over environmental contamination, especially from OCs, as well as vertebrate toxicity, mainly from OCs, OPs, and carbamates stimulated a resurgence in the use of pyrethrins (Valentine, 1990).

Pyrethrins are a unique botanical insecticide, consisting of six, closely related biologically active esters, collectively referred to as pyrethrins. These "first-generation insecticides" are a naturally occurring mixture of insecticidal, secondary metabolites that are found in perennial white chrysanthemum flowers (*Chrysanthemum cinerariaefolium* Vis.), which accumulate to high concentrations in the flower heads (Casida, 1980; Matsuda et al., 2005). Because the pyrethrins are localized within the secretory ducts of the seeds, they are protected from photodecomposition upon growth, and are further isolated so they are not toxic to insects feeding on or visiting pyrethrum flowers (Casida, 1980). Relative to the dry weight of the flower,

a pyrethrum daisy contains small proportions of pyrethrins, which are produced throughout the plant, with approximately 94% of the total yield concentrated within the seeds. The aromatic flower heads are harvested shortly after blooming and are dried and powdered, or the oils within the flowers are extracted using solvents (Atkinson et al., 2004). Individually dried flowers contain small amounts of pyrethrum, often as little as 0.9 to 1.3%, while the refined pyrethrum extracts contain 45-55% total pyrethrins, of which approximately 71% are pyrethrins, and approximately 21% and 7% are cinerins and jasmolins, respectively (Proudfoot, 2005). Results of extractions on the heads of these flowers have shown that these two pyrethrins do not occur in the flowers in equal proportions, with the amount of pyrethrin II ranging from 38 to 172% of the total pyrethrin I content (Gnadinger and Coral, 1930). Today, due to extensive manipulation of pyrethrin flowers, the typical extract contains pyrethrins, cinerins and jasmolins in the proportion 10:3:1 (Crombie, 1995), with the ratio of pyrethrin I to pyrethrin II around 1.0 (Morris, 2006). When used in milling and other food facilities, the total extract is diluted to 20%, which is the maximum concentration commercially available for applied purposes, within the United States (OSHA, 2007).

The pyrethrins are enantiomerically pure 4-oxo-cyclopent-2-enyl ester derivatives of chrysanthemic acid and pyrethric acid (Bicker et al., 2004). Pyrethrins have three chiral centers, allowing for the possibility of eight different optically active forms, as well as geometrical isomerism (E or Z) in the side chain of the alcohol (chrysanthemates), or in the acid and alcohol (pyrethrates), ultimately increases the number of possible stereoisomers (Casida, 1980). Pyrethrins are a naturally-occurring group of six chemically-related esters, each of which is insecticidally active, these include, cinerin I: (Z)-S-3-(but-2-enyl)-2-methyl-4-oxocyclopent-2-enyl (1R)-trans-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate, cinerin II: [(Z)-S-3-(but-2-enyl)-2-methyl-4-oxocyclopent-2-enyl (E)-(1R)-trans-3-(2-methoxycarbonylprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate], jasmolin I: (Z)-S-2-methyl-4-oxo-3-(pent-2-enyl)cyclopent-2-enyl (1R)-trans-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate and II: (Z)-S-2-methyl-4-oxo-3-(pent-2-enyl)cyclopent-2-enyl (E)-(1R)-trans-3-(2-methoxycarbonylprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate), pyrethrin I : (Z)-S-2-methyl-4-oxo-3-(penta-2,4-dienyl)cyclopent-2-enyl (1R,3R)-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropanecarboxylate; and pyrethrin II : (Z)-S-2-methyl-4-oxo-3-

(penta-2,4-dienyl)cyclopent-2-enyl (E)-(1R,3R)-3-(2-methoxycarbonylprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate (Matsuo et al., 1994). It is these six biologically active chemicals that are directly responsible for the lethality and the knockdown properties of pyrethrin insecticides (Casida, 1980).

Pyrethrins possessing an ester linkage are classified into two types, type I and type II (Matsuda et al., 2005). The pyrethrin esters are derived from a combination of three alcohols, cinerolone I, jasmolone I, and pyrethrolone I, as well as two acids, chrysanthemic acid II and pyrethric acid II (Patenden et al., 1973). The two active constituents of pyrethrins are esters of the cyclic ketonic alcohol pyrethrolone. The type I pyrethrins are pyrethrin I (PI), cinerin I (CI) and jasmolin I (JI), esters of chrysanthemum monocarboxylic (or chrysanthemic) acid, which contain a chrysanthemoyl moiety; whereas the type II pyrethrins are pyrethrin II (PII), cinerin II (CII) and jasmolin II (JII), which are esters of methyl ester of chrysanthemum dicarboxylic acid (i.e., pyrethric acid), and have a pyrethroyl moiety, in addition to a specific ester linkage, a methoxycarbonyl group. Pyrethrin I and II are structurally related esters, both being viscous liquids that oxidize readily to become inactivated, and sharing a common structural feature, a cyclopropane ring core within the acid moiety (Matsuda, 2005; Debboun, 2007). The two pyrethrins differ by the oxidation state of one carbon (Debboun, 2007).

The biological activities of pyrethrum constituents depend on the structures and stereochemical characteristics of both the acid and the alcohol components (Casida, 1980). The chrysanthemates (PI, CI, JI) are the lethal components of the applied insecticide, whereas the pyrethrates (PII, CII, JII) cause the observed rapid knockdown (Rugutt et al., 1999). The most prominent component of the insecticidal mixture are the pyrethrins I and II (OSHA, 2007). Pyrethrin I, containing chrysanthemic acid esters, is more toxic than pyrethrin II. The increased insecticidal activity of pyrethrin I could be directly related to the chrysanthemic acid esters/pyrethric acid esters ratio and particularly to the pyrethrin I content (Bathomeuf et al., 1996). However, the knockdown activity of pyrethrin II is better than that of pyrethrin I (Matsuo et al., 1994). It is this structure of pyrethrins that helps to explain its low mammalian toxicity as well as its selective potency to insects, which are unable to detoxify them, leading to their subsequent penetration into the nervous system (Valentine, 1990).

Erratic supplies of natural pyrethrum fueled a search for synthetic analogues, which led to the discovery and commercial development of pyrethroids, including cyfluthrin, cypermethrin, deltamethrin, permethrin, etc (Arthur and Peckman, 2006). Unlike pyrethrins, which have a low photostability, as well as low mammalian toxicity, and are thus rapidly degraded after application, the pyrethroids are overall more stable. They are often more toxic to insects than pyrethrins (Satelle and Yamamoto, 1988). Pyrethroids are more stable in light conditions and can persist for longer periods in the environment (Saggar et al., 1997), and have shown deficiencies regarding incidences of acquired resistance (Collins, 1990). Although, they were not used commercially until 1980, by 1982, pyrethroid insecticides accounted for more than 30% of the world market (Barr et al., 2007). Like pyrethrins, most of the established pyrethroids are esters containing various acid and alcohol entities. Often the most potent knockdown pyrethroid agents have carbonyl groups within the acid, or alcohol moieties (Casida et al., 1983), and it is this substitution of the substituents that are most susceptible to photochemical or metabolic degradation (Casida, 1980). Chirality may reside not only in the acid component, which is most often a derivative of 2,2-dimethyl-cyclopropanoic acid, but also in the alcohol moiety. For this reason, applied pyrethroid insecticides are often a mixture of several stereoisomers (Bicker et al., 2004). Today natural pyrethrins have largely been replaced by these synthetic analogues, since the natural pyrethrins are inferior in stability, insecticidal efficacy and production costs (Katsuda, 1999; Bicker et al., 2004). However, natural pyrethrins have several advantages over synthetic insecticides, such that they can readily and effectively be combined with synergists (Chadwick, 1963; Paul et al., 1988), serve as repellents, and exert toxic effects on a wider range of agriculturally based pest insects (Saggar et al., 1997).

Increased Efficacy of Pyrethrins Through Synergism

Synergized pyrethrin is likely one of the safest of all insecticides for use in foodstuffs and plant materials (Walter, 2006). Technical-grade (concentrated) pyrethrins are mixed with carriers or solvents, to produce a commercial-grade formulated product containing inert ingredients that can increase the toxicity of the insecticide (Anonymous, 1955). Synergists are used to extend the economic usage of natural pyrethrins (Elliott, 1976). Without the addition of synergists, insects can become resistant to pyrethrins through enzymatic detoxifiers (Farnham, 1971). Synergism action upon pyrethrins involves inhibition of pyrethrin detoxification, which results in greater persistence in insects and higher potency (Casida, 1980). However, this boost in insecticidal

activity is obtained with no apparent increase in mammalian toxicity over that of pyrethrum alone (Beroza and Barthel, 1957). A mixture consisting of pyrethrins and a synergist can serve as a repellent, drastically increasing the toxic effects (i.e., “killing power”), allowing for higher concentrations of active insecticides that then remain in the target insect for a longer period, as well as providing a higher potency to the insecticide (Proudfoot, 2005). Though pyrethrum is rapidly metabolized, some insects can revive from initial poisoning. For this reason, pyrethrum is often mixed with a synergist (Yamamoto, 1973). However, the effectiveness of a synergized pyrethrin will depend upon (a) the insect and the stage of the targeted insect at the time of application (Saggar et al., 1997), (b) the method of administration, its compatibility with other ingredients of a formulation, and (c) its effects on other beneficial insects (Beroza and Barthel, 1957).

A beneficial synergistic compound used in combination with pyrethrins should (a) display a low mammalian toxicity, yet show an increased effectiveness against many insects in both knockdown and kill, (b) possess good solubility characteristics, (c) have the ability to remain highly stable upon storage and exposure, (d) should not emit an offensive odor or other irritating properties, and (e) should possess the ability to be produced at a low costs, so that it can become readily commercially available. Also important is that repeated usage of the pyrethrum-synergist does not evoke the high degree of resistance commonly encountered with most synthetic insecticides (Beroza and Barthel, 1957). Piperonyl butoxide (PBO) is a registered insecticide synergist, which when used by itself does not possess or display insecticidal properties; however, when used in combination with pyrethrum, the mixture possesses insecticidal activity. These synthetic synergists are present in commercially available formulations of pyrethrum, usually present at ratios 1:4 or 1:10 active ingredient/synergist ratio depending on the target insect (Minello et al., 2005; FAO, 2008). It is a moderately stable, semisynthetic derivative of safrole (Saggar et al., 1997), that combines many favorable aspects, including low mammalian toxicity, as well as being very effective over a wide range of insects (Kakko et al., 2000). PBO synergism was revealed as early as the 1940s and has since been used as a means of multiplying insecticidal effectiveness by up to three to five times (Anonymous, 1955), through its inhibition of metabolic degradation of the active ingredients. Today, PBO has become the most widely used synergist in the last decade (Proudfoot, 2005).

Pyrethrins contain multiple active ingredients, each of which may be synergized to a different degree. The best pyrethrum synergists may be divided into two main categories, amides (i.e., malondialdehyde) (MDA) and methylenedioxyphenyl (MDP) derivatives (Beroza and Barthel, 1957). MDP-based synergists originated in 1942, with the isolation of sesamin from sesame oil. Its activity as a pyrethrum synergist was demonstrated shortly thereafter (Beroza and Barthel, 1957). The MDP group has long been recognized as the active structural feature of PBO (Yamamoto, 1973), as it is this MDP group that provides the increased toxicity to the synergistic compound, while not providing it with the same knockdown toxicity of other synergistic compounds containing the MDA group (Scott et al., 2007). Acting as a potent synthetic MDP inhibitor of the cytochrome P-450 dependent polysubstrate monooxygenases (PSMOs), enzymes produced by microsomes bind enzymes that degrade the pyrethrin (Scott, 1999). PBO is responsible for preventing the metabolic degradation of the pyrethrin by inhibiting microsomal oxidation and esterase function, through the binding of these oxidative enzymes, thus preventing the degradation of the applied pyrethrin (Yamamoto, 1973; Proudfoot, 2005), and otherwise greatly enhancing the insecticidal activity of the pyrethrins (Elliot and Janes, 1973). It was thought that PBO is a specific inhibitor of microsomal oxidases (Sato et al., 2006), but it was recently shown that PBO also inhibits resistance-associated esterases (Young, 2003; Young et al., 2005). As new insecticides with novel modes of action emerge, they will be inevitably expensive, thus any extension of pyrethrin use is beneficial to those working in stored products, as well as those facing pest problems in developing countries (Young et al., 2005).

Physiological Effects of Using Pyrethrins

The active substances in pyrethrin are contact poisons that are highly toxic to insects because they rapidly penetrate into the nervous system. A few minutes after application, the insect cannot move or fly away (Narahashi, 1996). Synergized pyrethrins are usually utilized for knockdown and kill effect, as well as the fact that it possesses substantial inherent repellency of insects. This 'knock-down' effect often occurs within a few minutes after application, and results in early paralysis of the exposed insects, whereas the 'kill' effect occurs several hours *post-treatment* (Proudfoot, 2005). Pyrethrins induce a reversible paralysis, which suggests that insects may have detoxification systems (Agosin, 1963). Pyrethrins and pyrethroids are lipophilic molecules that generally undergo rapid absorption, distribution, and excretion. Their lipophilicity and inert chemical structures allow them to be stored in fatty tissues of organisms rather than

being metabolized and eliminated, further rendering them susceptible to mammalian detoxification systems. These detoxification systems metabolize and detoxify pyrethrins by oxidation and ester cleavage, allowing them to be rapidly converted to more polar compounds and thus excreted in the urine or feces. This mode of action will help to prevent an effective concentration from being reached within mammals (Elliot, 1976).

The cellular target of pyrethrins within insects is the neuronal insect voltage-gated sodium channels. These channels are responsible for generating action potentials in insect nerve cells. Pyrethrins affect these channels by creating multiple potentials across the membranes, to delay the sodium channel inactivation (Type I), (i.e., membrane depolarization), which leads to longer prolongation of the sodium influx along the axon, which then leads to persistent nerve depolarization and blockage of axonal conduction (Type II) (Kueh et al., 1985; Valentine, 1990; Ling et al., 2001). This protracted sodium influx results in repeated and extended firings of the nerves, which if sufficiently large and longer than 0.5 seconds, lowers the threshold, causing repetitive firing (Lund and Narahashi, 1983; Proudfoot, 2005). The type II pyrethrins affect the nerve cells in a similar manner to the Type I pyrethrins, but may also block inhibitory pathways through binding and altering GABA receptor-mediated chloride channels (Ling et al., 2001), leading to hyperexcitation of the entire nervous system. It is this disruption of the electrical transmission of the insects nerve impulses that lead to the muscle system being rapidly paralyzed (Kakko, 2000). Natural pyrethrins still remain a widely used material in stored product pest control. After application, the compounds break down rapidly with no residue persistence, induce a rapid knock down effect on the exposed insect pests (Silcox and Roth, 1994), are rapidly metabolized within the insect (Yamamoto et al., 1969), and do not result in building up of resistance in the exposed insect populations (Fine, 1963; Megaw, 1984; Bicker et al., 2004).

Pyrethrin Use In Stored Product Environments

Over the past few decades, the fumigants phosphine and methyl bromide have played a considerable role in the control of stored product insects, especially *Tribolium* spp. (Osteen, 2003). However, the methyl bromide phaseout has significantly reduced its availability, and has severely limited its usage as a control regime for stored product insects (Small, 2007). Pyrethrum is an effective repellent to insects, even at very low concentrations, and has a very high degree of flushing power that disturbs insects so that they move out of their hiding places and expose

themselves to the insecticidal spray (Pieper and Rappaport, 1982). Pyrethrins have gained a general acceptance of their usage associated with foodstuff, as well as gaining an established codex of tolerance, which remains the principle reason for its increased usage (FAO, 2008). Due to the limited persistence of pyrethrins, pyrethrin treated commodities are safe for human consumption, keeping minimum residues on foodstuffs far below the acceptable daily intake (ADI) value, which is 0.04 mg/kg body weight per day (Leng et al., 2006). These properties specifically allow pyrethrins to be used against insects in houses, on crops (even prior to harvest), and on stored foods, because their active components quickly degrade in sunlight (Roest, 1976; Pieper and Rappaport, 1982). In stored grains, nearly 50% of the applied pyrethrins disappear during the first three or four months of storage, and at least 80% of what remains is removed by handling, processing and cooking (Vettorazzi, 1979). Atkinson et al., (2004) reported the effects of temperature, moisture and oxygen as well as microbial degradation on prolonged storage of a pyrethrum crop. An initial rapid loss of the natural pyrethrins observed before the pyrethrins content stabilized around 65% remaining content, with the majority of the loss attributed to the pyrethrin I and pyrethrin II esters, suggested that the plant structure might provide chemical or physical protection to the pyrethrin molecules. Caboni et al. (2007) studied pyrethrin levels during a postharvest treatment on stored durum wheat, at both single and double the recommended label rates. The initial pyrethrin residue deposition was determined to be 3 mg/kg, and results indicated that the fate of the pyrethrins at both application rates were similar, with the total pyrethrin content remaining largely unchanged for 22 days, followed by a complete dissipation at 8 months. The single dosage experiments showed that the half-life times of pyrethrins I and II were 46 and 72 days, while the double dosage experiments showed half-lives of pyrethrins I and II at 41 and 53 days, respectively.

Stored product environments, such as mills and other food processing/storage facilities, contain barriers to aerosol treatments, including large pieces of equipment, shelving, pallets, etc. Toews et al. (2006b) report results from tests that examined the influence of flour accumulation, open versus closed exposure levels, exposed life stage (eggs, larvae, pupae and adults) and insecticide type (a pyrethroid, esfenvalerate versus synergized pyrethrins) on the efficacy of aerosol applications for management specifically of *T. castaneum*. The insecticide treatments were applied on five separate occasions, at the labeled rates for each of insecticide (0.25%

esfenvalerate and a 1% pyrethrin + 2% PBO mixture). After exposure and a 24 hour reentry period, mortality (live, dead or moribund) for each of the exposed insect stages was assessed at days 3, 7 and 21, post-treatment, while individuals exposed as eggs were assessed on day 21. Results of these tests revealed evidence of adult recovery only in replicates treated with the synergized pyrethrins. Results also showed that immature stages were always more susceptible than exposed adults, and that the addition of flour decreased mortality significantly, ~20-50% throughout the post-treatment observation period. Mortality of *T. castaneum* will likely decrease about 10% in environments where equipment exists, allowing residual populations to persist, as the applied aerosol is not effective at depositing droplets in or around these barriers.

Pyrethrin Use On Stored product Insects

The effect of pyrethrin and synergized-pyrethrin aerosols on the life stages of both *T. castaneum* and *T. confusum*, have been conducted by Arthur and Campbell (2008) and Arthur (2008). The presence of flour food material increased survival of adults exposed to the aerosol insecticidal mixture, with *T. castaneum* being the more susceptible of the two species (Arthur 2008). Results of these tests reiterate the importance of sanitation and cleanliness within milling and other food processing facilities, especially when attempting to control adult insect pests. The effects of aerosolized pyrethrin has also been evaluated against other stored product insect species, including the granary weevil, *Sitophilus granarius* L. (Biebel 2003) and the Indianmeal moth, *Plodia interpunctella* Hübner (Jensen, 2008).

Pyrethrin Persistence in the Environment

When pyrethrins are sprayed in the outside environment, rapid degradation can occur by direct photolysis (i.e., natural sunlight) and by reaction with hydroxyl, ozone and nitrate radicals, all of which prevent pyrethrins from persisting in the environment beyond a few weeks. Previous studies have shown that under ultraviolet light irradiation, the pyrethrin components degraded faster than the cinerins, and the photodegradation products of the pyrethrins showed infrared spectral changes consistent with chemical modifications occurring on the side chain of the cyclopentenolone moiety, specifically implicating the alcohol moiety as that which undergoes the most severe photochemical attack (Freeman, 2006). Chen and Casida (1969) found that pyrethrin I was stable for around 24 hours under controlled low-light conditions, but was highly unstable in the presence of oxygen and light. The insecticidal esters in the pyrethrum extract

were unstable and converted to non-insecticidal products upon exposure to air and UV light. These natural esters are often nonpersistent, thus accounting for the ease with which they undergo photodecomposition (Chen and Casida, 1969).

Sasaki et al. (1970) and Ueda and Matsui (1971) showed that the carbon 1 and 3 of the main cyclopropane group are cleaved, which subsequently leads to the formation of a di-radical. However, of more importance is the photochemical degradation of the esters, which is rapid in the presence of oxygen and sunlight. Elliot (1976) suggested the possibility of two reactive positions, one on the isobutenyl side chain, and the other along the pentadienyl side chain of pyrethrins I and II that indicated that both reactive positions were attacked under light conditions. Based on these findings, they suggested that the presence of at least two photosensitive centers in the same molecule explains the observed instability in air and light of the pyrethrin I. Bullivant and Pattenden (1976) determined the photodecomposition products of PI and PII to be the trans isomers, which resulted from the cis/trans isomerization of the double bond (C8'-C9') in the butadiene side chain. Crosby (1995) showed under dark conditions, there is little degradation of pyrethrins over time, yet in natural light conditions, there is a rapid degradation from 100% to less than 1% within 5 hours. It is however, this non-persistence that makes pyrethrins widely accepted as safe and environmentally innocuous alternatives to other "hard pesticides" (Hitmi et al., 2000).

Pyrethrum has also been shown to be stable for long periods in water-based emulsions as well as in stabilized oil concentrations (OSHA, 2007). Temperature has also been shown to be a critical factor in the degradation of natural pyrethrins, whereas moisture, oxygen alone, and microbial activity did not play any major role. When stored pyrethrin crops were exposed to temperatures of 20, 60 and 100 °C, pyrethrin content was lost in the amounts of 26, 65 and 68%, respectively (Atkinson et al., 2004). Because these values did not reach zero concentration, it was suggested that the plant structure might be providing a chemical or physical protection to the pyrethrins within. The authors went on to show a similar pattern of percentage loss of the jasmolins and the cinerins, whereas, the pyrethrins were shown to degrade at a much greater extent (Atkinson et al., 2004). Because pyrethrins display such a great degree of instability to such a wide range of environmental conditions, there is substantial interest in improving the

biological activity and stability of the natural pyrethrins to increase their efficiency as insecticides. This improvement concerning increased efficiency and reduction of application rates by complexation with gamma-cyclodextrin, has been an aim for investigators for years (Kasaj et al, 1999). Cyclodextrins (CDs) were added to slow down the degradation of pyrethrum induced by exposure to light. They offer a hydrophilic exterior and a hydrophobic cavity, allowing them to form inclusion complexes with hydrophobic guest molecules, rendering them useful for a wide variety of purposes, as well as effective for use in a range of insecticides (Yamamoto, 1973). Because CDs are not toxic towards humans, mammals, or the environment, they prove to be a useful additive to pyrethrum. Biebel (2003) showed that the gamma-cyclodextrin had the highest complex-formation constant with pyrethrum, and that the stability of the pyrethrum against light could be greatly enhanced by this complexation. Complexes of sesamol, or tocopherols with the gamma-CD were prepared and analyzed for their synergistic action against the grain weevil, *Sitophilus granarius*. The addition of pyrethrum with the gamma-CD showed a slightly enhanced action, compared to that of a pyrethrum-free product.

Perceived Disadvantages of Applied Pyrethrins

The principle drawbacks of pyrethrum usage has been instability in air, heat and light, rapid loss of insecticide activity (Allan and Miller, 1990), and quick recovery from knockdown (Atkinson et al., 2004). It is this general instability that has somewhat restricted the development of pyrethrin as all-purpose crop protection agent and as a satisfactory insecticide against agricultural pests (Atkinson et al., 2004). However, while the rapid metabolism of pyrethrins is a drawback concerning the frequency of the application, as it will have to be applied more often, than with other insecticides, synergists help alleviate this disadvantage (Biebel et al., 2003). Despite their environmental friendliness, the application of pyrethrins has also been limited, because of their relative high costs when compared to other insecticides.

The inherent instability of pyrethrins often also triggers problems concerning proper and effective storage. After pyrethrum crops are harvested, they are stored for a given period of time, sometimes up to one year before being processed. It is during this time that substantial losses of the pyrethrins occur, often due to the environmental conditions of its storage, which creates major economic concerns for those within the pyrethrum industry (Atkinson et al., 2004). Morris et al. (2006) assessed the effects of the cutting and drying processes on mature pyrethrum field

crops and showed that these methods are actually the most cost effective means of preparing material for commercial pyrethrins extraction. During the initial drying period, the crops experienced significant moisture loss without any detrimental effect on pyrethrins content, reassuring the industry that the current practice of drying crop material prior to harvest was not deleterious to pyrethrins yield. It is also during this storage period, that heat, oxygen, moisture and microbial action can all increase further degradation of the natural pyrethrins (Gnadninger, 1936; Martin, 1949; Picone, 1999). Selection for resistance in pest populations is the principal threat to the continued efficacy of pyrethrin and pyrethroid insecticides for the control of agricultural pests (Soderlund and Knipple, 2003). Repeated usage of the pyrethrum-synergist combinations on insects does not evoke the high degree of resistance commonly encountered with most synthetic insecticides, where high levels of resistance have been reported in field-derived strains of *T. castaneum* (Collins, 1990). However, these distinct disadvantages of high cost, poor stability, inadequate toxicity to some species, selection for resistance and lack of ovicidal action have also been offset somewhat by the use of synergists (FAO, 2008).

Since 1995, a decline in the African production of pyrethrins, as well as increasing worldwide demands that are exceeding the supply, has caused a significant increase in the market price of pyrethrum. However, it has also encouraged research methods that can improve yields and aid in the development of alternative methods of production (Hitmi et al., 2000). Due to the complexity of the active molecules comprising pyrethrins, chemical synthesis is not economically efficient. However, large-scale cultivation of plant cells remains one of the most viable options (Barthomeuf et al., 1996), allowing pyrethrum to be extracted from high-productive clones derived from selected genotyping and collected by tissue culture (Hitmi et al., 2000). This development of an efficient tissue culture system for the rapid production of pyrethrum, can alleviate problems with its availability, and associated costs for mill applications. The increasing demand for natural pyrethrins has persuaded the use of these cultivated plant cells, as an alternative to obtaining pyrethrins from the *C. cinerariaefolium* flowers. It has been shown that the callus these flowers are able to synthesize pyrethrins at much higher concentrations, often more than 30 mg/100g in dry biomass (Wambugu and Ranagan, 1981; Ikahu and Ngugi, 1993, Barthomeuf et al., 1996). The advent of stricter environmental legislation and the mounting industrial research and development costs of new chemical

insecticides have thus encouraged the use and a resurgence of natural pyrethrin usage (Van Latum and Gerrits, 1991; Matsuda, 2005).

Chemical Control with Insect Growth Regulators

Insect Growth Regulators

Insect growth regulators (IGRs), also called “third-generation insecticides”, include juvenile hormone (JH) mimics and chitin synthesis inhibitors, which interfere with larval cuticle deposition and disrupt the molting process by inhibiting the synthesis of chitin. IGRs are synthetic analogues of naturally occurring hormones in insects, otherwise known as juvenile hormone analogues, ecdysone agonists or molt inhibitors. IGRs with juvenile hormone activity, otherwise known as juvenile hormone analogues (JHA), are nonpoisonous and do not bioaccumulate, therefore they generally do not persist for prolonged periods in the environment. IGRs have been shown to generally have a good margin of safety for most non-target biota, as they display a very low toxicity for humans and other mammals, are readily biodegradable (i.e., very low persistence in the environment), highly toxic to target insects, and leave no hazardous residues, making JHAs very useful in food preservation and storage (Tunaz and Uygun, 2004). There has been a renewed interest in IGR usage, specifically in the capacity as grain protectant treatments, surface treatments, as well as aerosol and fogging treatments in the interior of food storage structures (Niño et al., 2009).

JH is a highly effective, sesquiterpenoid hormone that regulates growth and development in insects. Since JH signaling is specific to insects and other arthropods, compounds disrupting JH action in insects are an ideal target for pest management, due to their low toxicity to non-target organisms outside the Arthropoda (Minakuchi and Riddiford, 2006). Early work suggested that JH-active compounds could be used specifically to disrupt the normal activity of the endocrine (i.e., hormone) system of insects (Williams, 1967), by mimicking the hormones that control the processes of molting, which in turn ultimately affects the processes of development, and interferes with reproduction, as well as the regulation of metamorphosis within the targeted insect (Riddiford and Truman, 1978; Mondal and Parween, 2001). Considerable effort has been made to develop synthetic JHAs that are both stable in the environment and resistant to metabolism (Minakuchi and Riddiford, 2006). The concept of using an insect’s own hormones to

control pest populations was introduced in 1956 (Williams, 1956; Williams, 1967). Upon the suggestion of Williams (1956) to make use of the JH compound in insect control, many programs were initiated to synthesize these compounds, as well as additional analogs of varying chemical structure (Tunaz and Uygun, 2004; Minakuchi and Riddiford, 2006). JHAs exhibit similar effects to those of naturally secreted juvenile hormone (JH), but are structurally different than their JH precursors (Novák 1971). Most JHAs resemble JH in their basic terpenoid structure. The most active of the JHAs, such as methoprene and hydroprene however, lack the epoxide function present in JH (Staal, 1975). JHAs have been shown to be very effective in disrupting both insect embryonic development (Riddiford and Williams, 1967; Staal, 1975) and metamorphosis (Staal, 1975; Lim and Lee, 2005), thus actively preventing recurring infestations (Daglish, 2008).

JHAs are most effective at the beginning stages of metamorphosis in insects (i.e., freshly ecdysed last larval and pupae instars and newly deposited eggs), where embryogenesis is easily disrupted. Application to early last instar larvae often results in the development of supernumary instars, whereas treatment at the later stages tends to result in abnormal pupation, and development of larval-pupae mosaics, or intermediates (Tunaz and Uygün, 2004). IGRs do not kill adult insects, but help to eliminate infestations by inhibiting development of immature insect stages, which ultimately reduces subsequent adult emergence (Arthur, 2003). The most effective IGRs against insect pests have been shown to cause rapid death of the targeted insect through failures of key regulatory processes (Tunaz and Uygün, 2004). Compounds developed to disrupt metamorphosis ensure that very few, if any reproductive adults are formed. Those that specifically interfere with reproduction may promote the development of adults with certain morphogenetic abnormalities that reduce their reproductive potential. These adults are often sterile or possess abnormally developed genitalia, which may ultimately hinder the mating process, as well as their capacity to produce fertile offspring (Tunaz and Uygün, 2004).

JHAs, have been readily integrated into pest management programs, in the form of aerosol spray treatments to control stored product insects in bulk grain, as well as in milling, processing, and storage facilities (Ishaaya, 2007). Three possible modes of exposure exist for integrating IGRs into existing pest management strategies: surface exposure/contact; vapor

action; and, ingestion (Tunaz and Uygun, 2004). The most common IGRs used for stored product insect pest management purposes, include S-hydroprene, S-kinoprene, and S-methoprene, all of which disrupt the normal development of a wide array of insects (Wolterink et al., 2001; Peckman and Arthur, 2006). Methoprene and S-methoprene, are labeled for uses both indoors and outdoors, and can be applied directly to both food supplies as well as other nonfood entities. S-kinoprene and S-hydroprene are currently registered for use, only for indoor purposes and are not labeled for direct application to food products (Glare and O'Callaghan, 1999). Unlike S-methoprene, S-hydroprene, which delivers a visible, dissipating foam, is not registered for space treatments, and is conveniently used for spot-treating and crack-and-crevice applications, and most often labeled for cockroaches (Kawada et al., 1989; Koehler and Patterson, 1991), and stored product insects (Arthur, 2003). Although IGRs have been available for over 30 years, there has been a renewed interest in their application for stored product insect pest control purposes (Oberlander et al., 1997), as reduced-risk insecticides that can replace older chemicals currently being used in pest management programs (Arthur, 2004). Today, IGRs represent one of the newest of all utilized approaches to operational and commercial insect control (Tunaz and Uygun, 2004). It is possible for reduced risk insecticides such as IGRs to be used as potential replacements for conventional neurotoxins that are currently facing the possibility of losing their registration through the FDA certification process (López et al., 2005).

JH compounds may prove more useful as grain protectants (i.e., preventative) rather than infestation control measures (i.e., curative). When applied to insect free grain or grain containing relatively low numbers of pest insects, they can be effectively used to prevent an infestation buildup. Under these conditions, prolonging the development of small numbers of immature larvae would prove insignificant, as little contamination or product loss would likely occur. However, when used in combination with more deleterious control measures, lower application rates could be utilized, and would perhaps prove more effective in environments where JHAs applications are applying stress to the existing populations (Semple, 1992). The potential of IGRs has been documented in laboratory trials and undoubtedly will play a significant role in future strategies involving integrated approaches for control of stored product insect pests. Previous documented research, as well as modern insect management have shown that the application of IGRs possessing JH activity to larvae, will help to prevent their subsequent

development into the adult stage, thus reducing the reproductive potential of a given pest population. Thus, it becomes clear that the usefulness of IGRs in pest control is under those conditions where the adult is the harmful stage of the insect pest (Semple, 1992).

Morphological Abnormalities Associated with Insect Growth Regulators

Development and reproduction in insects is affected by a number of endogenous, biogenic hormones that influence both metamorphosis and the subsequent development of targeted insects (Glare and O'Callaghan, 1999). The physiological changes associated with metamorphosis, are regulated by both the ecdysones that initiate molting, and by the JHs that regulate growth and development of the immature stages, under normal physiological conditions (Semple, 1992; Tunaz and Uygun, 2004). JH is necessary for insects to molt and reach the next stage in their development. With each subsequent stage, the amount of JH is reduced, allowing insects to eventually develop and emerge into adults (Niño et al., 2009). JH is found in relatively high concentrations in the hemolymph during certain larval stages, where its function is to maintain the larvae in their current state, and thus prevent the progression of metamorphosis. During normal insect development, the concentration of the JH decreases in the final larvae instar stage, at which time the gene expression of the steroid hormone 20E is activated, allowing progressive development through subsequent life stages (Glare and O'Callaghan, 1999; Wu et al., 2006). JHs expressed at precise times leads to metamorphosis, however if present at non-essential times, morphogenetic abnormalities can occur which are usually irreversible, and are easily the most readily observed effect of IGRs. The extent and character of these responses vary between insect developmental stages, but generally occurs in the larval and pupae stages (Sehnal, 1971; Staal, 1975; Glare and O'Callaghan, 1999), often, disturbing the process of ecdysis, which most notably interferes with the formation of new cuticle (Hajjar, 1985).

The presence of JHs and JHAs can initiate arrested development and cause species-specific larval and pupae mortality, as well aiding in the development of arrested individuals at each developmental stage (Tunaz and Uygun, 2004). Most insect species will respond to IGR treatment by producing extra larval or pupae forms, which may range from almost perfect to intermediates between the immature and adult forms. When applied directly to larvae this can result in a disruption of both pupae development and adult emergence, leading to the formation of various intermediate forms between the larval and pupae stages, as well as various

intermediate forms between the pupae and the adult stages, upon which reproduction is often inhibited and normal ecdysis is not readily achieved. The intermediates always succumb earlier to the effects of the JH and JHA, as this exposure to extraneous JH causes ecdysis to be impaired (Tunaz and Uygun, 2004). It has been proposed that the period of greatest sensitivity for metamorphic inhibition is during the last larval stage. Retnakaran (1973) demonstrated that altering the titer of JH in the last larval instar results in the production of larval-pupae intermediates that seldom survive. If the JH is applied on the first day of the last larval instar, prior to the ecdysteroid programming peak, the larva molts into a supernumary larva. If applied during the peak, larval-pupae intermediates are formed, and if the JH is applied after this programming is over, there were no observed effects. These supernumary larvae consume additional food, and therefore can cause increased damage to the infested commodity. Though it is the larval and adult stages that feed upon the infested resource, it was proposed that this extra damage is limited, to a degree, even when several additional stages are formed (Sehnal, 1971). The extent and character of these developmental responses to the application of IGRs depends on the exposed species, the time of the application, the dose, the mode of administration and the specific compound, or formulation utilized in the application process. Because these formative processes occurring in an insect are not all sensitive at exactly the same time, the longer duration of exposure, to the life stage that is most susceptible, yields a more complete inhibition across all other subsequent life stages (Sehnal and Meyer, 1968; Stall, 1975).

Williams (1967) proposed that timely applications of JH and JHA could be employed to control insects because of their ability to disrupt normal physiological functions. The addition of a JHA, such as methoprene, to an insect at a time when its natural hormone level is low, allows for disruptions in the normal morphogenesis. These developmental abnormalities, due to the effects of applied IGRs were initially demonstrated in stored products insects by Metwally and Sehnal (1973), Strong and Diekman (1973), Amos et al. (1974), and Loschiavo (1975). Results from these studies suggested that if treated larvae were unable to complete development, they might ultimately give rise to morphologically deformed adults. A range of adult deformities were recognized upon exposure of *T. castaneum* larvae to IGRs, including aberrations of the tarsi, legs reduced to unchitinized stump-like appendages, lack of differentiation and poorly chitinized antennae clubs, crumpled and greatly diverging wings and elytra and developmental

intermediates including larval-pupae intermediates, and pupae-adult intermediates (i.e., “adultoids”), due to incomplete metamorphosis, resulting in the premature eversion of the elytra and the membranous wing.

Most published studies regarding IGR treatments on stored product insects have been performed using either the egg stage, or by exposing adults on treated grain, allowing efficacy to be assessed by observing inhibition of progeny development. Research studies in which IGRs have been tested against both *T. castaneum* and *T. confusum* have largely involved mixing the chemicals components with the insect diet and seeding the diet with eggs (Oberlander et al., 1997). Often, this technique has proven to be effective, because the earlier that an insect is exposed to IGRs in the larval life cycle, the less likely it is to reach the adult stage (Arthur, 2003). When eggs of stored product beetles were exposed on grain treated with hydroprene, adult emergence was severely reduced, and a great percentage of adults that emerged exhibited morphological abnormalities (Rup and Chopra, 1984; Amos et al., 1974). Amos et al. (1974) exposed eggs (0 - 2 days old) of *T. castaneum* and *T. confusum* to a range of JHAs. Exposure to hydroprene applied at 20 ppm in combination with S-methoprene at both the 5- and 20 ppm amounts, revealed a prolonged developmental time of immature stages. Most notably, they observed a significant increase in the developmental period between the larval and pupal stages. They commented that, "prolonging this developmental trophic stage is likely to result in more food being consumed or contaminated, or both. For control of existing insect infestations, it may be more desirable to use a JH compound, which does not appreciably increase the larval life span, which preferably should be reduced. In this situation, the compound should ideally either inhibit the development of the egg or young larva, or affect the prepupae and pupae stages so that metamorphosis is either prevented or the adults produced are sterile. Amos et al. also observed a complete inhibition of development at both the 5 and 20 ppm application rates, such that all individuals either died in the larval stage, or as adultoids. This inhibition of adult emergence indicated that a combination of the two IGRs were in fact more effective in reducing developmental survival. In addition, both of the exposed *Tribolium* spp. showed inherently different responses and sensitivity to the applied JHAs (Amos et al., 1974; Williams and Amos, 1977). However, these particular studies involving the exposure of eggs to IGRs, while often

effective at suppressing pest populations, might not accurately simulate exposure under actual field conditions.

Only a few published studies have reported the morphological effects of exposing later larval instar stages of stored product insects to IGRs and thus observing their development through subsequent life stages. The application of certain IGRs to larvae has been shown to increase developmental mortality, while others completely inhibited the development of adults, depending upon the applied concentration. The effectiveness of JHAs depends on the timing of application and the specific stage of insect development (Tunaz and Uygin, 2004). The application to larvae during a given period in which some cells are sensitive, while others are not, often leads to the production of various intermediate forms. When last instar *T. castaneum* were exposed on wheat flour treated with increasing concentrations of methoprene, several physiological effects were described, these included dead adults, live deformed adults with twisted elytra and/or wings, dead adults that failed to completely emerge from the puparium, and larvae that failed to pupate (Hoppe, 1981). Hoppe (1981) assessed the effectiveness of S-methoprene against several insecticide-resistant strains of *T. castaneum*, when used over a range of applied concentrations, and found when larvae were exposed to lower concentrations of S-methoprene, adults were able to effectively emerge. However, when treated to moderate concentrations of S-methoprene, the larvae were more likely to induce development of 'adultoids'. These concentrations were sufficiently low enough to allow pupation of larvae, but the development of normal adults was still prevented. Applying increasing concentrations of S-methoprene produced a gradual reduction both in the number of adultoids being formed, and in their mortality, within the larger population. Upon exposure to these increased concentrations, the number of surviving 'superlarvae' increased, and it was during induced prolongation of the larval stage that the weight of these superlarvae were shown to increase with time (Hoppe, 1981).

Loschiavo (1976) reported the effects of methoprene on the survival, development and reproduction of six species, including both *T. castaneum* and *T. confusum*. Methoprene was applied to insects over a concentration range of 1-20 ppm, maintained at 30 °C and 63 ± 3% r.h., noting the number of F1 progeny. An application rate of 20 ppm largely prevented the

subsequent development of *T. castaneum* larvae to the pupal stage, and significantly reduced pupation of larval *T. confusum*. At application rates of 5 ppm or higher, oviposition was inhibited in both species. Furthermore, the presence of methoprene at any application rate increased the time required for complete larval development, often resulting in the production of superlarvae. Fully developed larvae of both species, which had failed to pupate upon exposure to concentrations ranging between 5-20 ppm of treated flour, had more sclerotized integuments, and were larger than those not exposed to methoprene. Similar results were reported by El-Sayed (1984) in a study whereby methoprene was applied to flour at application rates of 0.5-10 ppm. Pupation of *T. castaneum* was substantially reduced on the flour treated at 10 ppm concentration, whereas at the application rate of 0.05 ppm oviposition was inhibited, and both pupae-adult intermediates and malformed adults were observed. Results of Leftwich (1976) showed a disruption within both pupae development and adult emergence, as well as the characteristic formation of various intermediates between the larvae and the pupae, and the pupae and the adult stages, when late-instar larvae were exposed to IGR-treated diets. Upon further observation of the morphologically deformed adults, impaired reproductive physiology was often observed in these studies. This sterilizing effect has also been observed in the khapra beetle *Trogoderma granarium* Everts (Metwally et al., 1972), as well as *T. castaneum*, when adults were reared on a flour source treated with either methoprene or hydroprene (Amos and Williams, 1977).

The mechanisms of JHA action on *T. castaneum* have been recently investigated and are reported by Parthasarathy and Palli (2009), and provide a basis for understanding the molecular mechanisms of hormonal regulation of metamorphosis in *Tribolium* spp. Application of JHAs, specifically S-methoprene, during both the penultimate and final instar larval stages, both blocked larval-pupae metamorphosis and induced supernumary larval molt. Disruption of the normal hormonal pattern of either of these stages can severely alter the normal developmental sequences of the exposed insects. Insects exposed to varying dosages of S-methoprene during their final instar stages showed a nearly complete block in pupae metamorphosis, especially in doses in excess of 5 ppm. At an application rate of 1 ppm, S-methoprene blocked larval to pupae metamorphosis in 85% of treated larvae, while the remaining 15% pupated and subsequently died. The authors stated that the presence of JHA during this final larval instar stage, likely blocked midgut remodeling and suppressed the expression of genes involved in 20E action

within the midgut, which effectively blocked further proliferation and differentiation of the imaginal cells, ultimately preventing normal development. However, when both the final and the penultimate larval stages were treated to 0.01 ppm of S-methoprene, 50-60% of treated insects molted into supernumary larvae, while the remaining larvae pupated and emerged as adults. The mechanism of this specific and selective toxicity, both within different insect species, as well as various insect stages lays in differential actions on the JH target receptors (Narahashi, 1996; Ishaaya, 2007). The action of JHAs is accomplished by competing with JH in binding to the JH receptors, or to the JH carrier proteins, which then interferes with normal JH biosynthesis, effectively acting as feedback inhibitor of this JH biosynthesis (Ishaaya, 2007).

Methoprene Use In Stored product environments

Methoprene was first introduced into the market as Altosid™ (EPA Reg. No. 2724-393) in 1975 (Tunaz and Uygun, 2004), then later re-registered as Diacon™ (EPA Reg. No. 2724-788) in the 1980's. Methoprene (isopropyl (E,E)-(RS)-11-methoxy-3,7,11-trimethyldodeca-2,4-dienate) is a JHA that specifically disrupts normal insect development. S-methoprene (isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate), known as Diacon II™ (EPA Reg. No. 2724-298), was registered in 2002. As a synthetic analog of the naturally occurring insect JH, it contains a long chain hydrocarbon ester, which has shown great potential as a grain protectant and as such, has been widely used to control emergent insect pest populations (Staal, 1975; Glare and O'Callaghan, 1999). S-methoprene is a terpenoid, and considered to have higher potency, as well as better field stability than do naturally occurring JHs (Henrick et al., 1976), and has been shown to be extremely stable, particularly when applied to commodities stored in darkened conditions (Crosby, 1995). A supplemental label was issued by the EPA in 2004, which permitted Diacon II™ to be used as both an aerosol and surface treatment inside mills, warehouses and other food storage facilities.

Methoprene Use to Control Stored product Insects

Methoprene and S-methoprene are generally very effective in selectively controlling a wide range of stored product insect pest species, especially those that feed externally. Arthur (2008b) reported results of a series of field trials in which *T. confusum* larvae were exposed to methoprene aerosol. This study showed that adult emergence resulting from these exposures was less than 2%. Other reported studies have observed the effect of methoprene on stored product

insects, including *Tribolium castaneum* (Herbst) (Loschiavo, 1976; Amos et al., 1977; Daghli et al., 1995; Hoppe, 1981), *Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle (Mian and Mulla, 1982; Daghli, 2008), *Rhyzopertha dominica* (F.), the lesser grain beetle (Oberlander et al., 1997; Daghli, 1998; Arthur, 2003), *Sitophilus granarius*, the grain weevil (Loschiavo, 1976), *Sitophilus oryzae*, the rice weevil (Loschiavo, 1976; Daghli et al., 1995), as well as many others. However, methoprene does not give good control of *Sitophilus* spp. (Samson et al., 1990).

Perceived Disadvantages of Applied IGRs

The prolongation of larval life presents a distinct disadvantage to using IGRs as a means of controlling stored product insects, and many published studies have noted this effect, upon exposing larvae of stored products insects to IGR treated diets. Firstenberg and Silhacek (1976) found that when exposing *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) larvae to different IGR treated diets, on average the larval feeding period was nearly doubled from that of control larvae. Though the length of this feeding period was dependent on the concentration of applied IGR, it did not alter the rate of larval feeding. Metwally and Sehnal (1973) demonstrated that *Trogoderma granarium* Everts (Coleoptera: Dermestidae) treated within the first nine days of the final thirteen-day larval instar stage underwent up to six extra larval molts, increasing the longevity of the larval trophic phase by more than four months compared to control larvae. Most of these supernumerary larvae died, while those that pupated either formed adultoids, or were able to complete development into morphologically normal adults. These adultoids were also produced when the IGR compounds were applied within the first third of the pupae instar stage. When larvae were subjected to JHA vapors for 6 week periods, the last instar larvae experienced 1-4 extra molts, finally developing into either larvae-pupae intermediates or normal pupae, which were able to continue their development, producing again either adultoids or morphologically normal adults. Compared to the control pupae, the exposed pupae were shown to be able to continue their development into normal adults, however these adults had lower fecundity, and their eggs were shown to have a reduced hatchability, often by as much as 75%. The remaining 25% hatched into larvae, but died within the first two or three larval instars. Upon exposure of *T. castaneum* to IGR spiked flour, Ishaaya and Yablonski (1976) demonstrated that the exposed *T. castaneum* larvae showed a 10x prolonged larvae feeding period, and a doubling in larval weight, when compared to the untreated control larvae. Loschiavo (1975) observed a delayed developmental period, of up to 5 days in *T. confusum*, and up to 6.5 day delay within *T.*

castaneum, when reared on diets containing hydroprene and S-methoprene at 10 ppm concentrations. Results from this study implicated the period between the fourth instar larvae and the time of pupation as the critical level for the JH effect. Alternatively, triprene or kinoprene were shown to have no effect on larval survival or development in either of *Tribolium* spp.

The timing of an IGR application with regards to the exposed insect stage presents a challenge to the management of *Tribolium* pest populations within a milling or warehouse facility. Brieger (1973) suggested that most effective JHAs target insects early in the last larval instar stage, as well as demonstrate a level of stability that will allow it to be maintained for the length of time in which the larvae continue to consume treated commodity resources. JHAs (i.e., juvenoids) exert their influence only within a limited time and often do not stop the development of early larval stages of most insect species, often the most destructive insect stage. Thus, a major limitation of IGR usage is that they generally do not kill adult insects; however, targeting a specific stage has been proposed as the most ideal means of controlling insect pest populations. An ideal targeted insect, for IPM purposes was defined by Henrick (2007) as one that can be characterized as having several short life cycles per year, a species that lives in confined environments (i.e., storage areas), and a species in which the larval stage does little to no damage. For example bugs, locusts and other hemimetabolous insects are usually only sensitive JHAs or mimics shortly after the last larval ecdysis, (i.e., the first third of the last larval instar), or after adult emergence, at which time JH can exert an ovicidal effect. Alternatively, the larvae of most holometabolous insects such as Lepidoptera and Coleoptera are sensitive only at the end of the last larval instar, while the pupae are sensitive for several hours or at most a few days after the last larval ecdysis. Therefore to be effective in the field, JHAs must be developed such that they can be applied at critical times, and possess the stability to persist in the environment for long enough periods of time to expose all members of the population during their periods of sensitivity to JH (Schneiderman, 1971; Brieger, 1973).

In the early stages of IGR-focused research, it was suggested that insects would not develop resistance to this specific control agent (Williams, 1967), due to JHs unique mode of action (Monandass et al., 2006). Over time, it became evident that particular species were developing cross-resistance to a number of JHAs (Dyte, 1972; Jakob, 1973). IGR resistance was

shown to be readily induced under laboratory conditions in different insect species through selection, after only a small number of successive generations (Brown and Brown, 1974; Brown et al., 1978). The development of IGR resistance, as well as the resistance of pest species to other classes of insecticides has been verified (Collins, 1998). This concept of IGR resistance was realized early on concerning S-methoprene (Horowitz and Ishaaya, 1994; Dame et al., 1998; Cornel et al., 2000, 2002; Horowitz et al., 2002). Allanson and Wallbank (1994) determined the feasibility of protecting stored grain with S-methoprene as insects were beginning to display increased levels of resistance to commonly used OP compounds, as well as pyrethroids. This increased resistance to widely utilized insecticides, as well as the impending loss of additional chemical methods within the stored products industry has accelerating the need for developing effective, less resistant alternative grain protectants. These S-methoprene treated grain studies suggested that this insecticide is able to provide residual protection to a number of target species, even after grain/commodity was removed from storage. Increased usage of S-methoprene in stored product environments has been deemed useful in resistance management strategies, specifically directed at lessening the selection pressures from OP insecticides (Oberlander et al., 1997). To date, a limited resistance to S-methoprene has demonstrated, and only within a few insect species. Resistance to synthetic JHAs has been demonstrated in both *T. castaneum* and *T. confusum* species, as well as other stored product insects (Glare and O'Callaghan, 1999).

Any level of resistance observed in IGRs displaying chitin-inhibiting properties, indicates a level of multi-resistance factors, generally thought to be due to enzymatic detoxification methods. Insects able to metabolize various groups of insecticides may confer some level of cross-resistance to other IGRs (Biddinger et al., 1996). Dyte et al. (1975ab) had previously reported that JH and other synthetic JHAs are metabolized much more rapidly in resistant insects than in those susceptible to the effects of JHAs, such as S-methoprene. Because of the differing structure of these juvenoids, any observed IGR-resistance was not necessarily associated with an enhanced detoxification by one particular pathway. It was proposed in 1979. An altered enzyme or an increased amount of enzyme in the mixed-function oxidase system is involved in the resistance to juvenoids (Oppenoorth and Welling, 1979). More recently, Wilson and Ashok (1998) suggest that an insects' resistance to JHAs could be due to either degradation of these artificially applied JHAs before reaching their target sites, or due to modification of the target

site itself, resulting in reduce affinity of juvenile hormone binding proteins to the JHAs. At the molecular level, insect resistance to an insecticide, including JHAs, could largely be a result of point mutations and/or up regulation or amplification of detoxification genes. During their development, insects normally inactivate, sequester, or excrete additional JH and many JHAs. Thus, nature has endowed insects with a built-in mechanism to resist the artificial application of JH and JHAs, at certain stages, by ensuring mechanisms to inactivate them (Riddiford, 2008). The ability to successfully resist the juvenilizing effects of these compounds normally only function at specific times during an insects development, however the existence of such mechanisms guarantees that natural selection could produce populations of insects resistant to exogenous JHAs (Semple, 1992). Observed differences in sensitivity across insect species might stem from differences in the rates of penetration, breakdown, excretion, storage or differences in behavior feeding habits, etc. At the molecular level, insect resistance to an insecticidal chemical, including JHAs could be mainly due to point mutations and/or up regulation or amplification of detoxification genes (Wilson and Ashok, 1998; Mohandass, 2006). The fact that one group of insects respond to JHAs, whereas a closely related, yet genetically diverse group of insects fail to respond, suggests that resistance t JHAs could be selected for in nature (Schneiderman, 1971). Observed species or life stages that are more susceptible to a particular IGR compared to conventional insecticides may be more amenable to selective control through existing IPM programs (Tunaz and Uygun, 2004).

Treated surfaces

There is a growing awareness of the need for cost-effective and efficient methods to monitor insecticide and pesticide residues in the food supply. Aerosols are the product of choice in most circumstances for insect integrated pest management programs (Buckle, 2004). However, new insecticide regulations resulting from the Food Quality and Protection Act, as well as increased consumer concern over pesticide residues have limited the availability of insecticides for use in stored products (Arthur and Rogers, 2003). Residual structural sprays are applied to grain facilities as an integral part of pest control practice. Storage structures often contain residual food and refugia that support resident insect populations and provide opportunities for insects to escape exposure on treated surfaces (Barson, 1991). However, the use of chemical repellents on food packages can result in contamination of the product should the

repellent migrate from the treated surface through the package barrier and into the commodity. Often this migration can be reduced or prevented with the use of barrier plies that are positioned between the chemically treated ply and the packaged contents (Highland, 1974). Highland (1977a) reviews studies showing the effectiveness of treated package material, specifically kraft paper, shrink wrap and fabric bags. The surface of a substrate is an important factor in establishing the relative toxicity (i.e., persistence and efficacy) of applied residual insecticides, in which insects have direct physical contact with on a continual basis, allow tests to be performed that can assess the extent to which these insecticides are able to effectively control pest infestations (Arthur and Peckman, 2006). A residual insecticide, when applied to a surface, is deposited as a concentration with residual activity that continues to kill the targeted pests until the concentration of available residue has dropped to a sublethal level. Depletion of the residue takes place by a number of means, including oxidation, absorption into the substrate, volatilization, etc (Gudrups et al., 1994). Degradation of the insecticide residue may be accelerated by high temperatures, which might also affect the physiology and behavior of the targeted insect pests. At these elevated temperatures, insects respire more rapidly and may move around on the treated surface more extensively, resulting in a greater incidence of picking up the active ingredient (Gudrups et al., 1994). In addition, the volatilization of insecticides may also be increased by conditions of high temperature, due to the effects of changes in humidity, such that these lipophilic pyrethrins will be less readily volatilized (Gudrups et al., 1994). Several analytical methods have been adapted for the detection and quantification of insecticides, such as methoprene and pyrethrins, including GC/MS, HPLC/UV, LC/MS, etc, however, often the problem resides in determining the minute quantity of insecticides on a surface.

The packaging of products is the last line of defense against insect infestation of their finished products. These materials are often incredibly susceptible to attack by insects, and must be both constructed and treated as to not allow insect infestations when materials, as they undergo subsequent shipping and distribution. Synergized pyrethrins with PBO were an effective insect-resistant package treatment (Highland, 1977a, 1978). The effectiveness of treating a variety of packaging materials has been demonstrated by Highland (1977b) which reports the results of a variety of packaging materials treated with synergized pyrethrins. Because *Tribolium* spp. cannot penetrate intact packaging and thus must enter through existing holes, insect-resistant

packages must exclude even the smallest of openings, to be effective against invaders and penetrators. These packaging materials can be made resistant to penetration through the use of appropriate chemical treatments or physical barriers, such that penetrators are prevented from making holes in the packaging, through the altering of their behavior. Paper and cellophane are probably the least resistant to penetrating insects of all the flexible packaging materials. Depending on conditions, species of some insects can penetrate kraft paper in less than one day, and multi-ply construction often add little to the resistance (Highland, 1977). Treatment of the outer plies of multiwall paper bags with synergized pyrethrins, were shown to provide protection for up to 12 months, whereas bags of textile materials were shown to require much more aggressive management (Anonymous, 1955). However, with regards to insect-resistant packaged food products, often it is impossible to avoid all vulnerable spots in packaging due to high-speed production lines (Mullen and Mowery, 2006).

It has been shown that porous surfaces provide less control over time than less porous surfaces (Gudrups et al., 1994). On many of these porous substrates, the insecticide is carried into the pores, making it difficult and at times even unavailable for insects to absorb the residual insecticides, even though the persistence of the insecticide may be prolonged, as they are provided more protection from their environment. Webley and Kilminster (1980) showed that the loss of biological activity is not necessarily due to chemical breakdown of the insecticide, as evidenced by bioassay results on treated polypropylene (synthetic fabric) sacks as well as natural jute bags. Results of these tests showed that the deposits on polypropylene retained equal or greater activity against *Sitophilus zeamais* Motsch and *T. castaneum*, than did the deposits on the jute material. Residue analysis tests showed that the insecticides were found to have a much shorter persistence on polypropylene than on jute, and were coupled with results showing higher residues on the grain inside of these materials. Shahjahan et al. (1991) reported the effect of methoprene coated kraft paper, used as insect resistant packaging material against *T. castaneum*. Results of this study showed no observable penetration by adults or larvae. However, after a 3-4 month storage period, it was found that almost all exposed packages were infested, demonstrating the capability of the larvae to penetrate small gaps, over time, in the seal of the packaging. This methoprene treatment, even at low levels, was shown to be overall effective in reducing infestations in the experimental packages by preventing the development of immatures.

Often manufacturers are unaware of the exact stage in which finished products become infested (i.e., production, packaging, transportation or storage), affecting consumer confidence and the bottom line of business, the profit margins. Once infested, these products cannot be regenerated, often condemning a majority of these products to a very low economic threshold. This situation therefore calls for an improvement in packaging material and design, which are likely to increase costs for the manufacturing of these insecticide treated (insect-resistant) packaging materials.

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CHAPTER 2 - Residual efficacy of pyrethrin + methoprene aerosol to control *Tribolium spp.* (Coleoptera: Tenebrionidae).

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Abstract

Wheat flour was exposed in Petri dishes to aerosol formulations of either 1% pyrethrin + methoprene or 3% pyrethrin + methoprene at the label rates in a commercial milling facility. Residual studies were conducted by placing four-week old larvae of both *Tribolium castaneum* (Herbst), the red flour beetle, and *T. confusum* (DuVal), the confused flour beetle, along with flour on the treated concrete exposure arenas, every two weeks on different sets of arenas exposed to the aerosol. Morphological characteristics were assessed for a period of 30 days for species- and stage-specific effects of the aerosol insecticides. Both *T. castaneum* and *T. confusum* were susceptible to the aerosol insecticide, but *T. castaneum* appeared to be relatively more susceptible. Due to the high susceptibility of *T. castaneum* larvae to methoprene, very few larvae advanced to their pupal stage over the 16-week residual toxicity study. However, normal adult emergence was relatively higher in the 1% aerosol application than in the 3% application for *T. confusum* on the exposed wheat-flour substrate. *T. confusum* larvae were able to develop into normal adult stage. Toxic effects of the aerosol insecticides were mainly observed in the larval stage for *T. castaneum*, but in pupal stage for *T. confusum*. This phenomenon is most likely due to the lesser amount of susceptibility of *T. confusum*, to the aerosol insecticide, allowing many larvae to develop into pupal stage. Our results indicate that these aerosol formulations could be used to effectively control these insects.

Keywords: methoprene; synergized-pyrethrin; wheat flour; *Tribolium castaneum*; *Tribolium confusum*; aerosols

1. Introduction

Tribolium are considered serious cosmopolitan pests of stored grains worldwide (Fedina and Lewis, 2007). They attack stored grain products such as flour, cereals, meal, crackers, beans, spices, pasta, cake mix, dried pet food, dried flours, chocolate, nuts, and seeds (Via, 1999; Weston and Rattlingourd, 2000; Mahroof et al., 2003). *Tribolium* spp. are worldwide insect pests of mills, food warehouses, retail stores, and urban homes (Rees, 2004), and are often disseminated worldwide through transported grains (Ryan et al., 1970). Product contamination by whole insects, eggs, insect fragments, frass, and cast skins often occurs in *Tribolium* infested processing plants and warehouses (Baur, 1984). Both *T. castaneum* and *T. confusum* may be present in large numbers in damaged grain, but neither species is a primary feeder of undamaged grain. Flour beetles are often attracted to grain that has high moisture content and it is this preference in conjunction with their secretion of benzoquinones, which lead to persistent odors that often encourages mold growth in the infested commodities/products (Assié et al., 2007; Walter, 1990). This species readily moves among resource (flour) patches, can distribute eggs among multiple patches, and can complete development in very small patches (Campbell and Hagstrum, 2002; Campbell and Runnion, 2003).

Tribolium feed primarily externally on stored grains (i.e., 'secondary colonizers'), as such, they will often have a greater reproductive capacity than primary feeders, which increases their potential for both causing losses and damaging grain products (Weston and Rattlingourd, 2000). The female *Tribolium* adults are quite prolific, laying between 500-1500 eggs (Sokoloff, 1974), more or less continuously during their life spans (Fedina and Lewis, 2007). Their atypically long adult lifespan, combined with the prolonged amount of time in which they are reproductively active, is suggested as a coping strategy of sorts, enabling them to effectively contend with fluctuating conditions during characteristic colonization-overexploitation cycles (Fedina and Lewis, 2007), as would commonly be experienced in mills, food warehouses and other stored product facilities. As efficient colonizers, *Tribolium* spp. can often achieve a relatively high intrinsic population growth rate, fast maturation, rapid larval development, as well as rapid dispersal activity (Fedina and Lewis, 2008).

Stored product pests are economically important and are responsible for millions of dollars of loss every year, causing both quantitative and qualitative losses (Neethirajana et al., 2007). Consumers and regulators have little tolerance for insect-damaged or contaminated food products, which pose serious consequences and challenges for pest management professionals (Arthur and Peckman, 2006). Grain losses resulting from insect infestations of all post harvest product losses have a major economic impact on the food industry due to the costs associated with the treatment and monitoring, rejection and return of contaminated products, loss of consumer goodwill, and failure to pass inspection or meet regulations (Campbell and Arbogast, 2004). The direction and breadth of pest-control strategies in the food industry has changed significantly over the past few years, through more-restrictive regulatory positions both on the reduction of residual insecticides, and a reduced reliance on insecticide usage within food-processing and storage facilities. These regulatory positions stress the importance of implementation and integration of policies and procedures to better manage pest populations, as well as aiding in the development of more sophisticated methods for measuring insecticidal residues, and calling for the optimization of insecticides with reduced mammalian toxicity (Faustini, 2006). The methods currently in use to control *Tribolium* populations have proven to be necessary in preventing a contamination of food materials, primarily used for human consumption. These methods specifically address the issues of stored, handled or processed food materials, and aid in preventing revenue losses (Daglish, 2005).

Pest management professionals commonly utilize aerosolized liquid applications, also known as fogging, for the management of stored product insects (Toews et al., 2005a). When applying aerosols in large commercial structures, there are a number of factors that could be encountered that will affect final efficacy of the aerosol. Comprehensive integrated pest management programs designed for commercial food processing facilities typically rely on the target pest species, application of residual insecticides, the distribution of the aerosol, the specific insecticide formulation, and the level of sanitation in the facility (Toews et al., 2006). Sanitation as it relates to IPM purposes refers to cleaning and eliminating extraneous food materials within food related facilities. The presence of food materials often compromises the residual efficacy of insecticidal treatments (Arthur 1998, 2000; Arthur and Campbell, 2008), by providing nutrition to the targeted insect pests, which can mitigate the effect of the applied residual insecticides, as

well as diminishing the likelihood for the targeted insects to encounter and be successfully exposed to insecticides (Arthur and Peckman, 2006).

Wheat milling is a particular major food industry with a very low tolerance of insect infestation. Flour mills contain complex networks of storage bins, processing equipment, and machinery for moving grain and milled material (Campbell and Arbogast, 2004). Within a milling setting, grain spillages and other residues in machinery and empty silos can support these residual infestations of stored-product insects (Sinclair and White, 1980). Infestations can arise from within the supply chain, resulting in contaminated food products being shipped through the supply chain to the product manufacturer (Daglish, 2005; Faustini, 2006). It is this presence of insects in finished and packaged goods, which directly affects consumer confidence (Collins, 1998). Because residual infestations can be the primary source of insects infesting stored grain, industry places a heavy emphasis on managing these populations through the adoption and use of good hygiene practices, which call for an elimination of food sources that allow grain insects to survive and reproduce (Hagstrum and Flinn, 1996; Daglish, 2005). Federal laws strictly regulate the presence of insects in processing facilities, as well as the amount of insect fragments in processed goods sold to consumers, insect management remains an important tool used to uphold consumer confidence (Neethirajana et al., 2007).

Aerosol insecticides are becoming a rapidly commercialized technology that is gaining popularity as a means of targeting and managing stored product insect pests. These applications are commonly applied on a recurring basis, often in 2-3 week increments, in a variety of stored product facilities (i.e., food-processing and indoor storage facilities, flourmills, warehouses, and grocery stores) (Toews et al., 2005; Toews et al., 2006). Aerosols have been shown to work effectively in environments where pests are primarily hidden, as the extensive coverage and residual activity of the applied aerosol formulation could mean larger percentages of dispersers within a given population are exposed to the aerosol treatment, which would negatively impact colonization by eliminating these individuals, which would readily reduce the population growth rate. Aerosols offer some residual control, but the level of persistence can depend on the substrate. Additionally, aerosols have been highly regarded for IPM purposes because they provide good coverage of all exposed surfaces, they do not penetrate packaging materials or

other surfaces, but can disperse into relatively sheltered areas, and the short exposure/reentry time allows for flexibility in treatment timing and targeting. Aerosols can also target specific areas, and also prolong the time interval between structural fumigations or heat treatments, all of which allow aerosols to be more readily utilized, especially as the food, shipping and other agricultural industries are searching for effective fumigant replacement strategies (Toews et al., 2005a, 2006b).

Residual contact insecticides can be successfully used as part of an integrated approach to control pest insects, for control purposes. Pyrethrins act to delay the sodium channel inactivation (Type I), (i.e., membrane depolarization), which leads to a longer prolongation of the sodium influx along the axon, creating persistent nerve depolarization and blockage of axonal conduction (Type II) (Valentine, 1990; Ling et al., 2001). Pyrethrins are rapidly metabolized within insects via the microsomal oxidase system (Proudfoot, 2005). Due to this rapid metabolism, pyrethrum is often mixed with a synergist such as piperonyl butoxide (PBO) to enhance its insecticidal activity. Decreasing this rapid metabolism (i.e., inhibiting degradation of the active insecticidal ingredients), therefore increases the toxicity (both, persistence and potency) of the pyrethrin-based insecticide, to exposed insects (Yamamoto, 1973; Proudfoot, 2005). Development and reproduction in insects are affected by a number of endogenous hormones, which influence metamorphosis, as well as regulate growth and development (Glare and O'Callaghan, 1999), thus allowing these chemicals to be successfully employed to manage stored product insects (Mohandass et al., 2006). Methoprene is applied as grain protectants to control emergent insect populations and further prevent infestation buildup in stored foods, grain, animal feed, and other seed stock. Acting as a juvenile hormone mimic, methoprene disrupts normal insect development, disrupting normal physiological functions (Williams, 1967). IGRs do not kill adult insects; instead, they eliminate infestations by inhibiting development of immatures, often by causing abnormal morphological development and ultimately reduced adult emergence (Tunaz and Uygun, 2004).

Quantitative data are lacking on the residual efficacy for economically important stored product insects, when exposed to various aerosol concentration rates. Characterization of the response of stored product insects to aerosol insecticide exposure (i.e., fogging), can aid in

determining the most responsive applied concentration, as well as the most cost-effective reapplication interval used to maintain insect emergence below an economic threshold. The objectives of this study were to: determine the efficacy of two independently applied concentrations of an aerosol insecticide comprised of synergized pyrethrins, at 1% and 3% label-rate concentrations, plus a 1% methoprene component, upon *Tribolium* spp., when exposed to treated wheat flour; evaluate a series of post-exposure storage intervals, by examining the interaction between the two aerosol application rates and the developmental effects on the two exposed *Tribolium* spp.; and, evaluate insect emergence and related species- and stage-specific deformities of *Tribolium* spp. Bioassays were conducted with four-week old larvae of both *T. castaneum* and *T. confusum*.

2. Materials and Methods

2.1 *Tribolium* spp.

Tribolium castaneum and *T. confusum* are common insect pests associated with food-processing facilities worldwide (Sinha and Watters, 1985; Mills and Pedersen, 1990). The larvae of *T. castaneum* and *T. confusum* that were used in the following experiments described below were obtained from a laboratory-reared population reared on a diet of 95% whole wheat, bleached flour, and supplemented with 5% (w/w) brewer's yeast. These insecticide-susceptible colonies had been reared for more than twenty years at the USDA Grain Marketing Research and Production Center in Manhattan, KS. All the colonies were maintained in a low-light environmental chamber at $27 \pm 3^\circ\text{C}$ and $70 \pm 5\%$ r.h.

2.2 Experimental Design

The substrate used for bioassays was whole wheat, bleached flour, a food source commonly found on a variety of surfaces within a milling facility. Implementation of integrated pest management (IPM) practices often calls for increased facility hygiene, emphasizing elimination of any food sources that allow insects to survive and reproduce (Daglish, 2005). Residual food material may provide nutritional components that help reduce the efficacy of the applied insecticide (Arthur and Peckman, 2006). The concrete exposure treatment arenas that were used for all bioassay experiments, were constructed using a standard, plastic 100 x 15 mm plastic Petri dish and concrete patching material (Rockite®), purchased from a local hardware

store. A water-based slurry was prepared by mixing about 2000 g Rockite® with 1.0 L of tap water, and pouring approximately 20 mL into the bottom of a Petri dish to cover the surface and dried overnight to create individual treatment arenas used in all subsequent bioassays experiments. The two aerosol insecticidal formulations were purchased from Entech Systems Corp. (Kenner, LA, USA). Entech Fog-10® (EPA Reg. No. 73049-400-40391) is comprised of 1.0% pyrethrins, 2.0% PBO synergist, 3.33% N-octyl bicycloheptane dicarboximide and 93.67% refined petroleum solvent. Entech Fog-30® (EPA Reg. No. 73049-400-40391) is similarly contains 3.0% pyrethrins, 6.0% PBO synergist, 10% N-octyl bicycloheptane and 81% refined petroleum solvent. Each pyrethrin formulation was applied according to label directions, which is 29.6 mL/m³ of space. Methoprene was added to both 1% and 3% pyrethrin at the label rate for space applications of 90 mg of active ingredient per 28m³, such that it amounted to 1% of the overall aerosol concentration. For the purposes of this study, these combined aerosol formulations are referred to as “1% pyrethrin” and “3% pyrethrin”.

The insecticidal application portion of this overall study was conducted in an operational flourmill, located in western Kansas, which had been previously equipped with an automatic aerosol application system. Multiple stationary mechanical sprayers were positioned roughly 4.5 m above the floor, in multiple locations throughout the mill, such that each floor of the flourmill could be treated separately. One particular floor within this mill, measuring roughly 1,716.8m² was chosen for the exposure portion of this study, of which approximately 25-30% of this total surface area was occupied by milling equipment. The prepared concrete exposure treatment arenas (described previously) were placed directly on the floor of this chosen level, within a 6m x 6m (3.3m²) unobstructed area, such that no treatment arena was placed within 0.6m of a potential overhead barriers (i.e., milling equipment, walls, doorways, etc.). Each treatment arena contained a pre-measured 6 g (6000 mg) of wheat flour; during the aerosol treatment, the lids of each treatment arena were placed open side up, underneath each treatment arena. A re-entry period of 2.5 h post aerosol application was selected, based on the preliminary results indicating that this was the minimum amount of time required for the settling of the aerosol cloud/fog within the interior of this particular milling facility. All untreated controls were held in another on-site building that was not exposed to the aerosol treatment. After the exposure to the prescribed aerosol treatment, and the subsequent re-entry period had lapsed, each treatment

arenas containing the exposed wheat flour substrate were retrieved, lids were replaced and treatment arenas were bundled, secured and placed in dark storage containers and returned to the lab at Kansas State University. Once in the laboratory, all treated arenas and control arenas, were maintained continuously at very low light conditions, with temperature and r.h. being monitored by a HOBO data logger. Treated as well as untreated arenas were stored in a similar manner for the duration of the study.

The series of experiments being reported were done to evaluate the residual efficacy of an insecticide at two different concentration rates, at specified time points over a given four-month period. Therefore, estimating the amount of residual efficacy that may result from a single aerosol application through residual bioassay tests provides a means to assess the persistence and potency of the aerosol insecticide. The use of bioassays provides a quantitative, as well as qualitative means of assessing the effects of this insecticidal mixture on two *Tribolium* species, as time increases from the date of the initial aerosol application through the duration of the four-month storage period, during which degradation of the chemical residues is subsequently occurring. To aid in determining the degradability of this aerosol insecticide and in specifying how this degradation specifically effects the development and eventual emergence of morphologically and presumably viable, normal adults, of both exposed species of *Tribolium*, a series of ‘time’ factors were incorporated into the overall experimental design. Thus, for the purposes of reporting data for this study, ‘time’ will be defined in three ways. First, complementary sets of concrete treatment arenas containing the wheat flour substrate were exposed to each applied aerosol concentration rate, such these tests were each replicated a total of four times, at each aerosol concentration. Each four-replicate experiment was performed throughout different two month periods, with the 3% pyrethrin treatments executed between August and September, and the 1% pyrethrin treatments performed between November and January. The independent, four-replicate-based sets of exposed treatment arenas, were exposed to the two independent aerosol applications, such that four complementary sets of treatment arenas were exposed, per application rate, for a total of eight sets of exposed treatment dishes (i.e., replications), used directly in the subsequent bioassay experiments. These eight replications will consequently be referred to as “replications in time” (i.e., “1% pyrethrin replications in time”, and “3% pyrethrin replications in time”).

Second, to aid in estimating the residual efficacy over a four-month period of the two applied aerosol concentrations, a factor of “storage time(s)”, (or “storage periods”), was incorporated into the overall experimental design. This factor is representative of a series of nine, biweekly storage periods, representing increasing intervals of time from the initial aerosol application. These nine periods compose the overall 16-week storage experiment (i.e., 0, 2, 4, 6, 8, 10, 12, 14 and 16 weeks post aerosol treatment). Analyzing bioassay data at each two-week storage interval provides the opportunity to observe discernible trends or patterns of insect development (e.g., percentage of species-specific and stage-specific morphogenic abnormalities and developmental arrests, as well as percentage of species-specific normal, adult emergence), at both aerosol application rates. During this 16-week “storage time”, the treatment arenas were stored such that low-light, stable temperature and uniform r.h. conditions could be reasonably controlled and monitored. By establishing as close to ideal storage conditions as possible, potential degradation of insecticides was minimized. The final included factor of time was denoted as “observation days/period”. This factor encompasses a 30-day period, during which species-specific as well as stage-specific development of *Tribolium* species was monitored and recorded, comprising the quantitative dataset that will be used for statistical analysis. Under laboratory rearing conditions at 27°C, four-week old *Tribolium* spp. larvae progressively develop into pupae within 6-7 days, and emerge as adults 7-8 days later. Incorporating a 30-day observation period into this study provides roughly an additional two-week period in which development within each stage, at each post-treatment storage time, can be observed for stage-specific development.

For each independent replication treatment within both the “1% pyrethrin replications in time” and the “3% pyrethrin replications in time”, at each two-week post-treatment “storage time”, two treatment arenas were chosen randomly from a larger sample consisting of 20 previously exposed concrete treatment arenas. These two plates were then utilized for the subsequent individual bioassay experiments, which correspond to each two-week storage period, within the 16-week study. Each of the eight, independent replication, complementary sets of 20 concrete exposure treatment arenas contained 6 g of lightly-compressed wheat flour that was exposed to one of the two applied aerosol concentration rates. This 6g amount of treated flour within each of these two randomly chosen, previously exposed concrete treatment arenas were

divided into four-equal 3g amounts. Of these four amounts, two 3g samples, one from each of the previously chosen exposure arenas, were placed into capped glass vials, sealed, labeled and retained in darkened storage containers at -20 °C, to be used in conjunction with HPLC residue degradation analysis experiments (data not shown). To initiate the bioassay experiments, the two remaining 3g treated flour samples were each subdivided into six equal amounts of 0.5g and each was placed into the center of a freshly prepared, unexposed concrete treatment arenas. These 12 arenas correspond to a particular post-aerosol application storage time/interval, and will be referred to as ‘observation t arenas’. Of these 12 prepared bioassay observation arenas, six were used for tests with *T. castaneum*, and the other six for tests with *T. confusum*. Ten four-week old *T. castaneum* larvae were added into each of the three previously exposed treatment arenas and ten four-week old *T. confusum* larvae were added into each of three arenas, such that n=10 larvae/treated observation arena. This addition of larvae, denote ‘day 0’ of the 30-day observational experiment, in which these twelve observation arenas will be monitored by recording the morphological development/progression, every two-days, for 30-days.

Development and subsequent stage-specific morphological characteristics (i.e., dead or arrested individuals), stage-specific morphological deformities, and emergence of morphologically normal adults were recorded at each two-day observation period. The morphological deformity scoring system classified individual insects as normal, presumably reproductively viable adults (i.e., fully eclosed adults), or ‘affected’ (i.e., individuals displaying a distinct morphogenic malformation, those arrested between stages of development and, those unable to progress their development, thus persisting in a single stage for the duration of the 30 day observation period), including live adults displaying a twisted wing deformity, live adults with a deformity in which their wings were unable to fold, live adults unable to shed their pupae cuticle (i.e., incompletely formed elytra), live pupae, arrested pupae-adult intermediates (i.e., secondary pupae or supernumary pupae), dead pupae, live larvae, arrested larvae-pupae intermediates, and dead larvae (Kamaruzzaman et al., 2006; Arthur, 2001; Arthur and Hoernemann, 2004; Arthur and Campbell, 2008, Fig. 1). During each of the 15 observations, any individual recorded as having fully emerged as a normal adult, those with a characteristic morphogenic abnormality, those arrested, or those that had died at any stage were consequently removed from the given observation arena. However, any individuals continuing development

were left in the observation arena. The removal of these individuals was done so to avoid documented *Tribolium* spp. cannibalistic behavior, and as a measure of further encouraging the consumption of the treated wheat flour substrate (Ho and Dawson, 1966; Beno et al., 1998; Via, 1999; Wade, 1976). After each observation, the arenas were again tightly sealed and returned to a temperature and humidity controlled environmental chamber. Humidity within this chamber was regulated at roughly 75% using a .32 x .26 x .07 m plastic pan (Fisher Scientific, 13-361-10), which was kept filled with tap water.

A comparison of the proportions of morphologically normal, presumably reproductively viable adults able to emerge at each aerosol concentration, at each of the nine “storage times”, versus those that displayed an affected stage-specific deformity was done to estimate the residual efficacy of the two, independent aerosol concentrations upon a treated flour substrate, evaluate quantitative differences between the nine “storage times”, assess the most practical and efficient aerosol reapplication time point, noting both economic threshold levels and applicator costs and, evaluate the occurrence of the many stage-specific responses of the two exposed insect species. The probability of normal adult emergence, as well as other stage-specific development and deformities, is modeled at both aerosol concentrations, by accounting for these proportions for each *Tribolium* species. The proportion of ‘normal’ versus ‘affected’ individuals, effectively indicates the potential for each utilized aerosol concentration, to adequately control these *Tribolium* spp., by effectively preventing their development and eventual emergence into reproductively viable adults that would be able to further propagate a population within a given milling setting. Further experiments are being performed at Grain Marketing and Production Research Center, USDA-ARS, 1515 College Ave., Manhattan, KS 66502, USA, to evaluate the viability of each of the morphologically deformed adults (data not shown).

2.3 Statistical analysis

Morphological data from each of the eight replications in time, were analyzed for the main effects of the applied aerosol concentration rate (1% and 3% pyrethrin, *Tribolium* species (*T. castaneum* and *T. confusum*), and the interaction effect of the applied aerosol concentration rate x species. The response variable was the aerosol concentration by *Tribolium* species by “storage period” by “replication in time” by the accumulated counts of individuals within each

scored morphological deformity category, per observation arena, during the 30-day period. Data for normal adult emergence and morphological deformities were pooled over the 30 day observation period, then further pooled into an accumulated value over all six of the observation arenas, according to species (n=60 per species). Preliminary analysis revealed no discernable trend emerging across the entire 16-week storage experiment, upon comparing each two-week storage interval, according to each applied aerosol concentration, *Tribolium* species, and the exposed packaging material surface. Therefore, the data was again collapsed over the entire 16-week experiment. This comprehensive value was based on a model 2 species x 2 concentrations x 4 individual replications in time, for a total n=540, per each of the ten scored morphological categories. This collapsed value depicts the proportion of total individuals within each scored morphological category for the larger 16-week storage period. In assessing the data, using this overall collapsed value, it allows us to directly evaluate species differences with regards to emergence, developmental capabilities of the two exposed species when challenged to the aerosol insecticide at the two application rates, and to estimate the residual efficacy of this aerosol, based on the proportions of individuals emerging both unaffected and affected.

Preliminary analysis was performed with the SAS General Linear Model (GLM) procedure of the Statistical Analysis System (SAS Statistical Software, version 9.1.3), revealed that while a majority of the data possessed both equal variances and normality, however, large variations within each replication in time, produced negative confidence intervals. Therefore, data from the bioassay experiments were analyzed using the SAS Generalized Linear Model (GENMOD) procedure, by comparing the proportions of deformities to each of the applied aerosol concentration rates, by species, as well as the concentration by species interaction effect. Statistical significance of both the main effects and the interaction effects were tested using the type III analysis of a logistic ANOVA, and reported as a series of chi-squared (χ^2) values, along with their corresponding p-values. The GENMOD procedure allowed fitting a sequence of models and generated a type 3 analysis. This type 3 analysis consists of specifying a model and computing likelihood ratio statistics for all parameters in the given model. The model for this analysis consisted of modeling the proportion of each morphological category for the two main effects (concentration and species), as well as the interaction effect (concentration x species). Table 2.2 through 2-4 report upper and lower confidence limits (confidence intervals) for the

modeled parameters based on asymptotic normality. Within this SAS GENMOD analysis, the LS-MEANS statement was used, which computes the least squares means (LS-means) corresponding to the specified effects, allowing a direct comparison of the means. After all pairwise comparisons were made, χ^2 values as well as associated P-values are reported for each treatment level. A significant species by concentration interaction would indicate a level of inherent variability in the stage-specific development of each species when challenged with the aerosol insecticide, at both applied aerosol application rates, and would suggest that the main effects cannot be generalized with as much confidence, and are likely not statistically significant in isolation.

The proportion of 'affected' individuals was assessed for differences using the least squares means (LS-means) option and a chi-squared test, to determine any deviation from a 1:1 ratio. Results were reported on the probability scale and were calculated for both the main effects of concentration and species, and the corresponding interaction effect. Data for the percentage of each of the ten morphogenic categories, per species were plotted using equations generated from Sigma Plot software (Systat Software, Inc.). Significance between the reported treatment means, within each biweekly post-aerosol application storage period (0-16) were analyzed using unpaired *t*-tests (ProStat, Version 4.0.3, Poly Software International, Inc.). Unless otherwise noted, significance was determined at the 0.05 level.

3. Results

Table 2-1 highlights a comparison of morphogenic categories, at each of the four-flour treatment categories. The variation in the responses of both *Tribolium* spp., upon exposure to both applied aerosol concentration rates are reported as mean \pm SE. The exposure of *Tribolium* spp. to JHA's, has been shown to result in inherently different responses and susceptibilities (Amos et al., 1974; Williams and Amos. 1977). The bioassay results show that when *T. castaneum* were exposed to the applied aerosol formulations, the morphological categories that were observed at greater than 10% emergence were those of the arrested pupae-adult intermediates, individuals that died while in the larvae-stage, those that died while in the pupae-stage and those individuals that persisted throughout the 30-day observation period as larvae, likely undergoing several supernumerary molts. However, when *T. confusum* were exposed to

the applied aerosol formulations, the morphological categories that were observed with the greatest percentages were those of the emergence of normal, live adults, arrested pupae-adult intermediates, individuals that died while in the larvae-stage, those that died while in the pupae-stage and those individuals that persisted throughout the 30-day observation period as larvae. Table 2.1 further reveals a great deal of variation between across the two applied aerosol formulations, within each individual exposed species.

Table 2.1 effectively illustrates both a species-specific, as well as stage-specific variation within each treatment. A comparison of *T. castaneum* at both the 1% pyrethrin and the 3% pyrethrin treatments shows that the greatest percentages of individuals are retained in the larval and the pupal stages. Within both the 1%- and the 3% pyrethrin treatment, approximately two-thirds of the initially exposed individuals had either died while in the larvae stage, or simply persisted as larvae over the course of the 30-day observation period, while approximately 30% were characterized as pupae-stage characteristics, such that they were either reported as live, persistent pupae, dead pupae or arrested pupae-adult intermediates. Whereas a comparison of *T. confusum* at both the 1%- and the 3% pyrethrin treatments demonstrate that at both applied aerosol formulations, roughly a quarter of all individuals were characterized as either live, persistent larvae, or dead larvae. Results further show that *T. confusum* exposed to the 1% pyrethrin treatment resulted in roughly one third of the individuals characterized as pupae characteristics, while at the 3% pyrethrin treatment, roughly half of the overall individuals were characterized as such. However, a comparison of *T. confusum* at both applied aerosol formulations demonstrated that individuals were able to progress their development beyond the larval stages and greater percentages of individuals were subsequently found in the pupae and adult stages.

The adult is often considered to be the most harmful stage of the insect pest life cycle (Semple, 1992), as they feed, live and reproduce within the infested commodity (Sehna, 1971). *Tribolium* spp. have been known to cause significant damage in a number of stored commodities, within many stored product industry environments. The ease of their adaptability to survive harsh environments as well as developmental conditions, combined with their high reproductive capability, allow these insect pests the potential to cause significant product damage in and

around food storage facilities. As the infesting, feeding and reproducing stage of *Tribolium* spp., the adult stage deserves considerable examination. Normal, live adult development was much more profound in *T. confusum* bioassays, with roughly 2 times the percentages of normal adults observed in the 1% pyrethrin treated flour bioassays (29.3%), compared to the 3% pyrethrin treated bioassay (13.3%). Results for the *T. castaneum* demonstrated the percentages of normal, live adults to be less than 2% emergence, regardless of the applied aerosol formulation.

Both the 1%- and the 3% pyrethrin aerosol treatments generated distinctive percentages over a majority of the scored morphological categories ($P < 0.05$), as was determined in Table 2.1. As a result, there were few non-statistical differences in the percentages of individuals displaying each morphogenic characteristic, when compared across the two applied aerosol formulations (Table 2.2). This relatively uniform, high degree of significance across each morphological category suggests a differential response between the two applied aerosol formulations, when exposed to *Tribolium* spp. Table 2.3 reveals a uniform analysis in which statistical significance ($P < 0.001$) is achieved for each morphological category when *T. castaneum* and *T. confusum* are compared to one another. Further examination of the analysis within this table suggest that *T. confusum* consistently reported higher percentages of individuals emerging within each adult category, normal and the three morphogenic malformations, as well as the arrested pupae-adult intermediates. Whereas the remaining pupae- and larvae-stage specific categories demonstrated greater percentages of *T. castaneum*. This uniform account of significance demonstrates the vast differences between the two exposed species, as well as illustrates a significant stage-specific occurrence. These results further demonstrate the considerable difference in potential of each of the two exposed species to overcome developmental barriers, and to emerge as normal, live adults. These results demonstrate that less susceptible *T. confusum* individuals are better able to overcome the effect of the applied aerosol formulation and emerge as normal, presumably reproductively active adults, able to propagate successive generations of pests within this flour mill setting. Table 2.3 shows the inherent developmental limitations of *T. castaneum* individuals exposed to a treated flour substrate, regardless of a.i. aerosol formulation, whereas Table 2.4 corresponds to the interaction effect of aerosol concentration by *Tribolium* spp. in which each of the four treatment categories were analyzed for statistical significance. This interaction analysis reveals that only six of the ten morphological categories demonstrated statistical significance

($P < 0.05$). Again, the same pattern as was determined in Table 2.3 emerges for Table 2.4, in which *T. confusum* consistently develop through the pupal and adult stages much more readily than *T. castaneum*, which demonstrate greater percentages of individuals characterized as having larvae and pupae characteristics. These results suggest that the observed proportions of *Tribolium* spp. individuals within each statistically significant morphological categories, demonstrate a differential response upon exposure to the 1% pyrethrin treated flour, than when they were exposed to 3% pyrethrin treated flour.

T. castaneum individuals regardless of applied aerosol formulation were largely unable to complete development and emerge as normal, live adults (Fig 2.2B). The percentage of *T. castaneum* individuals able to emerge as normal live adults was $>2\%$, regardless of applied formulation. The *T. castaneum* was consistently shown to be the more susceptible of the two exposed *Tribolium* species. *T. confusum* exposure to both the 1%- and 3% pyrethrin + methoprene treated flour substrate resulted in a very different pattern of adult emergence. *T. confusum* exposed to the 3% pyrethrin treatments demonstrated a gradually decreasing percentage of individuals characterized as ‘affected’, or a increasing percentage of individuals characterized as normal, live adults, throughout the 16-week storage experiment. However, there is a much less discernable trend observed when *T. confusum* are exposed to the 1% pyrethrin treated flour substrate (Fig 2.2A). Bioassay results demonstrated that upon exposure to the 1% pyrethrin treatment, *T. confusum* experienced more breakdown of residual effectiveness over time, than did the *T. castaneum* (Fig 2.3A). A similar result is observed upon exposure to the 3% pyrethrin treatment, to a lesser degree (Fig 2.3B).

Analysis of the data from the untreated control observation arenas, revealed that the total adult emergence for each species was roughly 90-100%. No individuals emerged that displayed any of the scored morphological deformities, at any stage. The affected emergence observed within these arenas was that of dead larvae and pupae. Also, no significant differences were reported in the proportions of normal, adult emergences, at each of the two applied aerosol concentration rates. Adult emergence of the untreated controls for *T. castaneum* at both 1% pyrethrin and 3% pyrethrin treatments were 98.75 ± 0.80^a and $88.75 \pm 0.80\%^b$, respectively, whereas this percentage of emergence within *T. confusum*, exposed to the treated flour substrate

at 1% pyrethrin and 3% pyrethrin were 96.7 ± 2.04^a and $87.9 \pm 0.80\%^b$, respectively. However, a significant species-specific difference was observed at each applied concentration rate.

4. Discussion

Results of this study show the potential for using a pyrethrin plus a methoprene aerosol insecticide to control two particular species of stored product insect pests in an active milling facility. In this study, we observed a clear distinction between the developmental ability of both exposed *Tribolium* species, at both of the applied aerosol concentrations. When used in combination with the synergized pyrethrin, this insecticidal combination proved to be an effective control agent, even applied at very low concentrations. The timing of an IGR application with regards to the exposed insect stage, presents a challenge to the management of *Tribolium* pest populations within a milling or warehouse facility (Brieger, 1973). Methoprene is generally used against larvae, although the extent and character of the response varies between insects and is often stage-specific, with the majority of the deformities being observed in both the larvae and the pupae stages (Tunaz and Uygün, 2004). Because the formative processes occurring in an insect are not all susceptible at exactly the same time, the longer duration of exposure to the life stage that is most susceptible, yields a more complete inhibition across all other subsequent life stages (Sehnal and Meyer, 1968; Stall, 1975). Morphogenetic abnormalities are usually irreversible and the most readily observed effect of IGRs.

It has been proposed that the period of greatest susceptibility for metamorphic inhibition is during the last larval instar. The effectiveness of JHAs depends on the timing of application, about the specific stage of insect development. The application to larvae during a given time in which some cells are sensitive, whereas others are not, often leads to the production of various intermediate forms (Tunaz and Uygün, 2004). Retnakaran (1973) demonstrated that upsetting the titer of JH in the last larval instar results in the production of larval-pupae intermediates that seldom survive. Arthur (2008b) published results showing that immature stages were most susceptible to the effects of the aerosol, as well as revealing higher survival rates in *T. confusum*, than in *T. castaneum*, suggesting that *T. confusum* were far less susceptible to the effects of an applied pyrethrin aerosol formulation, when these individuals were provided a flour food source. The methoprene component within the aerosol mixture prolongs specific insect stages,

particularly the larvae stages, beyond the time normally required to complete development and seemingly affects different developmental stages in both of the exposed *Tribolium* species. Data reported in this study, offers clear evidence that the more susceptible species, *T. castaneum*, are affected by the IGR component to a greater degree than are the less susceptible *T. confusum* individuals, which are more readily able to develop beyond the larvae and pupae stages, or overcome the effects of the applied aerosol insecticide, thus ensuring greater percentages of both individuals displaying later stage morphogenic abnormalities, as well as those emerging as normal adults. These morphologically deformed adults may or may not be viable, perhaps possessing slightly decreased fitness. Once these adults emerge, they might be able to reproduce, adding to the population of stored product insect pests in a milling setting, and perhaps increasing the selection for resistance to these aerosol insecticides, or their components.

Results of this study further indicate a variable response between the two exposed *Tribolium* species, in regards to how both are influenced (i.e., developmental rates, proportions of normal adults, proportion of individuals displaying morphogenic abnormalities, etc.) by the two applied aerosol concentration rates, and how the two aerosol formulations differentially affect the two exposed *Tribolium* spp. The 1% pyrethrin aerosol treatment was shown to provide superior control against *T. castaneum*, almost fully prohibiting the development and eventual emerge of adults, on the treated wheat flour substrate. Bioassay test results revealed only one normal, adult individual emerging at the post application storage treatment week 2, and two normal, adults emerging at week 6, as opposed to almost 30% normal, adult *T. confusum* emergence. The 3% pyrethrin aerosol treatment also provided adequate control against *T. castaneum* larvae; however, at this concentration, surprisingly more normal, live adults emerged, than under the 1% pyrethrin treatments. *T. castaneum* adult emergence was shown to occur at weeks 4, 8, 12, 14 and 16 in which 13, 20, 17, 23 and 17%, respectively, of individuals initially exposed as larvae were able to complete development and emerge as normal, live adults. Though these results for *T. castaneum* normal adult emergence are unexpected, there are a number of potential explanations as to why we might have observed this increased adult emergence, over the course of the 16-week storage experiment when exposed to the 3% pyrethrin treatment, including the possibility of environmental conditions within the mill, such as r.h. or temperature differences during the application, variability of environmental conditions within each of the

laboratory storage container (i.e., UV-light, temperature, r.h., etc) thereby decreasing residual efficacy, the potential of varying environmental chamber conditions, varying moisture content of the flour either at the time of the aerosol application, or during the time that the beetles were exposed to the treated flour substrate, or increased UV-light exposure throughout the 30-day observation period, which could account for less persistency as well as reduced efficacy.

Although susceptibilities at various developmental stages of both *T. castaneum* and *T. confusum* were not directly examined in this study, our bioassay results indicated that at 27°C, the length of time required for the four-week-old larvae stage to complete development and emerge as normal adults was about 19 d for *T. castaneum*, with the majority of the pupation occurring between days 3-10 post larvae addition, whereas this emergence was achieved within about 23 d for *T. confusum*, with the majority of its pupation occurring between days 4-12 post larvae addition. Similar results have been reported by White (1987), Arthur (2001), and Arnaud et al. (2005). The increased incidence of persistent pupae and larvae 30-days after their initial addition to each of the treated observation arenas, demonstrates the juvenilizing effect of the applied aerosol insecticide formulations and further implicates that *T. castaneum* is a more susceptible species to the aerosol insecticide.

Upon comparing each of the treatment categories, we had expected to observe a discernable shift in the percentages of deformities arising at each post-treatment storage increment. It was initially assumed that each independent replication in time, would model the assumption that as time, defined as the biweekly storage increments increased from the time of the initial aerosol application, through week 16, the insecticidal components would gradually degrade, portraying a uniform pattern of decrease in affected individuals over each incremental storage time, or a trend in which we observe an increase in the percentages of normal adult emergence. However, this trend was only semi-apparent when *T. confusum* were exposed to 1% pyrethrin treated flour, and was less apparent when *T. confusum* were exposed at 3% pyrethrin. No apparent trend was observed upon *T. castaneum* exposure to wheat flour treated with either 1% pyrethrin or 3% pyrethrin. As has been established throughout this study, *T. castaneum* is indeed the much more susceptible species as demonstrated in Fig 2.2B, which illustrates that the ‘affected’ emergence within *T. castaneum* at both aerosol application rates is roughly 90-100%

across all post-aerosol treatment storage weeks. This lack of variation among the affected emergence further reveals an overall significant effect of the two applied aerosol concentrations at each post-application storage period. However, because so few adults emerged as a result of this insecticidal pressure, it is difficult to assess a specific trend upon which the percentage of unaffected individuals can be directly compared to the percentage of affected individuals, as was able to be done with the less susceptible *T. confusum*. Thus, an evaluation of the developmental patterns and the overall trends of the ‘affected’ individuals appear to be the most logical approach to analyzing this particular data. Analysis revealed that upon contrasting the percentages of emergence per each scored deformity, for *T. castaneum* at both 1% pyrethrin and 3% pyrethrin applied aerosol treatments, both datasets showed similar trends within many of the morphological categories defined for the purpose of this study (refer to Appendix Table 2.2 through Appendix Table 2.4).

The application rate and frequency are important factors to consider when assessing the effectiveness of any chemical control method. This interval of aerosol reapplication will depend on the target insect pests. Previous studies have been conducted in which *Tribolium* species have been exposed to insecticide treated flour (Arthur 2000, 2001; Arthur and Hoernemann, 2004). However, little has been reported on the morphological effects of an aerosol insecticide mixture containing both synergized pyrethrin and Diacon IITM. This study demonstrates that effective residual control is achieved for *T. castaneum* pest populations, where it is much less pronounced for *T. confusum*. Although no correlation between normal adult emergence and each post-treatment storage interval can be established, our results do show that after the 4-month storage period, exposure of *T. castaneum* to both the 1% pyrethrin and the 3% pyrethrin aerosol treatments, are effective at prohibiting adult development and emergence, at less than 3% and 11%, respectively. Upon exposure to 3% pyrethrin aerosol treated substrate, the percentage of *T. confusum* normal adult emergence does not surpass 30%, whereas upon exposure to the 1% pyrethrin treatment, *T. confusum* reach almost 47% normal adult emergence, by week 16. Though both 1% and 3% pyrethrin aerosol treatments were less effective for controlling *T. confusum* adult emergence, than that observed in the *T. castaneum* bioassays, this study does reveal that the exposure of *T. confusum* individuals to the 3% pyrethrin aerosol treated flour substrate was more effective in preventing the adults from completing development and

subsequent emergence, than those exposed to the 1% pyrethrin aerosol. It should be further noted that even though *T. confusum* emergence at both concentrations is relatively high, each stage in development is significantly delayed as compared to developmental rates of larvae in the non-treated controls, likely due to the effect caused by Diacon II™. This delayed development should ultimately confer sterility and reduced fecundity (Tunaz and Uygun, 2004).

Based on results from this study, it is difficult to assess the effects of the two, insecticide components comprising this aerosol insecticide formulation, the synergized pyrethrin and the methoprene, as well as the precise targeted effects of both components on stage-specific and species-specific development, or to what extent both are driving the formation of the observed morphogenic malformations. Under UV-light conditions, pyrethrin degrades quickly, and is often stable for less than 24 h. This instability, as well as its inherent instability in air and heat, causes pyrethrin to rapidly lose its insecticidal activity, upon environmental exposure (Allan and Miller, 1990). In this study, we determined that the manner in which each exposed *Tribolium* species was differentially affected by the presence of the aerosol mixture, regardless of the applied formulation rate. We observed roughly 0 to 40% normal adult emergence at the 16-week post aerosol application storage increment, indicating that residual effectiveness of the applied aerosol formulation. Due to this persistence over a four-month period, as well as the observed toxicity to exposed insects, at both aerosol concentrations throughout the each biweekly post-aerosol application storage period, it can be assumed that the methoprene is likely the chemical component providing this residual control throughout.

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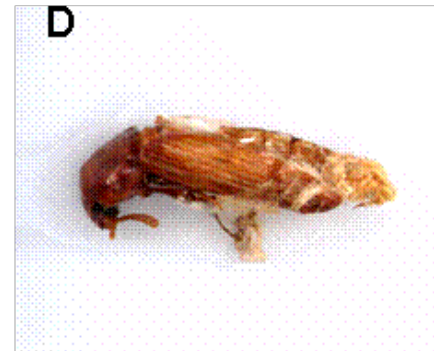
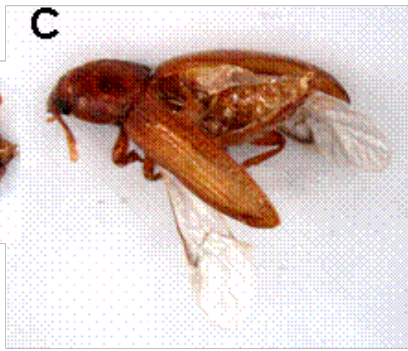
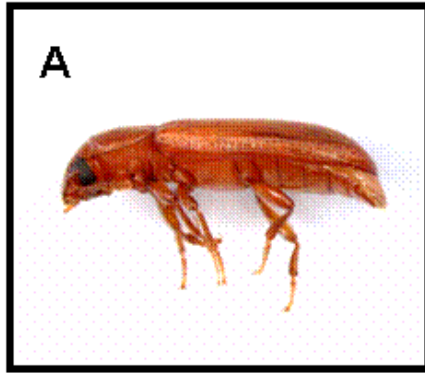
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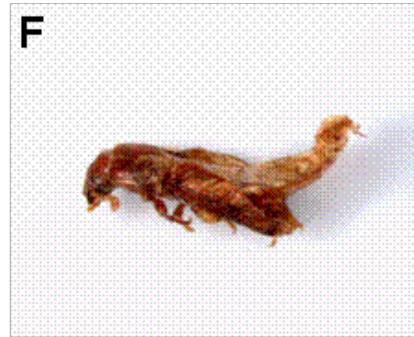
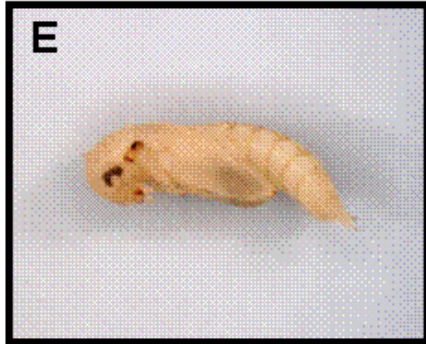
Figures and Tables

Figure 2.1. *Tribolium* spp. morphological deformities characteristic of Diacon II™. (A) Normal, live adults (i.e., fully eclosed adults), (B) Live adults displaying a twisted wing deformity, (C) Live adults with a deformity in which their wings were unable to fold, (D) Live adults unable to shed their pupae cuticle (i.e., incompletely formed elytra), (E) Live pupae (F) Arrested pupa-adult intermediates (i.e., secondary pupae), (G) Live larvae and (H) Arrested larvae-pupae intermediates

Adults:



Pupae:



Larvae:

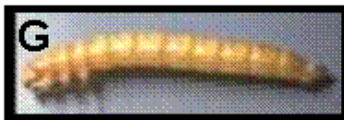


Table 2.1. Comparisons of mean \pm standard error of morphological categories, corresponding to each treatment category. *T. castaneum* exposed to 1% pyrethrin and 3% pyrethrin and *T. confusum* exposed with applied aerosol concentrations of 1% pyrethrin and Entech Fog-30[®] + Diacon II[™].

Wheat Flour	<i>T. castaneum</i>		<i>T. confusum</i>		
	1% pyrethrin + methoprene	3% pyrethrin + methoprene	1% pyrethrin + methoprene	3% pyrethrin + methoprene	
Normal, Live Adults	0.1 \pm 0.1 ^C	1.3 \pm 0.6 ^C	29.3 \pm 2.7 ^A	13.3 \pm 1.1 ^B	*
Adults Displaying Twisted Wing Deformity	0.6 \pm 0.1 ^C	0.0 \pm 0.0 ^D	2.5 \pm 0.2 ^B	2.9 \pm 0.1 ^A	
Adults with Unfolded Wing Deformity	0.9 \pm 0.2 ^B	0.1 \pm 0.1 ^B	6.2 \pm 0.6 ^A	5.8 \pm 0.7 ^A	
Adults Unable to Shed Pupal Cuticle	0.8 \pm 0.3 ^B	0.2 \pm 0.1 ^B	4.8 \pm 0.3 ^A	5.3 \pm 0.3 ^A	
Arrested Pupae-Adult Intermediates	8.1 \pm 0.7 ^C	10.5 \pm 0.8 ^C	19.0 \pm 1.4 ^B	27.4 \pm 1.0 ^A	*
Live Pupae	4.9 \pm 0.4 ^{AB}	6.3 \pm 1.1 ^A	3.4 \pm 0.4 ^B	4.6 \pm 1.2 ^{AB}	
Dead Pupae	16.3 \pm 0.8 ^A	17.1 \pm 0.9 ^A	8.8 \pm 0.4 ^B	15.1 \pm 0.5 ^A	*
Arrested Larvae-Pupae Intermediates	1.5 \pm 0.1 ^A	1.4 \pm 0.1 ^A	0.2 \pm 0.1 ^B	0.5 \pm 0.2 ^B	
Live Larvae	36.5 \pm 1.0 ^B	39.8 \pm 0.6 ^A	7.1 \pm 0.4 ^C	6.8 \pm 0.8 ^C	
Dead Larvae	30.4 \pm 1.1 ^A	23.3 \pm 1.3 ^B	18.7 \pm 0.7 ^C	18.2 \pm 0.4 ^C	

(*) Denotes a statistically significant difference (unpaired t-test) between the two reported means across the two applied aerosol concentrations

(A-D) Means with different letters are significantly different by experiment-wise t-tests (P<0.05 Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

Figure 2.2 (A-B). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the wheat flour substrate, within each biweekly storage period. Resultant graph depicts how the concentration of the applied aerosol formulation consequently affects development within both exposed *Tribolium* spp. The proportion of (A) *T. castaneum* and (B) *T. confusum* adversely affected following a 30-day exposure with Entech Fog-30[®] + Diacon II[™] or Entech Fog-10[®] + Diacon II[™], applied directly to the wheat flour substrate. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ABCD) correspond to the Entech Fog-10[®] + Diacon II[™] applied aerosol formulation treatments, whereas means (abcd) correspond to the applied 3% pyrethrin formulation treatments, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

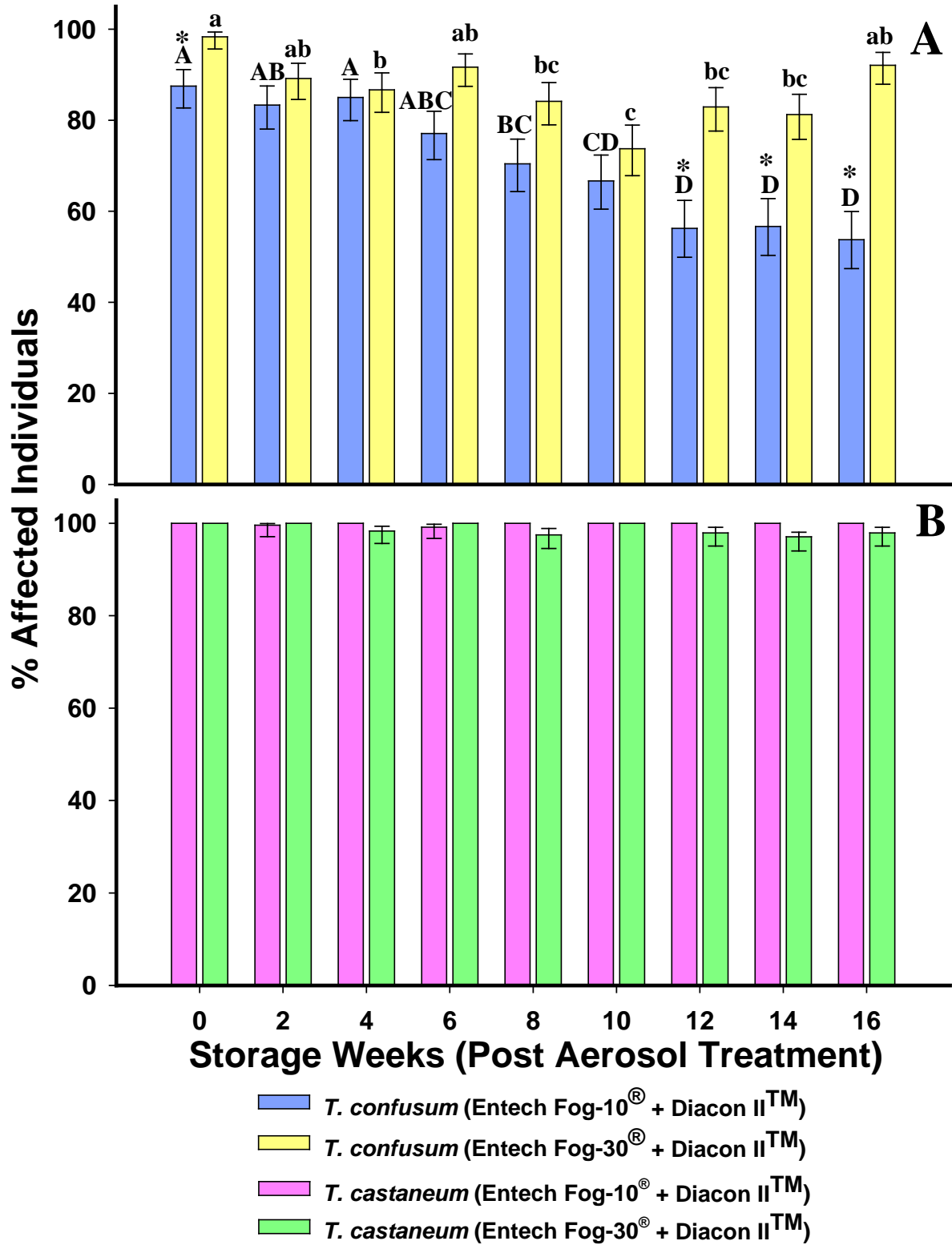


Figure 2.3 (A-B). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the wheat flour substrate at both the 1% pyrethrin and the 3% pyrethrinaerosol formulations, within each biweekly storage period. Resultant graph depicts the species-specific response, at both applied aerosol concentrations. Comparison of means for differences in the percentage of *T. castaneum* and *T. confusum* adversely affected following a 30 day exposure with 3% pyrethrin or with 1% pyrethrin (A and B, respectively) applied directly to wheat flour. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrinaerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

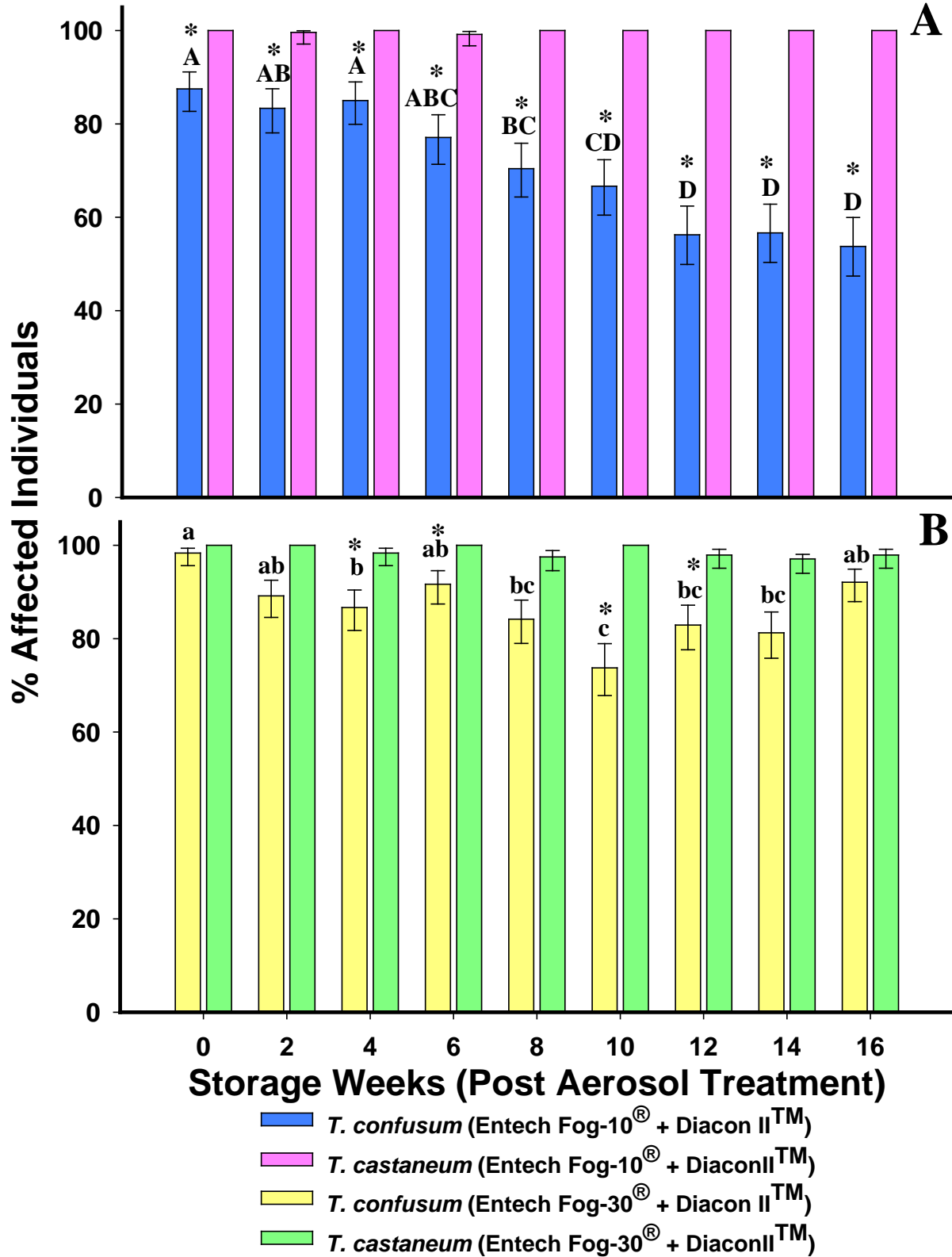


Table 2.2. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of total individuals that emerged in each morphological category, at each aerosol concentration (1% pyrethrin and Entech Fog-30[®] + Diacon II[™]) on treated wheat flour. Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	1% pyrethrin + methoprene	1.341	2.342	4.061	5.33	0.0209
	3% pyrethrin + methoprene	3.488	4.226	5.113		
Live Adults Displaying Twisted Wing Deformity	1% pyrethrin + methoprene	0.9108	1.2307	1.661	9.48	0.0021
	3% pyrethrin + methoprene	0.1387	0.3716	0.9922		
Live Adults w/ Unfolded Wing Deformity	1% pyrethrin + methoprene	1.8587	2.3562	2.9828	12.93	0.0003
	3% pyrethrin + methoprene	0.5205	0.9197	1.6201		
Live Adults Unable to Shed Pupal Cuticle	1% pyrethrin + methoprene	1.516	1.9541	2.5156	0.0099	0.0099
	3% pyrethrin + methoprene	0.6161	1.0111	1.6551		
Arrested Pupae-Adult Intermediates	1% pyrethrin + methoprene	11.583	12.582	13.655	34.97	<0.0001
	3% pyrethrin + methoprene	16.192	17.358	18.59		
Arrested Larvae	1% pyrethrin + methoprene	0.367	0.5872	0.9383	1.13	0.2888
	3% pyrethrin + methoprene	0.562	0.8029	1.1458		
Dead Larvae	1% pyrethrin + methoprene	22.786	24.057	25.376	13.98	0.0002
	3% pyrethrin + methoprene	19.485	20.671	21.91		
Dead Pupae	1% pyrethrin + methoprene	11.105	12.064	13.094	28.3	<0.0001
	3% pyrethrin + methoprene	15.018	16.085	17.212		
Live Larvae	1% pyrethrin + methoprene	16.025	17.318	18.693	0.49	0.4849
	3% pyrethrin + methoprene	16.656	18.005	19.437		
Live Pupae	1% pyrethrin + methoprene	3.5286	4.0836	4.7215	7.92	0.0049
	3% pyrethrin + methoprene	4.7378	5.3758	6.0942		

Table 2.3. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated wheat flour. Data collapsed over the 16-week storage period.

Morphological Category	Spp.	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	<i>T. castaneum</i>	18.927	20.144	21.418	1053.21	<0.0001
	<i>T. confusum</i>	0.23	0.418	0.757		
Live Adults Displaying Twisted Wing Deformity	<i>T. castaneum</i>	2.2564	2.7005	3.2292	103.24	<0.0001
	<i>T. confusum</i>	0.0605	0.1672	0.4611		
Live Adults w/ Unfolded Wing Deformity	<i>T. castaneum</i>	5.3233	5.9933	6.7417	252.94	<0.0001
	<i>T. confusum</i>	0.1906	0.3501	0.6421		
Live Adults Unable to Shed Pupal Cuticle	<i>T. castaneum</i>	4.4249	5.0391	5.7334	200.4	<0.0001
	<i>T. confusum</i>	0.2219	0.3822	0.6575		
Arrested Pupae-Adult Intermediates	<i>T. castaneum</i>	21.709	22.95	24.24	306.29	<0.0001
	<i>T. confusum</i>	8.384	9.215	10.118		
Arrested Larvae	<i>T. castaneum</i>	0.1915	0.3274	0.5592	32.07	<0.0001
	<i>T. confusum</i>	1.1198	1.4344	1.8359		
Dead Larvae	<i>T. castaneum</i>	17.342	18.471	19.656	83.68	<0.0001
	<i>T. confusum</i>	25.4	26.705	28.051		
Dead Pupae	<i>T. castaneum</i>	10.69	11.607	12.616	15.3	<0.0001
	<i>T. confusum</i>	15.603	16.685	7.827		
Live Larvae	<i>T. castaneum</i>	6.222	6.943	7.741	1298.6	<0.0001
	<i>T. confusum</i>	36.697	38.135	39.594		
Live Pupae	<i>T. castaneum</i>	3.419	3.9643	4.5925	11.72	0.0006
	<i>T. confusum</i>	4.8889	5.5351	6.261		

Table 2.4. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated wheat flour, at each applied aerosol concentration (1% pyrethrin and Entech Fog-30[®] + Diacon II[™])Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Spp.	Lower C.L.	Mean	Upper C.L.	X ²	p
Normal, Live Adults	1% pyrethrin + methoprene	<i>T. castaneum</i>	27.378	29.259	31.214	50.85	<0.0001
		<i>T. confusum</i>	0.045	0.139	0.43		
	3% pyrethrin + methoprene	<i>T. castaneum</i>	11.964	13.333	14.833		
		<i>T. confusum</i>	0.859	1.25	1.817		
Live Adults Displaying Twisted Wing Deformity	1% pyrethrin + methoprene	<i>T. castaneum</i>	1.9196	2.5	3.2501	12.88	0.0003
		<i>T. confusum</i>	0.3498	0.6019	1.0337		
	3% pyrethrin + methoprene	<i>T. castaneum</i>	2.2849	2.9167	3.7165		
		<i>T. confusum</i>	0.0065	0.0463	0.3279		
Live Adults w/ Unfolded Wing Deformity	1% pyrethrin + methoprene	<i>T. castaneum</i>	5.2183	6.1574	7.2526	11.24	0.0008
		<i>T. confusum</i>	0.5618	0.8796	1.3749		
	3% pyrethrin + methoprene	<i>T. castaneum</i>	4.92	5.8333	6.039		
		<i>T. confusum</i>	0.0448	0.1389	0.4297		
Live Adults Unable to Shed Pupal Cuticle	1% pyrethrin + methoprene	<i>T. castaneum</i>	3.9461	4.7685	5.752	9.39	0.0022
		<i>T. confusum</i>	0.4898	0.787	1.2623		
	3% pyrethrin + methoprene	<i>T. castaneum</i>	4.4529	5.3241	6.3543		
		<i>T. confusum</i>	0.0695	0.1852	0.4923		
Arrested Pupae-Adult Intermediates	1% pyrethrin + methoprene	<i>T. castaneum</i>	17.427	19.028	20.738	2.24	0.1347
		<i>T. confusum</i>	7.023	8.102	9.33		
	3% pyrethrin + methoprene	<i>T. castaneum</i>	25.567	27.407	29.328		
		<i>T. confusum</i>	9.241	10.463	11.826		
Arrested Larvae	1% pyrethrin + methoprene	<i>T. castaneum</i>	0.0964	0.2315	0.5549	1.65	0.1987
		<i>T. confusum</i>	1.0495	1.4815	2.0875		
	3% pyrethrin + methoprene	<i>T. castaneum</i>	0.2493	0.463	0.8583		
		<i>T. confusum</i>	0.9727	1.3889	1.9795		
Dead Larvae	1% pyrethrin + methoprene	<i>T. castaneum</i>	17.115	18.704	20.404	9.92	0.0016
		<i>T. confusum</i>	28.467	30.37	32.344		
	3% pyrethrin + methoprene	<i>T. castaneum</i>	16.668	18.241	19.926		
		<i>T. confusum</i>	21.597	23.333	25.164		
Dead Pupae	1% pyrethrin + methoprene	<i>T. castaneum</i>	7.716	8.843	10.115	18.54	<0.0001
		<i>T. confusum</i>	14.754	16.25	17.866		
	3% pyrethrin + methoprene	<i>T. castaneum</i>	13.644	15.093	16.667		
		<i>T. confusum</i>	15.599	17.13	18.777		
Live Larvae	1% pyrethrin + methoprene	<i>T. castaneum</i>	6.075	7.083	8.245	1.78	0.1819
		<i>T. confusum</i>	34.522	36.528	38.581		
	3% pyrethrin + methoprene	<i>T. castaneum</i>	5.817	6.806	7.948		
		<i>T. confusum</i>	37.724	39.769	41.859		
Live Pupae	1% pyrethrin + methoprene	<i>T. castaneum</i>	2.7364	3.4259	4.2816	0.02	0.8872
		<i>T. confusum</i>	4.0304	4.8611	5.8526		
	3% pyrethrin + methoprene	<i>T. castaneum</i>	3.7779	4.5833	5.5506		
		<i>T. confusum</i>	5.3464	6.2963	7.4017		

Appendix Tables and Figures

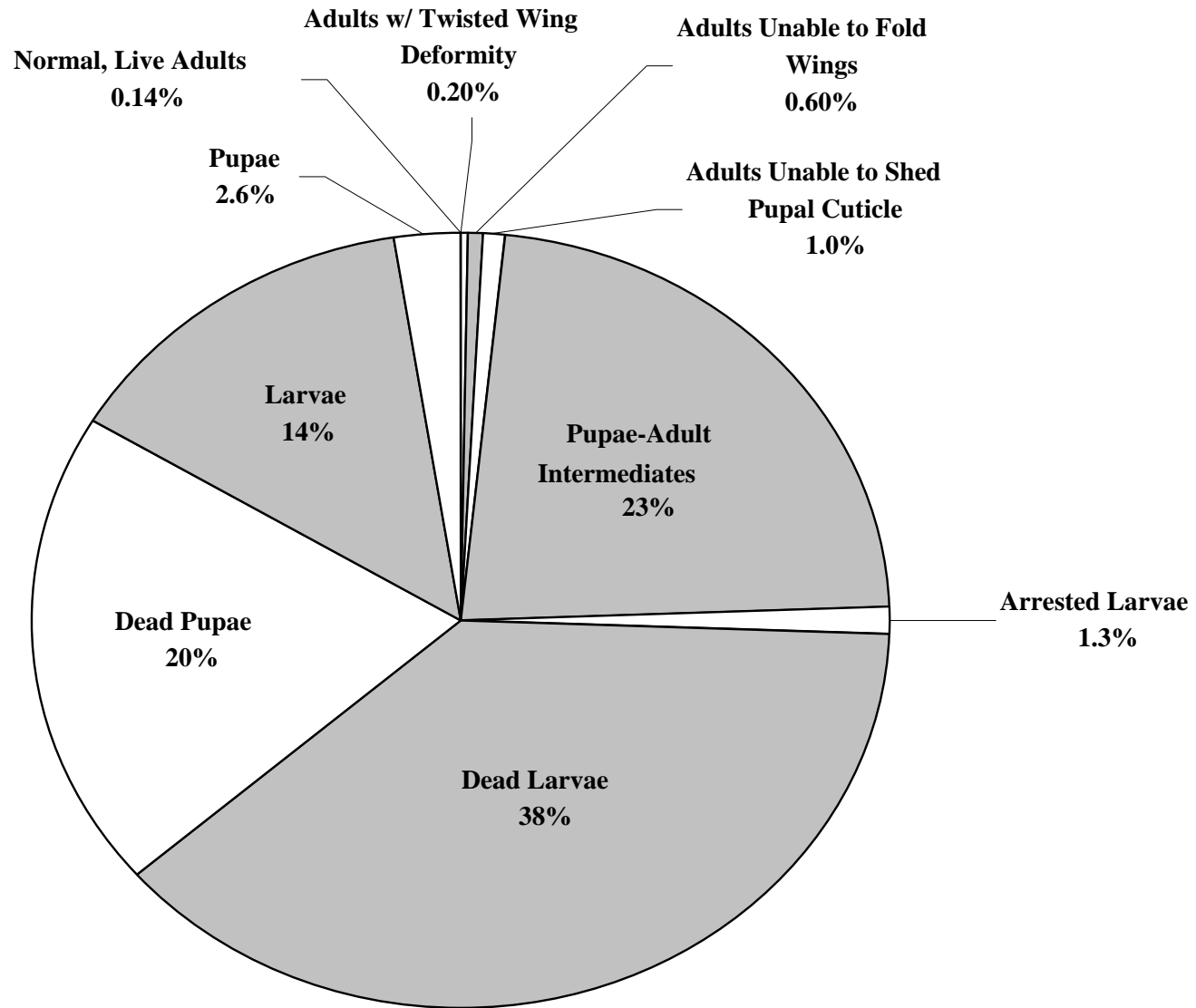
Appendix Table 2.1. No. of Normal, live adults emerging at each 2-week post-aerosol treatment storage increment, within the overall 16-week storage period.

Aerosol Conc.	Spp.	Rep	Ctrl.	wk0	wk2	wk4	wk6	wk8	wk10	wk12	wk14	wk16
1% pyrethrin + methoprene	<i>T. castaneum</i>	1	52	0	0	4	0	0	0	5	6	0
	<i>T. castaneum</i>	2	54	0	0	0	0	0	0	0	0	0
	<i>T. castaneum</i>	3	54	0	0	0	0	6	0	0	0	0
	<i>T. castaneum</i>	4	53	0	0	0	0	0	0	0	1	5
3% pyrethrin + methoprene	<i>T. castaneum</i>	1	59	0	0	0	0	0	0	0	0	0
	<i>T. castaneum</i>	2	58	0	0	0	2	0	0	0	0	0
	<i>T. castaneum</i>	3	60	0	1	0	0	0	0	0	0	0
	<i>T. castaneum</i>	4	60	0	0	0	0	0	0	0	0	0
1% pyrethrin + methoprene	<i>T. confusum</i>	1	54	4	3	4	5	18	21	5	6	7
	<i>T. confusum</i>	2	53	0	1	12	7	6	14	13	17	3
	<i>T. confusum</i>	3	52	0	9	7	3	12	16	14	20	5
	<i>T. confusum</i>	4	52	0	13	9	5	2	12	9	2	4
3% pyrethrin + methoprene	<i>T. confusum</i>	1	60	5	5	7	10	7	16	35	19	21
	<i>T. confusum</i>	2	57	11	19	13	25	27	22	24	28	28
	<i>T. confusum</i>	3	55	6	6	5	8	24	23	24	28	36
	<i>T. confusum</i>	4	60	8	10	11	12	13	19	22	29	34

Appendix Figure 2.1 (A-D). Observed percentages of *Tribolium castaneum* and *T. confusum* stage specific morphological deformities, exposed to each applied aerosol concentration. A) *T. castaneum* exposed to Entech Fog-10 ® + Diacon II™ B) *T. castaneum* exposed to Entech Fog-30 ® + Diacon II™ C) *T. confusum* exposed to Entech Fog-10 ® + Diacon II™ D) *T. confusum* exposed to Entech Fog-30 ® + Diacon II™.

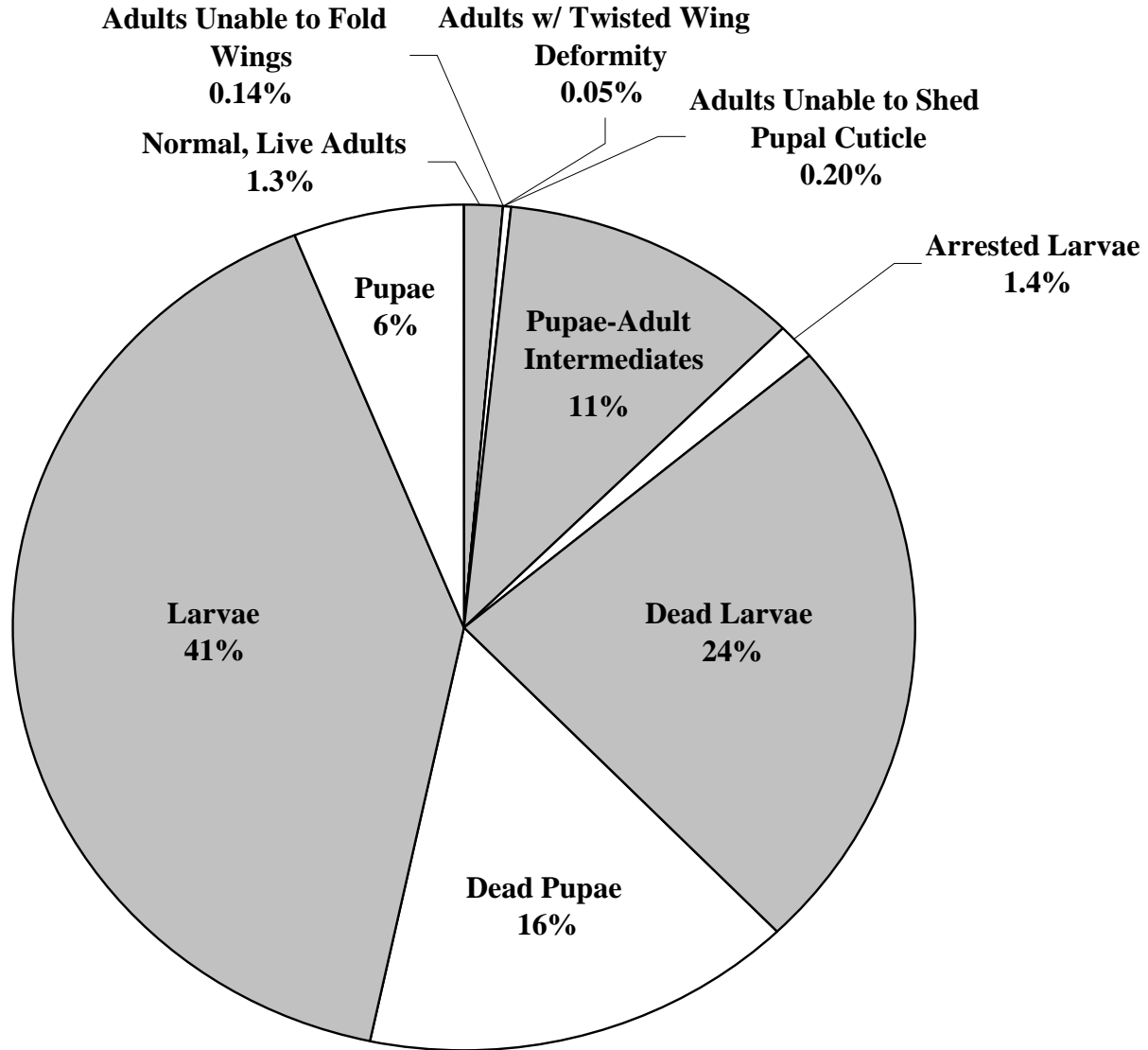
A

***T. castaneum* - 1% pyrethrin + methoprene**



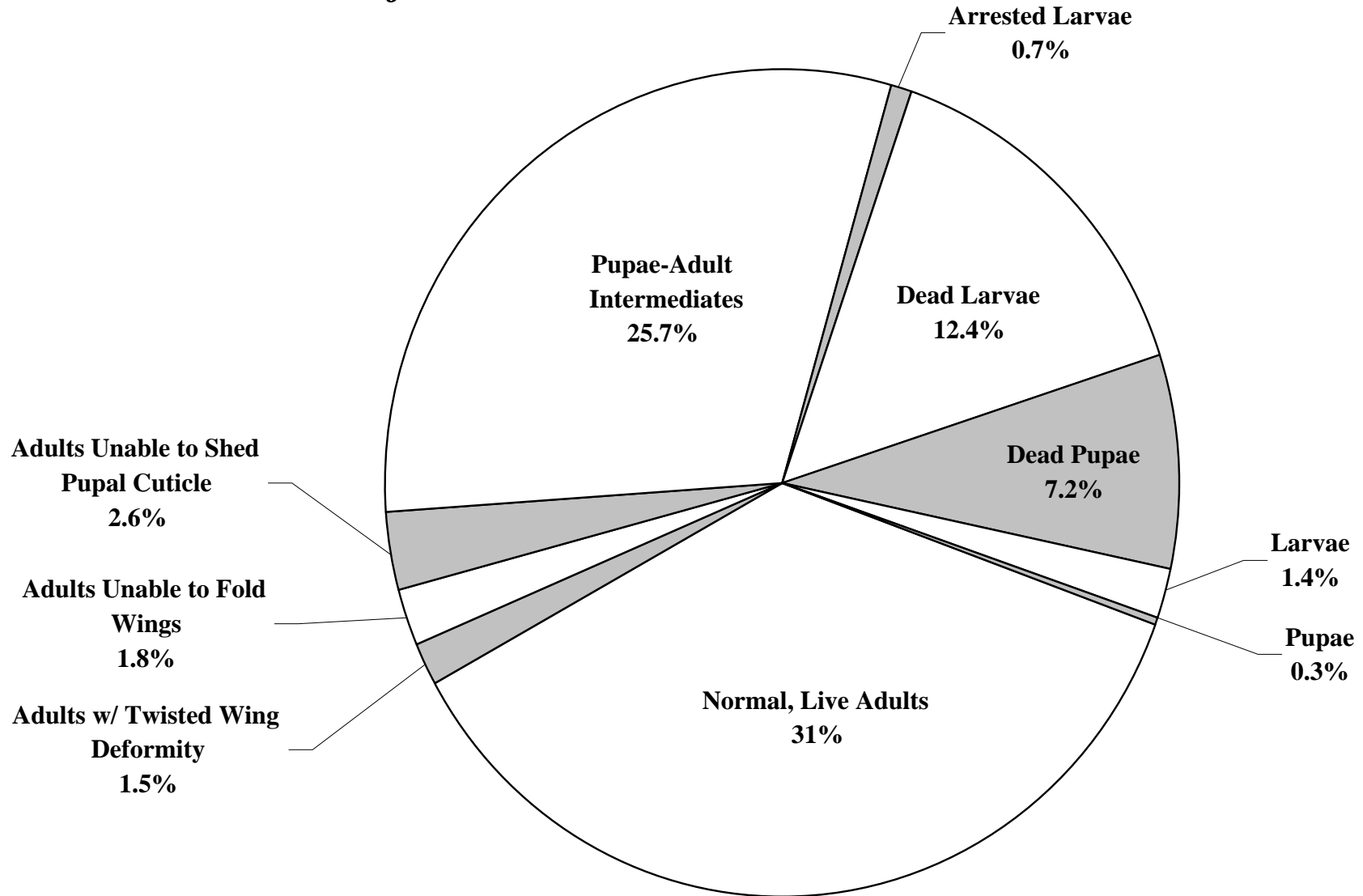
B

***T. castaneum* - 3% pyrethrin + methoprene**



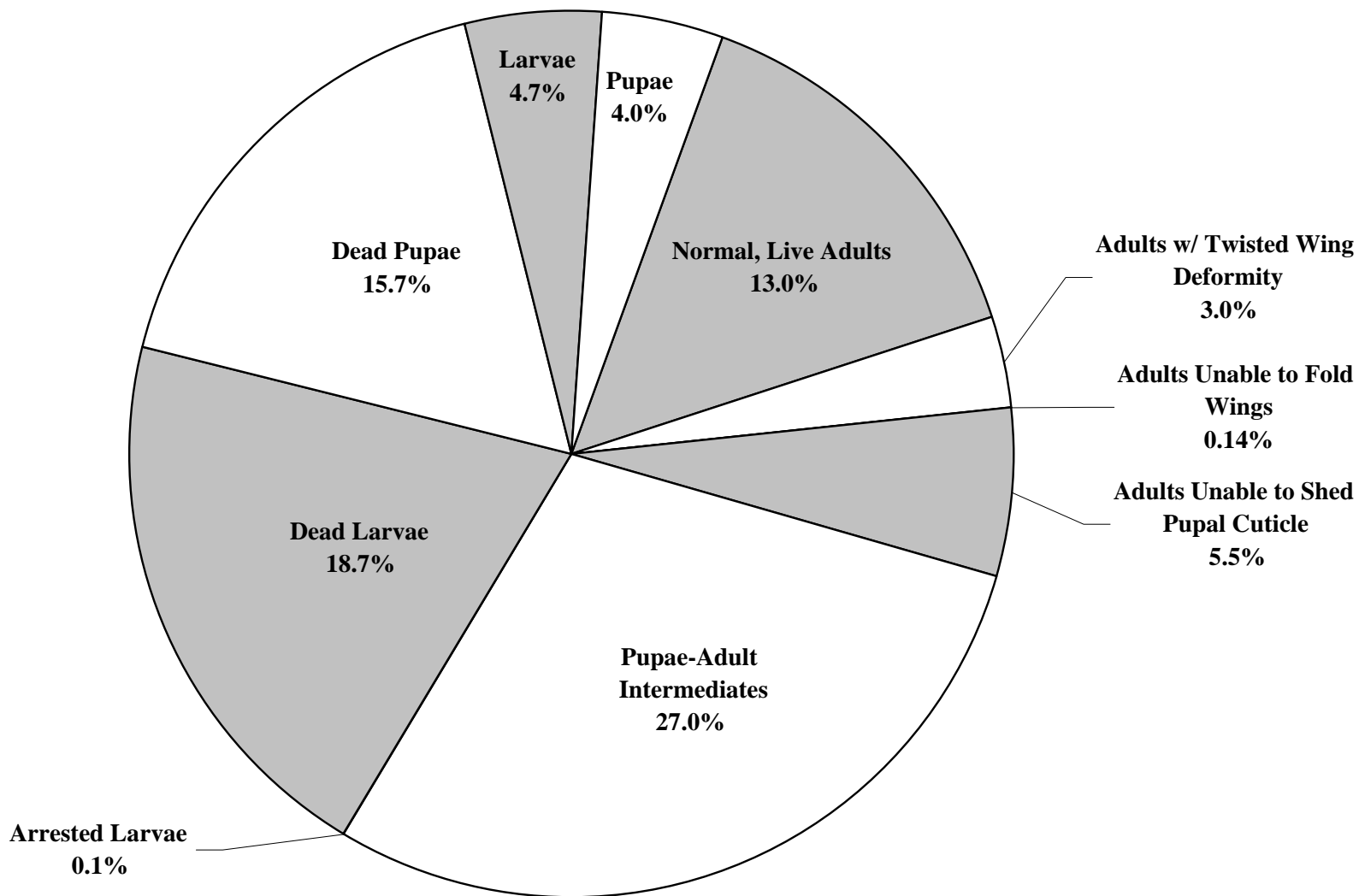
C

***T. confusum* - 1 % pyrethrin + methoprene**



D

***T. confusum* - 3% pyrethrin + methoprene**



Appendix Table 2.2. Percentage (mean \pm SE) of adult morphological characteristics: normal adult development; individuals displaying the twisted wing deformity, individuals unable to properly fold their wings beneath the elytra/wings; and adults unable to shed the pupae cuticle, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, for the two exposed species, at each post-application storage interval.

Storage Wks.	Normal Adult Development		Adults w/ Twisted Wing Deformity		Adults w/ Unfolded Wing Deformity		Adults Unable to Shed Pupal Cuticle	
	3% pyrethrin + methoprene		3% pyrethrin + methoprene		3% pyrethrin + methoprene		3% pyrethrin + methoprene	
	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>
Ctrl	88.75 ± 0.48a	87.92 ± 0.48A	b	b	b	b	b	b
0	b	1.67 ± 1.00D	b	2.50 ± 0.50AB	b	6.67 ± 1.08ABC	b	10.83 ± 2.33A
2	b	10.83 ± 2.75CD	b	4.17 ± 0.65A	b	7.50 ± 0.87AB	b	7.92 ± 1.11AB
4	1.67 ± 1.00b	13.33 ± 1.68C	b	2.50 ± 0.50AB	b	8.75 ± 1.03A	b	3.33 ± 0.91BC
6	b	8.33 ± 0.82CD	b	2.92 ± 1.11AB	b	7.50 ± 0.96AB	b	3.33 ± 0.41BC
8	2.50 ± 1.50b	15.83 ± 3.50BC	b	3.33 ± 0.41A	b	4.58 ± 0.25BCD	b	2.50 ± 0.29BC
10	b	26.25 ± 1.93AB	b	4.17 ± 1.19A	b	5.00 ± 0.41BC	b	2.50 ± 0.29BC
12	2.08 ± 1.25b	17.08 ± 2.0BC	b	1.67 ± 0.41AB	b	5.00 ± 0.41BC	b	5.83 ± 1.19ABC
14	2.92 ± 1.44b	18.75 ± 4.31BC	b	1.25 ± 0.25AB	1.25 ± 0.48a	3.75 ± 0.48CD	1.67 ± 0.71a	2.92 ± 0.48BC
16	2.08 ± 1.25b	7.92 ± 0.85D	0.42 ± 0.25a	3.75 ± 0.63AB	b	3.75 ± 0.48CD	b	8.75 ± 1.80BC
Storage Wks.	1% pyrethrin + methoprene		1% pyrethrin + methoprene		1% pyrethrin + methoprene		1% pyrethrin + methoprene	
	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>
	Ctrl	98.75 ± 0.48a	96.67 ± 1.22A	b	b	b	b	b
0	b	12.50 ± 1.32E	b	1.67 ± 0.41BC	b	7.92 ± 1.11AB	b	7.50 ± 0.65A
2	0.42 ± 0.25b	16.67 ± 3.19DE	0.42 ± 0.25a	2.92 ± 0.85AB	b	7.50 ± 0.65ABC	0.42 ± 0.25b	6.25 ± 0.48AB
4	b	15.00 ± 1.83E	0.42 ± 0.25a	1.67 ± 0.41AC	0.42 ± 0.25bc	8.75 ± 0.95AB	b	4.17 ± 0.29BC
6	0.83 ± 0.50b	22.92 ± 0.84CDE	0.42 ± 0.25a	2.08 ± 0.48ABC	b	4.58 ± 0.75CD	0.42 ± 0.25b	2.50 ± 0.65CD
8	b	29.58 ± 4.68CD	0.42 ± 0.25a	2.50 ± 0.29AB	0.42 ± 0.25bc	3.75 ± 0.25D	0.83 ± 0.50b	4.58 ± 0.63ABC
10	b	33.33 ± 1.58BC	0.83 ± 0.29a	2.08 ± 0.25ABC	0.83 ± 0.29bc	5.83 ± 0.50BCD	0.42 ± 0.25b	5.42 ± 0.63ABC
12	b	43.75 ± 2.95B	1.25 ± 0.75a	3.33 ± 0.71AB	1.25 ± 0.48b	9.17 ± 0.96A	0.83 ± 0.29b	4.58 ± 1.03ABC
14	b	43.33 ± 2.35B	1.25 ± 0.48a	4.17 ± 0.65A	0.83 ± 0.29bc	3.75 ± 0.48D	0.42 ± 0.25b	5.42 ± 0.85ABC
16	b	46.25 ± 2.66B	0.42 ± 0.25a	2.08 ± 0.48ABC	4.17 ± 0.29a	4.17 ± 0.29D	3.75 ± 0.75a	2.50 ± 0.50CD

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 2.3. Percentage (mean \pm SE) of pupae morphological characteristics: pupae-adult intermediates, pupae not able to undergo further development, and dead pupae, as a percentage of the total number of individuals (four-week old larvae) initially to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, for the two exposed species, at each post-application storage interval.

Storage Wks.	Pupae-Adult Intermediates		Pupae		Dead Pupae	
	3% pyrethrin + methoprene		3% pyrethrin + methoprene		3% pyrethrin + methoprene	
	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>
Ctrl	b	b	b	b	5.42 ± 0.63d	7.08 ± 1.49C
0	0.83 ± 0.50e	41.25 ± 1.25A	10.00 ± 1.58ab	5.00 ± 1.47AB	26.25 ± 1.25a	14.17 ± 1.19ABC
2	10.00 ± 1.87bc	30.83 ± 1.04AB	6.25 ± 0.63bcd	4.17 ± 1.19AB	24.58 ± 1.25ab	12.08 ± 0.75ABC
4	9.58 ± 0.85c	27.92 ± 1.75B	8.33 ± 1.78abc	3.75 ± 1.31AB	10.00 ± 1.08cd	13.33 ± 1.08ABC
6	10.42 ± 0.63bc	25.00 ± 0.71B	13.33 ± 1.08a	6.67 ± 1.08A	12.92 ± 1.38cd	18.33 ± 0.91A
8	12.92 ± 1.65abc	24.17 ± 1.71B	6.25 ± 1.65bcd	4.17 ± 0.96AB	22.08 ± 1.55ab	17.50 ± 0.65AB
10	17.50 ± 1.76a	19.17 ± 1.55BC	3.33 ± 1.15cde	2.08 ± 0.63AB	16.67 ± 1.68bc	17.50 ± 2.33AB
12	6.67 ± 1.00cd	27.50 ± 1.55B	4.17 ± 1.66cde	5.00 ± 2.12AB	12.92 ± 1.38cd	8.75 ± 1.11BC
14	16.25 ± 2.17ab	24.17 ± 2.50B	2.08 ± 0.75de	5.00 ± 1.08AB	11.67 ± 1.96cd	15.00 ± 1.22ABC
16	10.00 ± 1.35bc	26.67 ± 2.12C	2.92 ± 1.11de	5.42 ± 1.49AB	17.08 ± 1.31d	19.17 ± 2.63BC
Storage Wks.	1% pyrethrin + methoprene		1% pyrethrin + methoprene		1% pyrethrin + methoprene	
	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>
	Ctrl	b	b	b	b	0.83 ± 0.29B
0	0.83 ± 0.29d	36.25 ± 1.31A	8.33 ± 0.41a	4.17 ± 0.29AB	25.83 ± 0.65a	10.83 ± 1.19A
2	7.08 ± 0.85c	29.58 ± 1.11B	5.00 ± 0.71bc	3.75 ± 0.85AB	22.08 ± 1.03a	9.58 ± 0.48A
4	7.08 ± 0.85c	27.92 ± 1.03B	5.83 ± 0.96ab	2.50 ± 0.65BC	12.92 ± 0.63bc	10.42 ± 0.85A
6	7.50 ± 0.65bc	15.83 ± 1.32C	7.08 ± 0.48ab	5.42 ± 0.48A	11.25 ± 0.95c	8.33 ± 0.91A
8	10.83 ± 1.32abc	12.92 ± 2.06CD	5.42 ± 0.85bc	3.33 ± 0.71AB	17.08 ± 1.25b	9.17 ± 1.50A
10	13.33 ± 1.47a	7.08 ± 0.85DE	2.92 ± 0.48cd	2.92 ± 0.48AB	14.58 ± 0.85bc	8.33 ± 1.29A
12	11.25 ± 0.48ab	10.42 ± 0.85CDE	4.58 ± 0.63bc	3.75 ± 0.95AB	14.58 ± 0.63bc	7.92 ± 1.38A
14	12.50 ± 0.87a	6.67 ± 0.41E	2.92 ± 0.48cd	3.33 ± 0.41AB	12.92 ± 1.11bc	8.33 ± 0.41A
16	2.50 ± 0.50d	24.58 ± 1.89B	1.67 ± 0.41de	1.67 ± 0.00BC	15.00 ± 1.08bc	6.67 ± 0.71A

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 2.4. Percentage (mean \pm SE) of larvae morphological characteristics: individuals remaining in the larvae stage, those that died while in the larvae stage and those that were arrested between larvae and pupae development, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, for the two exposed species, at each post-application storage interval.

Storage Wks.	Arrested Larvae		Larvae		Dead Larvae	
	3% pyrethrin + methoprene		3% pyrethrin + methoprene		3% pyrethrin + methoprene	
	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>
Ctrl	b	b	b	b	5.83 ± 0.96c	5.00 ± 1.47C
0	0.83 ± 0.29bc	0.42 ± 0.25AB	41.25 ± 1.11abc	2.92 ± 1.11DE	20.83 ± 1.04ab	14.58 ± 1.65AB
2	0.83 ± 0.29bc	0.42 ± 0.25AB	42.92 ± 1.80abc	5.00 ± 0.71CD	15.42 ± 0.25bc	17.08 ± 1.03A
4	0.42 ± 0.25bc	0.83 ± 0.29AB	50.83 ± 2.66a	5.42 ± 1.11BCD	19.17 ± 1.66abc	20.83 ± 0.65A
6	0.83 ± 0.29bc	b	49.58 ± 1.31ab	10.00 ± 1.08AB	12.92 ± 1.18bc	17.92 ± 0.63A
8	2.92 ± 0.63a	0.42 ± 0.25AB	32.08 ± 1.31c	7.08 ± 1.60ABCD	21.25 ± 2.29ab	20.42 ± 1.44A
10	1.25 ± 0.25abc	0.42 ± 0.25AB	37.08 ± 1.03bc	4.58 ± 1.18CDE	24.17 ± 1.85ab	18.33 ± 1.47A
12	2.08 ± 0.63ab	0.42 ± 0.25AB	42.08 ± 1.93abc	9.17 ± 0.65ABC	30.00 ± 2.80a	19.58 ± 1.31A
14	1.25 ± 0.25abc	1.25 ± 0.75A	31.25 ± 1.93c	11.25 ± 0.95A	31.67 ± 2.48a	16.67 ± 0.91AB
16	2.08 ± 0.25bc	b	30.83 ± 2.96d	5.83 ± 0.87DE	34.58 ± 1.80bc	18.75 ± 1.11BC
Storage Wks.	1% pyrethrin + methoprene		1% pyrethrin + methoprene		1% pyrethrin + methoprene	
	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>	<i>T. castaneum</i>	<i>T. confusum</i>
	Ctrl	b	b	0.42 ± 0.25f	0.42 ± 0.25F	0.83 ± 0.29f
0	1.67 ± 0a	0.42 ± 0.25AB	40.83 ± 1.44ab	4.17 ± 0.65DEF	22.50 ± 1.19de	14.58 ± 1.49DE
2	1.67 ± 0.41a	0.42 ± 0.25AB	45.00 ± 1.96a	5.42 ± 0.48CDE	17.92 ± 0.63e	17.92 ± 0.95CD
4	1.25 ± 0.48a	b	45.83 ± 1.71a	7.92 ± 0.85BCD	26.25 ± 1.03cd	21.67 ± 0.41ABC
6	1.67 ± 0.41a	0.83 ± 0.29AB	40.42 ± 0.63ab	10.42 ± 0.85B	30.42 ± 1.44bc	27.08 ± 1.11A
8	1.67 ± 0.41a	0.42 ± 0.25AB	24.58 ± 1.89e	10.42 ± 1.25BC	38.75 ± 1.75a	23.33 ± 1.08AB
10	1.25 ± 0.48a	b	28.33 ± 1.08de	20.83 ± 1.19A	37.50 ± 1.85a	14.17 ± 1.26DE
12	1.67 ± 0.58a	b	33.75 ± 0.48cd	0.8 ± 0.50F	30.83 ± 1.32bc	16.25 ± 1.65CDE
14	0.42 ± 0.25a	b	35.00 ± 1.00bc	2.50 ± 0.96EF	33.75 ± 1.11ab	22.50 ± 1.44AB
16	2.08 ± 0.63a	b	35.00 ± 0.91bc	1.25 ± 0.48F	35.42 ± 0.95ab	10.83 ± 0.87E

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

**CHAPTER 3 - Residual efficacy of a pyrethrin + methoprene
aerosol insecticide to control *Tribolium spp.* (Coleoptera:
Tenebrionidae) on packaging material surfaces.**

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Abstract

Seven commercially used packaging materials were exposed in Petri dishes to an aerosol formulation of either 1% or 3% pyrethrin + methoprene at the label rates in a commercial milling facility. The treated surfaces were stored in low-light conditions for 16 wks, and every two weeks the arenas were bioassayed by adding four-week old larvae of *Tribolium castaneum* (Herbst), the red flour beetle, and *T. confusum* (DuVal), the confused flour beetle, plus 0.5g of fresh, untreated flour, to the previously exposed arenas containing each of the seven packaging materials. The arenas were monitored for 30 days and examined every two days for stage-specific effects. Both insect species were susceptible to the aerosol insecticide, but *T. castaneum* appeared to be more susceptible. Results show that regardless of the treated surfaces, the critical stage in which the effects of aerosol exposures were most apparent for *T. castaneum* was the transition between the larval and the pupal stages. Whereas the most critical stage for *T. confusum* appeared to be the period between the pupal and the adult stage. There were variations in the efficacy among the seven pre-exposed packaging surfaces, but little difference in overall trends. Results showed nearly complete suppression of *T. castaneum* on all surfaces. In the efficacy studies after the 16-wk storage, *T. castaneum* showed approximately 2-3% normal adult emergence after exposure to each of the previously treated packaging material surfaces treated with 1 or 3% aerosol insecticide. In contrast, *T. confusum* exposed to the treated surfaces demonstrated a greater suppression of adult emergence upon exposure to the 3% pyrethrin aerosol than the 1% treatments. At the completion of the 16-week storage period, the percentages of individuals advanced to normal and live adults was 43% on cotton bag, 50% plastic, 56% cardboard, 63% pallet wrap, 68% polyester bag, 78% paper bag and 78% flour bag treated surfaces, after *T. confusum* larvae were exposed to the 1% aerosol application. However, when the packaging materials were exposed to the 3% aerosol, the insect emergence reduced to 22% on the cotton bag, 8% plastic, 11% cardboard, 18% pallet wrap, 21% polyester bag, 13% paper bag and 16% flour bag. These results further demonstrate that these aerosol formulations could be adequately used to effectively manage these insect pests. It appears that 1% pyrethrin aerosol formulation may be sufficient for adequate control of these insects, especially *T. castaneum*.

1. Introduction

The flour beetles, *Tribolium castaneum* and *T. confusum*, are worldwide insect pests of mills, food warehouses, retail stores, and urban homes (Rees, 2004), and can be major pests in structures used for the processing and storage of grain-based products (e.g., flourmills, warehouses, retail stores) (Campbell and Runnion, 2003). These insects constitute important primary and secondary pests in many cereal products (Arnaud et al., 2005). Both species may be present in large numbers in damaged grain, but neither species is a primary feeder of undamaged grain (Walter, 1990). *T. castaneum* has been shown to be highly attracted to processed food products, indicating that the processed and combined ingredients of this food product may yield an odor stimulus that represents an optimal food source for this species. Unlike many insects, the flour beetles subsist in an infested food resource that is shared by both the adult and the juvenile stages (Weston and Rattlingourd, 2000; Fedina and Lewis, 2007). *Tribolium* adults can survive, develop and reproduce under conditions that are not suitable for the development of other stored product insect pests, allowing high population densities to be easily built up (Champ and Dyte, 1976; Hill, 1990). As population density increases, the shared flour medium that *Tribolium* spp. occupy begins to lose its nutritional quality, often accumulating waste products and other ethyl- and methylbenzoquinones, produced by adults and released as defensive compounds (Sokoloff, 1974). In contrast to other stored product insects, *Tribolium* spp. often show a greater response to various grains, which may reflect the habitat preference of this species, which develops in older and damaged grain substrates (Mullen and Mowry, 2006).

Stored product pests are economically important and are responsible for millions of dollars of loss every year, causing both quantitative and qualitative losses (Neethirajana et al., 2007). Food and beverage packaging is a \$70 billion market in the United States and more than \$200 billion worldwide (Wilkinson, 1998). In lieu of this, consumer product packaging is inundated with a deluge of challenges, with which to avoid infestation by insects, maintain high quality and attract consumers. These challenges include the type of packaging material used, the ability of the packaging to maintain its products' quality, the cost and availability of these materials, as well as consumer acceptance/confidence (Mullen and Mowry, 2006). The packaging of products is the last line of defense against insect infestation of their finished products. These materials are often incredibly susceptible to attack by insects, and must be both

constructed and treated as to not allow insect infestations when materials, as they undergo subsequent shipping and distribution. There is a growing awareness of the need for cost-effective and efficient methods to monitor insecticide and pesticide residues in the food supply. Consumers and regulators have little tolerance for insect-damaged or contaminated food products, which pose serious consequences and challenges for pest management professionals (Arthur and Peckman, 2006). Grain losses resulting from insect infestations of all post harvest product losses have a major economic impact on the food industry due to the costs associated with the treatment and monitoring, rejection and return of contaminated products, loss of consumer goodwill, and failure to pass inspection or meet regulations (Campbell and Arbogast, 2004).

The management of most stored product insects is becoming more and more dependent on the application of insecticidal fogs, fumigants and sprays (Cox and Bell, 1991). Pest management professionals often utilize aerosolized liquid applications otherwise known as fogging or ultra-low volume (ULV), to manage stored product insects. Used as structural insecticides and applied in non gas-tight structures, these residual contact insecticides are commonly applied on a recurring basis, often at regular treatment intervals of 2-3 weeks (Toews et al., 2005b), and used to control insects and prevent serious infestations of many stored product insect pests inside food processing facilities, flourmills, warehouses, indoor storage facilities and grocery stores. Aerosols have been highly regarded for IPM purposes as they provide good coverage of all exposed surfaces, as well as providing a level of residual control, depending on the substrate(s) that they are applied to. Aerosols are also known for their ability to effectively target insect pests, as well as their potential to increase the time interval between costly and otherwise invasive structural fumigations or heat treatments, all of which allow aerosols to be more readily utilized, especially during a time in which the food, shipping and other agricultural industries are searching for effective fumigant replacement strategies (Toews et al., 2005a, 2006b). One of the biggest challenges to the processing, milling and food plant industries has been the loss of the fumigant methyl bromide (Arthur and Phillips, 2002). Used as a disinfestation, or quarantine measure against stored product insects, in structures such as flourmills, warehouses, silos and food stores, methyl bromide is widely employed for its ability to rapidly kill insects, mites, microflora, and nematodes (Bell, 2000). Therefore, the need exists

to further develop and test effective and safe compounds for chemical stored product insect control methods (Williams and Amos 1974).

Research has focused on the identification of potential new insecticides that can be used on stored products, as well as a thorough examination of the factors that can affect efficacy of these residual insecticides. A mixture of pyrethrin plus methoprene is a residual insecticide that can be used as a part of an integrated approach to target insect control purposes. The objectives of this study were to determine the residual efficacy of the aerosol formulations comprised of synergized pyrethrins at the concentrations of 1 and 3% pyrethrins, plus a 1% methoprene component, to determine if there were differences in susceptibility to aerosol residual exposures between the two *Tribolium* species, and to evaluate the residual efficacy of each of the seven packaging materials exposed to the aerosol insecticide at two different application rates against *T. castaneum* and *T. confusum*.

2. Materials and Methods

2.1 *Tribolium* spp.

Tribolium castaneum and *T. confusum* are common insect pests associated with food-processing facilities worldwide (Sinha and Watters, 1985; Mills and Pedersen, 1990). The larvae of *T. castaneum* and *T. confusum* that were used in the following experiments described below were obtained from a laboratory-reared population reared on a diet of 95% whole wheat, bleached flour, and supplemented with 5% (w/w) brewer's yeast. These colonies had been reared for more than twenty years at the USDA Grain Marketing Research and Production Center in Manhattan, KS. All the colonies were maintained in a low-light environmental chamber at $27 \pm 3^\circ\text{C}$ and $70 \pm 5\%$ r.h.

2.2 Experimental Design

The packaging materials used in this study were a paper bag material, a 2 mm thick commercial cardboard, heavy commercial plastic sheets, pallet wrap material, commercial flour bags, polyester woven bag material, and, a cotton muslin bag material. All of these materials are commonly used in milling and other food processing warehouses. These surfaces of these materials often come in contact with raw commodity, or finished and packaged products, and are

often susceptible to attack by insects through the process of shipping and distribution (Highland, 1978). The concrete exposure treatment arenas that were used for all bioassay experiments, were constructed using a standard, plastic 100 x 15 mm plastic Petri dish and concrete patching material (Rockite®), purchased from a local hardware store. A water-based slurry was prepared by mixing about 2000g Rockite® with 1.0 L of tap water, and pouring 20 mL into the bottom of a Petri dish to cover the surface and dried overnight to create individual treatment arenas that would be further utilized in all subsequent bioassays experiments.

The two aerosol insecticidal formulations that were used in this study were purchased from Entech Systems Corp. (Kenner, LA, USA). The Entech Fog-10® (EPA Reg. No. 73049-400-40391) is comprised of 1.0% pyrethrins, 2.0% PBO synergist, 3.33% N-octyl bicycloheptane dicarboximide and 93.67% refined petroleum solvent. The Entech Fog-30® (EPA Reg. No. 73049-400-40391) is similarly contains 3.0% pyrethrins, 6.0% PBO synergist, 10% N-octyl bicycloheptane and 81% refined petroleum solvent. Each pyrethrin formulation was applied according to label directions, which is 29.6 mL/m³ of space. Methoprene was added to both the 1% and the 3% pyrethrin at the label rate for space applications of 90 mg of active ingredient per 28m³ of space, such that it amounted to 1% of the overall aerosol concentration. For the purposes of this study, These combined aerosol formulations are referred to as “1% pyrethrin” and “3% pyrethrin”.

The insecticidal application portion of this overall study was conducted in an operational flourmill, located in western Kansas, which had been previously equipped with an automatic aerosol application system. Multiple stationary mechanical sprayers were positioned roughly 4.5 m above the floor, in multiple locations throughout the mill, such that each floor of the flourmill could be treated separately. One particular floor within this mill, measuring roughly 1,716.8m² was chosen for the exposure portion of this study, of which approximately 25-30% of this total surface area was occupied by milling equipment. The prepared concrete exposure treatment arenas (described previously) were placed directly on the floor of this chosen level, within a 6m x 6m (3.3m²) unobstructed area, such that no treatment arena was placed within 0.6m of a potential overhead barriers (i.e., milling equipment, walls, doorways, etc.). Each treatment arena, corresponding to each of the packaging material surfaces, contained a precut circular disc that

was fit into the bottom of each concrete treatment arena. The edges and rims were caulked to minimize to prevent larvae and subsequent developmental stages from gaining access underneath or behind the disc. Flour was not added to the concrete treatment arenas during this exposure. A re-entry period of 2.5 h post aerosol application was selected, based on the preliminary results indicating that this was the minimum amount of time required for the settling of the aerosol cloud/fog in this particular milling facility. All untreated controls were held in another on-site building that was not exposed to the aerosol treatment. After the aerosol treatment and the subsequent re-entry period had lapsed, each of the aerosol treatments were retrieved, lids were replaced and treatment arenas were bundled, secured and placed in dark storage containers and returned to the lab at Kansas State University. Once in the laboratory, all treated arenas and control arenas, were maintained continuously at very low light conditions, with temperature and r.h. being monitored by a HOBO data logger. Both aerosol-treated arenas and the untreated control arenas were stored in the same manner for the duration of the study.

To evaluate the degradability of this aerosol insecticide on each of the seven utilized packaging material surfaces, and examine how this degradation specifically affects emergence of adults of both species of *Tribolium* exposed to each of the treated packaging materials, a series of ‘time’ factors were incorporated into the overall experimental design. Thus, for the purposes of reporting data, ‘time’ will be defined in three ways. First, complementary sets of concrete treatment arenas corresponding to each exposed packaging material surface, were exposed to each applied aerosol concentration rate, such these tests were each replicated a total of four times, at each aerosol concentration. Each four-replicate experiment was performed throughout different two month periods, with the 3% pyrethrin treatments executed between August and September, and the 1% pyrethrin treatments performed between November and January. The independent, four-replicate-based sets of exposed treatment arenas, were exposed to the two independent aerosol applications, such that four complementary sets of treatment arenas were exposed, per application rate, for a total of eight sets of exposed treatment dishes (i.e., replications), used directly in subsequent bioassays. These eight replications will consequently be referred to as “replications in time” (i.e., “1% pyrethrin replications in time”, and “3% pyrethrin replications in time”) in this paper.

Second, to aid in estimating the residual efficacy over a four-month period of the two applied aerosol concentrations, a factor of “storage time(s)”, (or “storage periods”), was incorporated into the overall experimental design. This factor is representative of a series of nine, biweekly storage periods, representing increasing intervals of time from the initial aerosol application. These nine periods compose the overall 16-week storage experiment (i.e., 0, 2, 4, 6, 8, 10, 12, 14 and 16 weeks post aerosol treatment). Analyzing bioassay data at each two-week storage interval provides the opportunity to observe discernible trends or patterns of insect development (e.g., percentage of species-specific and stage-specific morphogenic abnormalities and developmental arrests, as well as percentage of species-specific normal, adult emergence), at both aerosol application rates, as well as make comparisons between the various packaging material surfaces. During this 16-week “storage time”, the treatment arenas were stored such that low-light, stable temperature and uniform r.h. conditions could be reasonably controlled and monitored. By establishing as close to ideal storage conditions as possible, potential degradation of insecticides was minimized. The final included factor of time was denoted as “observation days/period”. This factor encompasses a 30-day period, during which species-specific as well as stage-specific development of *Tribolium* species was monitored and recorded, comprising the quantitative dataset that will be used for statistical analysis. Under laboratory rearing conditions at 27°C, four-week old *Tribolium* spp. larvae progressively develop into pupae within 6-7 days, and emerge as adults 7-8 days later. Incorporating a 30-day observation period into this study provides roughly an additional two-week period in which development within each stage, at each post-treatment storage time, can be observed for stage-specific development.

For each independent replication treatment within both the “1% pyrethrin replications in time” and the “3% pyrethrin replications in time”, at each two-week post-treatment “storage time”, a set of 6 companion treatment arenas were chosen randomly from each of the larger samples of 72 previously exposed treatment arenas per treated packaging material surface. These six arenas were then utilized for the subsequent individual bioassay experiments, which correspond to each two-week storage period, within the larger 16-week study. Each of the eight, independent replication, complementary sets of 72 concrete exposure treatment arenas contain the exposed, caulked packaging material that was exposed to one of the two applied aerosol concentration rates. To initiate the bioassay experiments, at ‘day 0’ of the 30-day observation

period, 0.5g of untreated, freshly prepared flour mixture was added to the center of each of the 6 previously exposed treatment arena per each of the seven packaging materials being examined, which will now be referred to as ‘observation arenas’. These 42 observation arenas correspond to a particular biweekly post-aerosol application storage time/interval. Of these newly prepared bioassay observation arenas, 6 of which are correlated to each of the seven exposed packaging material surfaces, three of an alike surface arenas were used to monitor *T. castaneum* development, whereas the other three were used to monitor *T. confusum* development over the course of 30-days. Ten, four-week old *T. castaneum* or *T. confusum* larvae were added into each of the three corresponding observation treatment arenas, such that n=10 larvae/treated observation arena/exposed packaging material surface. This initial addition of the 10 *Tribolium* spp. larvae, denoted ‘day 0’ of the 30-day observational experiment, in which these six observation arenas will be monitored, noting the morphological development/progression of these ten larvae, every two-days, over the 30-day observation period, for a total of 15 observations (i.e., 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30) at each post-aerosol application storage interval. It is important to reiterate that a flour substrate will not be added to the exposed packaging material surfaces until ‘day 0’. As part of the experimental design, we directly assessed the residual efficacy upon the packaging material surfaces over a four-month period of time. The flour that was added to each treatment arena was meant to sustain the larvae, and would likely absorb the insecticide on each surface, allowing the larvae to come in direct contact with the remaining amounts of chemical components at each post-aerosol application storage interval.

Development and subsequent stage-specific morphological characteristics (i.e., dead or arrested individuals), the characteristic stage-specific morphological deformities, and emergence of morphologically normal adults were recorded at each two-day observation period. The morphological deformity scoring system classified individual insects as normal, presumably reproductively viable adults (i.e., fully eclosed adults), or ‘affected’ (i.e., individuals displaying a distinct morphogenic malformation, those arrested between stages of development and, those unable to progress their development, thus persisting in a single stage for the duration of the 30 day observation period), including live adults displaying a twisted wing deformity, live adults with a deformity in which their wings were unable to fold, live adults unable to shed their pupae

cuticle (i.e., incompletely formed elytra), live pupae, arrested pupae-adult intermediates (i.e., secondary pupae or supernumary pupae), dead pupae, live larvae, arrested larvae-pupae intermediates, and dead larvae (Kamaruzzaman et al., 2006; Arthur, 2001; Arthur and Hoernemann, 2004; Arthur and Campbell, 2008, Fig. 1). At each of the 15 observations, any individual recorded as fully emerged as a normal adult, those with a characteristic morphogenic abnormality, those arrested between any two stages in development, or those that had died at any stage were consequently removed from the given observation arena however, any individuals continuing development were left in the observation arena. The removal of these individuals was done so to avoid documented *Tribolium* spp. cannibalistic behavior, and as a measure of further encouraging the consumption of the added untreated wheat flour substrate (Ho and Dawson, 1966; Beno et al., 1998; Via, 1999; Wade, 1976). After each observation, the arenas were again tightly sealed and returned to a temperature and humidity controlled environmental chamber. Humidity within this chamber was regulated at roughly 75% using a .32 x .26 x .07 m plastic pan (Fisher Scientific, 13-361-10), which was kept filled with tap water.

The use of a morphological scoring system allowed us to assess the developmental capabilities of the exposed insects to each applied aerosol concentration and assess both stage-specific and species-specific developmental patterns. A comparison of the proportions of morphologically normal, presumably reproductively viable adults able to emerge at each aerosol concentration, at each of the nine “storage times”, versus those that displayed an affected stage-specific deformity was done to estimate the residual efficacy of each of the two aerosol concentrations on each treated surface, as well as to evaluate quantitative differences between the nine “storage times” on each treated surface, plus to aid in assessing the most practical and efficient aerosol reapplication time point that would most effectively prevent the development of *Tribolium* adults on each surface, noting both economic threshold levels and applicator costs and lastly to evaluate the occurrence of the many stage-specific responses of the two exposed insect species again with regards to the individually treated surfaces. The probability of normal adult emergence, as well as other stage-specific development and deformities, is modeled at both aerosol concentrations, by accounting for these proportions per *Tribolium* species. The proportion of ‘normal’ versus ‘affected’ individuals, effectively indicates the potential for each utilized aerosol concentration, to adequately control these *Tribolium* spp. by effectively

preventing their development and eventual emergence into reproductively viable adults that would be able to further propagate a population within a given milling setting.

2.3 Statistical analysis

Morphological data from each of the two *Tribolium* species at each of the eight replications in time intervals were analyzed for the main effects of the aerosol insecticide at 1 and 3% pyrethrin and the interaction effect of the applied aerosol concentration rate x species. The response variable was the aerosol concentration by *Tribolium* species by “storage period” by “replication in time” by the accumulated counts of individuals within each scored morphological deformity category, per observation arena, during the 30-day period. Data for normal adult emergence and the morphological deformities were pooled over the 30 day observation period, then further pooled into an accumulated value over each of the three observation arenas, according to species (n=30 per species). Preliminary analysis revealed no discernable trend emerging across the entire 16-week storage experiment, upon comparing each two-week storage interval, according to each applied aerosol concentration, *Tribolium* species, and the exposed packaging material surface. Therefore, the data was again collapsed over the entire 16-week experiment. This comprehensive value is based on a model 2 species x 2 concentrations x 4 individual replications in time, for a total n=270, per each of the ten scored morphological categories. This collapsed value depicts the proportion of total individuals within each scored morphological category for the larger 16-week storage period. In assessing the data, using this overall collapsed value, it allows us to directly evaluate species differences with regards to emergence, developmental capabilities of the two exposed species when challenged to the aerosol insecticide at the two competing application rates, and to estimate the residual effectiveness of this aerosol, based on the proportions of individuals emerging both unaffected and affected.

Data analyzed using the SAS General Linear Model (GLM) procedure of the Statistical Analysis System (SAS Statistical Software, version 9.1.3) revealed that while a majority of the data possessed both equal variances and normality however, large variations within each replication in time, produced negative confidence intervals. Therefore, data from the bioassay experiments were analyzed using the SAS Generalized Linear Model (GENMOD) procedure by comparing the proportions of deformities to each of the applied aerosol concentration rates by

species as well as the concentration by species interaction effect. Statistical significance of both the main effects and the interaction effects were tested using the type III analysis of a logistic ANOVA, and reported as a series of chi-squared (χ^2) values, along with their corresponding p-values. The GENMOD procedure allowed fitting a sequence of models and generated a type 3 analysis. This type 3 analysis consists of specifying a model and computing likelihood ratio statistics for all parameters in the given model. The model for this analysis consisted of modeling the proportion of each morphological category for the two main effects (concentration and species), as well as the interaction effect (concentration x species). Table 3-2 through 3-4 report upper and lower confidence limits (confidence intervals) for the modeled parameters based on asymptotic normality. Within this SAS GENMOD analysis, the LS-MEANS statement was used, which computes the least squares means (LS-means) corresponding to the specified effects, allowing a direct comparison of the means. After all pairwise comparisons were made, χ^2 values as well as associated P-values are reported for each treatment level. A significant species by concentration interaction would indicate a level of inherent variability in the stage-specific development of each species when challenged with the aerosol insecticide, at both applied aerosol application rates, and would suggest that the main effects cannot be generalized with as much confidence, and are likely not statistically significant in isolation.

The proportion of 'affected' individuals was assessed for differences using the least squares means (LS-means) option and a chi-squared test, to determine any deviation from a 1:1 ratio. Results were reported on the probability scale and were calculated for both the main effects of concentration and species, and the corresponding interaction effect. Data for the percentage of each of the ten morphogenic categories, per species, per packaging material surface were plotted using equations generated from Sigma Plot software (Sysstat Software, Inc.). Significance between the reported treatment means, within each biweekly post-aerosol application storage period (0-16) were analyzed using unpaired *t*-tests (ProStat, Version 4.0.3, Poly Software International, Inc.). Unless otherwise noted, significance was determined at the 0.05 level.

3. Results

The suite of tables (Tables 3.1 through 3.7) report the variation in the responses of both *Tribolium* spp. upon exposure to both applied aerosol concentration rates for each exposed

packaging material surface: Paper bag (Table 3.1); Cardboard (Table 3.2); Plastic (Table 3.3); Pallet wrap (Table 3.4); Flour bags (Table 3.5); Polyester bag (Table 3.6); Cotton bag (Table 3.7). Each table (Table 3.1 through 3.7) reveals a great deal of variation between across the two applied aerosol formulations, within each individual exposed species. The variation in the responses of both of the exposed *Tribolium* spp. to both of the applied aerosol concentration rates (i.e., the four treatment categories) is reported as mean \pm S.E. of each of the ten scored morphological categories. The exposure of *Tribolium* spp. to JHA's, has been shown to result in inherently different responses and susceptibilities (Amos et al., 1974; Williams and Amos. 1977). *T. castaneum* bioassay results demonstrate that upon exposure to the applied aerosol formulations the arrested pupae-adult intermediates, individuals that died while in the larvae-stage and those that died while in the pupae-stage were consistently reported at above 10% emergence across all seven packaging material surfaces. However, larvae that persisted throughout the 30-day observation period, likely undergoing several supernumerary molts, were observed above 10% only upon exposure to the 3% pyrethrin + methoprene treatment for all but the muslin bag treated surface, which reported this developmental delay at roughly 7.5%. In contrast to *T. castaneum*, *T. confusum* treated bioassays showed considerable emergence of normal, live adults that are presumably reproductively active and thus able to propagate further generations at greater than the 10% in all treated packaging material when exposed to the 1% pyrethrin + methoprene aerosol formulation, and was observed above this 10% threshold, upon exposure to the 3% pyrethrin + methoprene, only on the muslin bag treated surface. The arrested pupae-adult intermediates were consistently observed above this 10% at both applied aerosol concentrations across all treated surfaces. The dead larvae, dead pupae and those individuals persisting as larvae throughout the 30-day observation period showed a more variable pattern.

Each of these tables effectively illustrates both a species- and stage-specific variations in each treatment. A comparison of *T. castaneum* at both 1% pyrethrin and 3% pyrethrin treatments shows that the greatest percentages of individuals are retained, on average, in the larval and the pupal stages. The arrested pupae-adult intermediates of *T. castaneum* individuals are observed in the greatest percentages when exposed to the 1% pyrethrin + methoprene aerosol formulation, for five of seven exposed packaging material surfaces except for the polyester bag and the muslin bag materials, in which the dead larvae were observed in the greatest percentages. When

exposed to 3% pyrethrin + methoprene, on average, the greatest percentage of individuals were observed in the dead larvae morphological category, except for cardboard on which larvae were most abundant. A comparison of *T. confusum* bioassays upon exposure to the 1% pyrethrin aerosol formulation demonstrated that the normal, live adults were consistently shown to be the morphological category that restricted the greatest percentages of individuals. The category with the second greatest percentage of observance was the arrested pupae-adult intermediates were observed with roughly 20-30%. Upon exposure to the 3% pyrethrin + methoprene aerosol concentration, the greatest percentages of individuals were observed, on average, in the arrested pupae-adult intermediate category, with only one surface, the muslin bag, showing over 10% of normal, live adult emergence.

The two *Tribolium* species showed similar patterns in different morphological categories among all seven packaging materials exposed to the aerosol insecticide at each of the two concentrations. Within the *T. castaneum* treated bioassays exposed to either the 1%- or the 3% pyrethrin formulation, the earliest stages of development, the larvae, pupae and the pupae-adult intermediate stages were the categories observed with the greatest percentages of individuals, after the 30-day observation period. The bioassays in which the *T. castaneum* were exposed to the 1% pyrethrin formulation were observed to have on average, almost 2x the percentage of arrested pupae-adult intermediates than was observed in the 3% pyrethrin formulation treated bioassays. The percentages of dead larvae, live pupae, arrested larvae-pupae intermediates, as well as dead pupae were usually statistically similar between the two applied aerosol concentrations, whereas the live, persistent larvae were often observed on average, roughly 3x more often in the bioassays treated with the 3% pyrethrin + methoprene aerosol formulation. Regardless of the applied formulation, the observed percentages of individuals able to complete development and emerge into adults, regardless of whether they were deemed normal, or somehow deformed, was consistently less than 3%. Whereas *T. confusum* treated bioassays saw a slightly less significant, yet similar pattern emerge than was observed in the *T. castaneum* bioassay analysis. Again, the earliest stages of development were observed to have the greatest percentages of individuals, after the 30-day observation period, however, this percentage was on average, often observed to a much greater degree within *T. confusum* bioassays in which the larvae were treated with the 3% pyrethrin formulation (please refer to Tables 3.1 through 3.7),

for all treated surfaces. Also within *T. confusum* bioassays, we observed much greater percentages of individuals characterized as having one of the three adult stage deformities, often this percentage was observed in greater percentages within the 1% pyrethrin treated bioassays. And lastly, the percentages of normal, live adults were observed to a much more significant degree in *T. confusum* bioassays, than was observed in the *T. castaneum* bioassays.

The adult is often considered to be the most harmful stage for the *Tribolium* species (Semple, 1992) as they feed, live and reproduce in the infested commodity (Sehna, 1971). Often insects are disseminated worldwide through transported grains (Ryan et al., 1970). Infestations can arise from within the supply chain, resulting in contaminated food products being shipped through the supply chain to the product manufacturer (Daglish, 2005; Faustini, 2006). However, once all precautions have been taken to ensure the processing and packaging of insect-free food, the food processor has little control over subsequent post-harvest shipping and storage. In developed countries, total food costs are increased with infestations because of the amount of food that is lost after incurring all costs of growing, harvesting, processing, packaging, shipping, warehousing and retailing (Highland, 1977a). The situation is significantly magnified by the presence of insects in finished and packaged goods, which directly affects consumer confidence (Collins, 1998). Normal, live adult development was much more profound in *T. confusum* bioassays across all of the treated packaging material surfaces. Emergence at the 1% pyrethrin + methoprene aerosol concentration was noted between 30% for the muslin bag treated surface up through 50% for the paper bag surface; whereas within the 3% aerosol concentration, this emergence was observed between roughly 3% in the treated cardboard bioassays through 14% for the muslin bag (refer to Tables 3.1 through 3.7). Results for the *T. castaneum* demonstrated the percentages of normal, live adults to be less than 1% emergence for all treated packaging material surfaces, regardless of the applied aerosol formulation. Statistical analysis of the normal, live adult morphological characteristic, demonstrated that though a statistically significant interaction was reported, upon simple effects analysis, the species main effect was determined to have a more prominent consequence, than the applied aerosol concentration main effect, in the paper bag, cardboard, plastic, flour bag, and the polyester bag treated surfaces. While the pallet wrap and the cotton bag surfaces did not report a significant interaction, both main effects were significant, which implies that the effect of either the main effects on the

normal, live adult response is independent of the other main effect (i.e., they are acting independently of one another).

Tables 3.8 (A-C) through 3.14 (A-C) account for the percentage of affected emergence, within each morphological category, for both of the main effects, applied aerosol concentration (1% pyrethrin and 3% pyrethrin) (A) and *Tribolium* species, both *T. castaneum* and *T. confusum* (B), as well as the interaction effect, applied aerosol concentration vs. *Tribolium* species (C). Both 1%- and 3% pyrethrin aerosol treatments generated distinctive percentages over a majority of the scored morphological categories ($P < 0.05$). Tables 3.8C through 3.14C corresponds to the interaction effect of aerosol concentration by *Tribolium* spp., in which each of the four treatment categories were analyzed for statistical significance. This suite of tables report a combination of statistical significance for a majority of morphological categories, within each treated surface analysis, such as normal, live adults, as well as a many categories that do not display statistical significance, across the majority of the surfaces. This suite of tables shows between three to five non-statistically significant morphological categories, for all surfaces except that of muslin bag, which demonstrate only two statistically significant categories. The high degree of significance, achieved for the many of the morphological categories, in each surface analysis, reported in Tables 3.8C through 3.14C suggests that an interpretation of both main effects, species and concentration, are needed to assess how each exposed species specifically responds to the two applied aerosol formulations. Overall, this interaction analysis suggests that the observed proportions of *Tribolium* spp. individuals within each statistically significant morphological category demonstrate a differential response upon exposure to the 1% pyrethrin treated surfaces, than when they were exposed to 3% pyrethrin treated surfaces.

Table 3.8A demonstrates a high degree of significance across each morphological category, in which nine of the ten were significant for the treated paperbag surface, which suggests a highly differential response between the two applied aerosol formulations, when exposed to *Tribolium* spp. Table 3.11A demonstrates a high degree of significance in which eight of the categories report statistical significance. Table 3.9A reports the statistical significance of the cardboard treated surface, in which seven categories demonstrated statistical significance, while Table 3.10A and Tables 3.12A through 3.14A reveal that six of the ten scored

morphological categories are reported as highly statistically significant, representing the plastic, the flour bag, the polyester bag material and the muslin bag material, respectively. These relatively high accounts of statistical significance achieved within each specific *Tribolium* stage, demonstrates the differences observed when each species was exposed to the aerosol treated surfaces, and further suggest vast differences between the two applied aerosol formulations, as well as illustrates a significant stage-specific occurrence within *Tribolium* spp. upon exposure to either the 1%- or the 3% pyrethrin concentration. These non-significant morphological categories were reported to be either normal, live adults, adults displaying either the twisted deformity, or the deformity in which their wings were not properly folded, or either of the two arrested intermediates, across the treated surfaces. These non-significant values establish that the concentration variable likely plays a minor role in determining the significance of the interaction effect of applied aerosol concentration by exposed *Tribolium* spp.

Response comparisons reported in Tables 3.8B through 3.11B reveal a completely uniform analysis of statistically significant values being achieved within each morphological category, when *T. castaneum* and *T. confusum* are compared to one another, within the paper bag, cardboard, plastic and pallet wrap surfaces, respectively. Analysis of these particular treated surfaces demonstrate that each category was reported as statistically significant. Whereas analysis of the flour bag treated surface (Table 3.12B) revealed that eight of the ten categories reported statistical significance, whereas analysis of both the polyester bag (Table 3.13B) and muslin bag (Table 3.14B) showed that nine of the categories were statistically significant. The categories that failed to demonstrate statistical significance were that of the arrested pupae-adult intermediates, live, persistent larvae, as well as live, persistent pupae. Further examination of the analysis within these tables suggest that *T. confusum* consistently reported higher percentages of individuals emerging within each adult category, normal and the three morphogenic malformations, as well as the arrested pupae-adult intermediates. However, the remaining pupae- and larvae-stage specific categories demonstrated greater percentages of *T. castaneum*. This highly uniform account of significance demonstrates the vast differences between the two species, as well as illustrates a significant stage-specific occurrence, as observed within each treated packaging material surface. A comparison of the reported mean values show the inherent developmental limitations of *T. castaneum* individuals exposed to a treated flour substrate

regardless of a.i. aerosol formulation. Comparison of the mean values within Tables 3.8 (A-C) through 3.14 (A-C) demonstrate the considerable difference in potential of each of the two exposed species, regardless of the treated surface, to overcome developmental barriers, and to emerge as normal, live adults (Table 3.8C through 3.14C). These results demonstrate that less susceptible *T. confusum* individuals are better able to overcome the juvenilizing effects of the applied aerosol formulation and emerge as normal, presumably reproduce successive generations in the flour mill setting.

The suite of graphs presented in Fig. 3.2 through Fig. 3.8 (A-B) and (C-D) report the mean \pm confidence limits (asymmetrical) of the proportion of ‘‘affected’’ *Tribolium* spp. individuals, corresponding to each of the four treatment categories, at each biweekly post-aerosol application storage interval. These graphs show that regardless of the treated surface and the applied aerosol formulation, *T. castaneum* individuals were largely unable to complete development and emerge as normal, live adults (Fig. 3.2A through Fig. 3.8A). The percentage of *T. castaneum* individuals able to emerge as normal live adults was $>2\%$, regardless of applied formulation. As such, the *T. castaneum* were consistently shown to be the more susceptible of the two exposed *Tribolium* species. *T. confusum* exposure to both the 1- and the 3% pyrethrin + methoprene treated surfaces resulted in a very different pattern of adult emergence. Exposure to the 3% pyrethrin demonstrated a gradually decreasing percentage of individuals characterized as ‘affected’, or a increasing percentage of individuals characterized as normal, live adults, throughout the 16-week storage experiment, for all treated surfaces. This emergence was observed to be between 7% (plastic) and 21% (polyester bag and muslin bag). However, there is a much less discernable trend observed when *T. confusum* are exposed to the 1% pyrethrin treated surfaces. Upon exposure to the 1% pyrethrin normal, live adult emergence ranged between 43% (muslin bag) and 77% (paper bag). Bioassay results for each exposed surface demonstrated that upon exposure to the 1% pyrethrin treatment, *T. confusum* experienced more breakdown of residual effectiveness over time, than did the *T. castaneum* (Fig. 3.2C through Fig. 3.8C). A similar result is observed upon exposure to 3% pyrethrin, to a lesser degree (Fig. 3.2D through Fig. 3.8D).

Analysis of the untreated, control observation arenas, revealed that normal, live adult emergence at each species was on average, roughly 90-100%. No individuals emerged that displayed morphological deformity, and the 'affected' emergence that was observed within these arenas was that of dead larvae and dead pupae. Statistical analysis revealed that no significant differences were reported within the species-specific proportions of normal, adult emergences, within each aerosol treatment. Within each treated surface, a significant species-specific difference was observed, upon exposure of the two *Tribolium* species to each applied concentration rate. Due to the lack of statistical differences between the treatments, the data for the untreated controls were eliminated from any further statistical analysis.

4. Discussion

Results of this study show the potential for using a pyrethrin plus a methoprene aerosol insecticide to control two particular species of stored product insect pests in an active milling facility. Results of this study show a clear distinction between the developmental ability of both exposed *Tribolium* species, at both of the applied aerosol concentrations, on each exposed packaging material. Aerosol insecticides have been adopted in response to the growing level of government and consumer concern over insecticide residues in food, these regulations have led to reduced availability of traditional chemical insecticides, placing increased importance on the continued development of alternative IPM-based methods (Arthur and Peckman, 2006). IGRs do not kill adult insects, but help eliminate infestations by inhibiting development of immature insect stages, which ultimately reduces subsequent adult emergence (Arthur, 2003). JHAs are most effective at the beginning stages of metamorphosis in insects (i.e., freshly ecdysed last larval and pupae instars and newly deposited eggs), where embryogenesis is easily disrupted. Compounds developed to disrupt metamorphosis ensure that very few, if any reproductive adults are formed. Those that specifically interfere with reproduction may promote the development of adults with certain morphogenetic abnormalities that reduce their reproductive potential. These adults are often sterile or possess abnormally developed genitalia, which may ultimately hinder the mating process, as well as their capacity to produce fertile offspring (Tunaz and Uygin, 2004). When utilized with a synergized pyrethrin, this aerosol mixture proves to be an effective management tool that can be used to target stored product insects.

These results support previous findings that the more susceptible species is more likely to achieve incomplete development when exposed to an IGR treated substrate (Arthur, 2008). Each packaging material bioassay demonstrates that the *T. confusum* are less susceptible to the effects of the applied aerosol application, at either concentrations, whereas the *T. castaneum* are the most susceptible to the same exposure. Regardless of the applied aerosol formulation, the normal, adult emergence in *T. castaneum* was extremely low (about 1%) in any of the treated packaging material surfaces, clearly demonstrating the inherent susceptibility of *T. castaneum*. However, analysis showed that upon exposure of *T. confusum* to the 1% pyrethrin + methoprene aerosol treated bioassays, analysis consistently demonstrated greater occurrences of normal, live adults, than in the 3% pyrethrin + methoprene treated bioassays. A bi-species analysis, in which the two aerosol formulations were compared, consistently resulted in the *T. confusum* being the prevalent species detected in each of the seven treated packaging material surface bioassays. These results indicated that *T. confusum* was less susceptible; these individuals were better able to overcome the juvenilizing effects of the applied aerosol formulation and to complete development thus emerging as normal, presumably reproductively active adults.

Analysis of the packaging material bioassay data indicated a varied response between the two exposed *Tribolium* species, in regards to how both are influenced by the aerosol formulations (i.e., developmental rates, proportions of normal adults, proportion of individuals displaying morphogenic abnormalities, etc.), as well as how the two aerosol formulations differentially affect the exposed *Tribolium* species. The 1% pyrethrin + methoprene aerosol treatment was shown to provide superior control against *T. castaneum*, almost fully suppressing the development and eventual emerge of adults, on the treated packaging material substrates. The 3% pyrethrin aerosol treatment also provided adequate control against *T. castaneum* larvae, largely preventing their development and establishment as adults. At a time when mill and facility managers are combating large overhead costs, these results suggesting that the lower a.i. formulation, as well as being the less expensive alternative can adequately control *T. castaneum* pest populations lowers bottom-line costs, making production, transportation and storage more effective.

A comparison of the seven packaging material surfaces, when averaged across all post-aerosol application treatment storage weeks, shows that the treated plastic and muslin bag surfaces provide the highest efficacy against development and subsequent emergence (approximately 30%) of normal, live adults in *T. confusum* exposed to 1% pyrethrin + methoprene aerosol insecticide. The treated polyester bag, pallet wrap and cardboard surfaces permit roughly 35% emergence, followed by the flour bag surface with roughly 45% emergence and lastly, the paper bag surface which permits nearly 50% emergence. The effects of the 3% pyrethrin + methoprene treated surfaces showed a somewhat different pattern of adult emergence. The plastic surface permits only 7% normal adult emergence followed by the treated cardboard and paper bag surfaces which permit 10% and 12%, respectively. The flour bag and pallet wrap treated surfaces allow for only 16% and 17% normal, live adult emergence, respectively. And lastly, the polyester bag and muslin bag surfaces both permit roughly 21% emergence. Results from these increased active ingredient aerosol formulation treated bioassays do not show significant statistical differences among the various treated packaging material surfaces, likely indicating that any observed differences are due to biological variation. However, these results did show that within the 1% pyrethrin treated bioassays, there is a more pronounced variations among the treated surfaces. This observed inconsistency of trends between the two applied aerosol formulations suggests that active ingredients of the aerosol insecticide may have an effect on the efficacy of the insecticide, to a degree, likely having its greatest impact on the absorption of the insecticide into the treated surface. Overall, the results of this study demonstrate improved efficacy on *Tribolium* spp. upon exposure to the 3% pyrethrin + methoprene aerosol formulation. Because the methoprene component was equally applied in both aerosol treatments, this increased efficacy is likely due to an increased concentration of pyrethrin.

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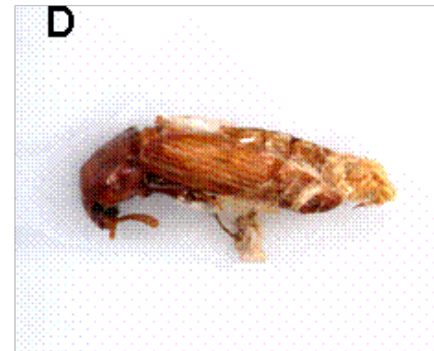
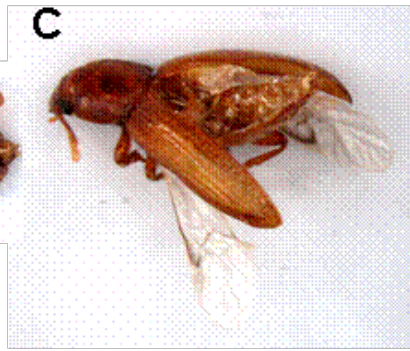
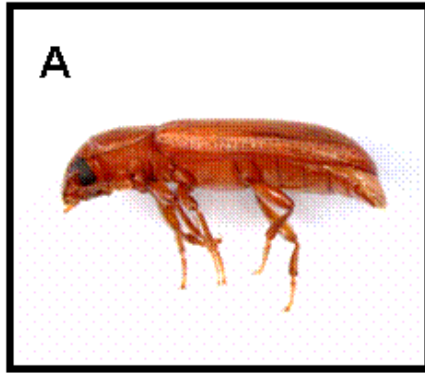
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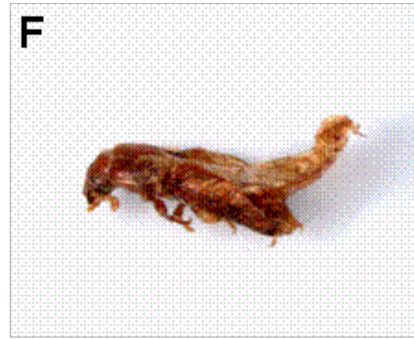
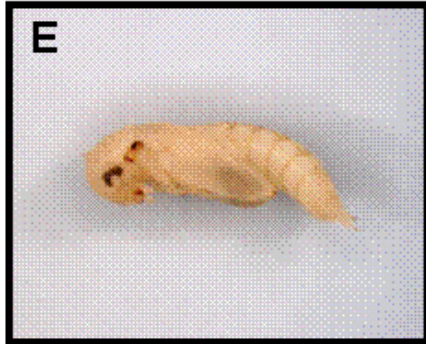
Figures and Tables

Figure 3.1. *Tribolium* spp. morphological deformities characteristic of Diacon II™. (A) Normal, live adults (i.e., fully eclosed adults), (B) Live adults displaying a twisted wing deformity, (C) Live adults with a deformity in which their wings were unable to fold, (D) Live adults unable to shed their pupae cuticle (i.e., incompletely formed elytra), (E) Live pupae (F) Arrested pupa-adult intermediates (i.e., secondary pupae), (G) Live larvae and (H) Arrested larvae-pupae intermediates

Adults:



Pupae:



Larvae:

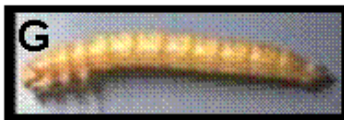


Table 3.1. Comparison of mean \pm standard error (S.E.) of the scored morphological categories corresponding to each individual treatment category, when the aerosol formulations are applied to a paper bag surface. *T. castaneum* and *T. confusum* exposed to both of the applied Entech Fog-30[®] + Methoprene and 1% pyrethrin aerosol formulations.

Paper Bag	<i>T. castaneum</i> Entech Fog-10 [®] + Diacon II [™]	<i>T. castaneum</i> Entech Fog-30 [®] + Diacon II [™]	<i>T. confusum</i> Entech Fog-10 [®] + Diacon II [™]	<i>T. confusum</i> Entech Fog-30 [®] + Diacon II [™]
Normal, Live Adults	0.09 \pm 0.001	0.28 \pm 0.002	48.98 \pm 0.07	5.19 \pm 0.01
Adults w/ Twisted Wing Deformity	0.28 \pm 0.002	0.00 \pm 0.00	3.33 \pm 0.01	1.02 \pm 0.002
Adults Unable to Fold Wings	1.02 \pm 0.01	0.09 \pm 0.001	5.37 \pm 0.01	2.50 \pm 0.01
Adults Unable to Shed Pupal Cuticle	1.67 \pm 0.01	0.28 \pm 0.001	3.80 \pm 0.01	3.43 \pm 0.01
Arrested Pupae-Adult Inermediates	39.91 \pm 0.04	18.33 \pm 0.02	20.65 \pm 0.03	25.93 \pm 0.01
Arrested Larvae-Pupae Inermediates	1.67 \pm 0.002	2.69 \pm 0.003	0.28 \pm 0.001	0.83 \pm 0.002
Dead Larvae	19.72 \pm 0.03	26.76 \pm 0.03	6.94 \pm 0.01	19.81 \pm 0.01
Dead Pupae	23.06 \pm 0.02	24.63 \pm 0.01	7.96 \pm 0.02	25.56 \pm 0.02
Live Larvae	7.69 \pm 0.02	20.37 \pm 0.02	1.76 \pm 0.01	8.89 \pm 0.02
Live Pupae	4.91 \pm 0.01	6.57 \pm 0.005	0.93 \pm 0.005	6.85 \pm 0.001

Table 3.2. Comparison of mean \pm standard error (S.E.) of the scored morphological categories corresponding to each individual treatment category, when the aerosol formulations are applied to a cardboard surface. *T. castaneum* and *T. confusum* exposed to both of the applied Entech Fog-30[®] + Methoprene and 1% pyrethrin aerosol formulations.

Cardboard	<i>T. castaneum</i> Entech Fog-10 [®] + Diacon II [™]	<i>T. castaneum</i> Entech Fog-30 [®] + Diacon II [™]	<i>T. confusum</i> Entech Fog-10 [®] + Diacon II [™]	<i>T. confusum</i> Entech Fog-30 [®] + Diacon II [™]
Normal, Live Adults	0.00 \pm 0.00	0.28 \pm 0.001	35.28 \pm 0.03	2.87 \pm 0.004
Adults w/ Twisted Wing Deformity	0.00 \pm 0.00	0.09 \pm 0.001	2.78 \pm 0.01	0.65 \pm 0.003
Adults Unable to Fold Wings	0.09 \pm 0.001	0.19 \pm 0.002	5.74 \pm 0.005	2.31 \pm 0.01
Adults Unable to Shed Pupal Cuticle	0.74 \pm 0.005	0.02 \pm 0.001	6.57 \pm 0.01	1.76 \pm 0.004
Arrested Pupae-Adult Inermediates	32.69 \pm 0.05	13.06 \pm 0.02	25.93 \pm 0.03	33.70 \pm 0.01
Arrested Larvae-Pupae Inermediates	2.50 \pm 0.003	1.67 \pm 0.004	0.00 \pm 0.0000	0.46 \pm 0.002
Dead Larvae	28.89 \pm 0.06	25.74 \pm 0.02	8.15 \pm 0.01	17.50 \pm 0.02
Dead Pupae	26.30 \pm 0.02	22.50 \pm 0.02	10.93 \pm 0.02	27.50 \pm 0.02
Live Larvae	6.11 \pm 0.01	30.65 \pm 0.04	3.06 \pm 0.01	9.54 \pm 0.01
Live Pupae	2.69 \pm 0.01	5.48 \pm 0.00	1.57 \pm 0.01	3.70 \pm 0.01

Table 3.3. Comparison of mean \pm standard error (S.E.) of the scored morphological categories corresponding to each individual treatment category, when the aerosol formulations are applied to a plastic surface. *T. castaneum* and *T. confusum* exposed to both of the applied Entech Fog-30[®] + Methoprene and 1% pyrethrin aerosol formulations.

Plastic	<i>T. castaneum</i> Entech Fog-10 [®] + Diacon II [™]	<i>T. castaneum</i> Entech Fog-30 [®] + Diacon II [™]	<i>T. confusum</i> Entech Fog-10 [®] + Diacon II [™]	<i>T. confusum</i> Entech Fog-30 [®] + Diacon II [™]
Normal, Live Adults	0.09 \pm 0.001	0.19 \pm 0.002	29.72 \pm 0.03	3.24 \pm 0.01
Adults w/ Twisted Wing Deformity	0.00 \pm 0.00	0.00 \pm 0.00	3.15 \pm 0.01	0.37 \pm 0.002
Adults Unable to Fold Wings	0.19 \pm 0.001	0.19 \pm 0.001	6.76 \pm 0.01	1.67 \pm 0.01
Adults Unable to Shed Pupal Cuticle	0.74 \pm 0.002	0.09 \pm 0.001	7.59 \pm 0.01	1.94 \pm 0.003
Arrested Pupae-Adult Intermediates	28.98 \pm 0.04	12.22 \pm 0.03	26.67 \pm 0.03	23.70 \pm 0.02
Arrested Larvae-Pupae Intermediates	2.69 \pm 0.005	2.31 \pm 0.003	0.28 \pm 0.002	0.28 \pm 0.002
Dead Larvae	30.37 \pm 0.02	33.70 \pm 0.02	10.00 \pm 0.02	23.43 \pm 0.02
Dead Pupae	25.00 \pm 0.02	21.67 \pm 0.01	11.76 \pm 0.02	28.80 \pm 0.03
Live Larvae	9.35 \pm 0.02	24.07 \pm 0.03	2.78 \pm 0.01	12.13 \pm 0.01
Live Pupae	2.59 \pm 0.01	5.56 \pm 0.01	1.30 \pm 0.002	4.44 \pm 0.005

Table 3.4. Comparison of mean \pm standard error (S.E.) of the scored morphological categories corresponding to each individual treatment category, when the aerosol formulations are applied to a pallet wrap surface. *T. castaneum* and *T. confusum* exposed to both of the applied Entech Fog-30[®] + Methoprene and 1% pyrethrin aerosol formulations.

Pallet Wrap	<i>T. castaneum</i> Entech Fog-10 [®] + Diacon II [™]	<i>T. castaneum</i> Entech Fog-30 [®] + Diacon II [™]	<i>T. confusum</i> Entech Fog-10 [®] + Diacon II [™]	<i>T. confusum</i> Entech Fog-30 [®] + Diacon II [™]
Normal, Live Adults	0.56 \pm 0.002	0.19 \pm 0.001	34.44 \pm 0.03	7.31 \pm 0.01
Adults w/ Twisted Wing Deformity	0.28 \pm 0.002	0.00 \pm 0.00	2.69 \pm 0.01	1.57 \pm 0.002
Adults Unable to Fold Wings	1.02 \pm 0.004	0.09 \pm 0.001	7.31 \pm 0.01	4.72 \pm 0.004
Adults Unable to Shed Pupal Cuticle	2.13 \pm 0.01	0.56 \pm 0.002	6.57 \pm 0.002	2.13 \pm 0.004
Arrested Pupae-Adult Intermediates	34.91 \pm 0.05	15.37 \pm 0.02	23.15 \pm 0.03	42.96 \pm 0.04
Arrested Larvae-Pupae Intermediates	2.59 \pm 0.003	2.13 \pm 0.004	0.65 \pm 0.004	0.83 \pm 0.01
Dead Larvae	25.93 \pm 0.05	27.87 \pm 0.03	8.98 \pm 0.02	14.44 \pm 0.01
Dead Pupae	24.35 \pm 0.01	24.35 \pm 0.02	10.19 \pm 0.02	16.48 \pm 0.02
Live Larvae	4.81 \pm 0.02	20.46 \pm 0.04	4.17 \pm 0.03	7.41 \pm 0.01
Live Pupae	3.43 \pm 0.01	8.98 \pm 0.01	1.85 \pm 0.004	2.13 \pm 0.01

Table 3.5. Comparison of mean \pm standard error (S.E.) of the scored morphological categories corresponding to each individual treatment category, when the aerosol formulations are applied to a flour bag surface. *T. castaneum* and *T. confusum* exposed to both of the applied Entech Fog-30[®] + Methoprene and 1% pyrethrin aerosol formulations.

Flour Bag	<i>T. castaneum</i> Entech Fog-10 [®] + Diacon II [™]	<i>T. castaneum</i> Entech Fog-30 [®] + Diacon II [™]	<i>T. confusum</i> Entech Fog-10 [®] + Diacon II [™]	<i>T. confusum</i> Entech Fog-30 [®] + Diacon II [™]
Normal, Live Adults	0.00 \pm 0.00	0.19 \pm 0.001	43.52 \pm 0.04	6.57 \pm 0.01
Adults w/ Twisted Wing Deformity	0.00 \pm 0.00	0.00 \pm 0.00	3.61 \pm 0.01	1.48 \pm 0.004
Adults Unable to Fold Wings	0.46 \pm 0.004	0.65 \pm 0.003	5.65 \pm 0.01	2.50 \pm 0.004
Adults Unable to Shed Pupal Cuticle	2.50 \pm 0.01	0.46 \pm 0.002	4.32 \pm 0.01	2.31 \pm 0.01
Arrested Pupae-Adult Inermediates	33.70 \pm 0.05	19.91 \pm 0.02	18.43 \pm 0.01	30.00 \pm 0.01
Arrested Larvae-Pupae Inermediates	1.20 \pm 0.005	1.20 \pm 0.004	0.65 \pm 0.002	0.09 \pm 0.001
Dead Larvae	27.69 \pm 0.05	29.26 \pm 0.04	9.91 \pm 0.02	19.91 \pm 0.02
Dead Pupae	26.57 \pm 0.01	28.52 \pm 0.02	8.61 \pm 0.01	20.46 \pm 0.01
Live Larvae	4.63 \pm 0.01	12.22 \pm 0.03	3.89 \pm 0.01	12.31 \pm 0.01
Live Pupae	3.15 \pm 0.01	7.59 \pm 0.01	1.39 \pm 0.01	4.35 \pm 0.01

Table 3.6. Comparison of mean \pm standard error (S.E.) of the scored morphological categories corresponding to each individual treatment category, when the aerosol formulations are applied to a polyester bag surface. *T. castaneum* and *T. confusum* exposed to both of the applied Entech Fog-30[®] + Methoprene and 1% pyrethrin aerosol formulations.

Polyester Bag	<i>T. castaneum</i> Entech Fog-10 [®] + Diacon II TM	<i>T. castaneum</i> Entech Fog-30 [®] + Diacon II TM	<i>T. confusum</i> Entech Fog-10 [®] + Diacon II TM	<i>T. confusum</i> Entech Fog-30 [®] + Diacon II TM
Normal, Live Adults	0.00 \pm 0.00	0.19 \pm 0.002	34.17 \pm 0.03	7.13 \pm 0.01
Adults w/ Twisted Wing Deformity	0.19 \pm 0.001	0.09 \pm 0.001	2.59 \pm 0.004	0.83 \pm 0.002
Adults Unable to Fold Wings	0.56 \pm 0.001	0.37 \pm 0.002	4.26 \pm 0.01	2.69 \pm 0.00
Adults Unable to Shed Pupal Cuticle	1.57 \pm 0.01	0.04 \pm 0.002	3.07 \pm 0.01	2.13 \pm 0.01
Arrested Pupae-Adult Inermediates	28.98 \pm 0.03	12.59 \pm 0.02	21.02 \pm 0.02	26.94 \pm 0.02
Arrested Larvae-Pupae Inermediates	1.30 \pm 0.002	2.13 \pm 0.003	0.65 \pm 0.002	0.19 \pm 0.001
Dead Larvae	38.70 \pm 0.03	37.22 \pm 0.04	13.24 \pm 0.03	24.35 \pm 0.01
Dead Pupae	23.33 \pm 0.07	25.74 \pm 0.03	13.61 \pm 0.01	23.52 \pm 0.01
Live Larvae	2.69 \pm 0.004	14.72 \pm 0.01	4.72 \pm 0.02	7.87 \pm 0.003
Live Pupae	2.69 \pm 0.01	6.57 \pm 0.01	2.04 \pm 0.01	4.17 \pm 0.01

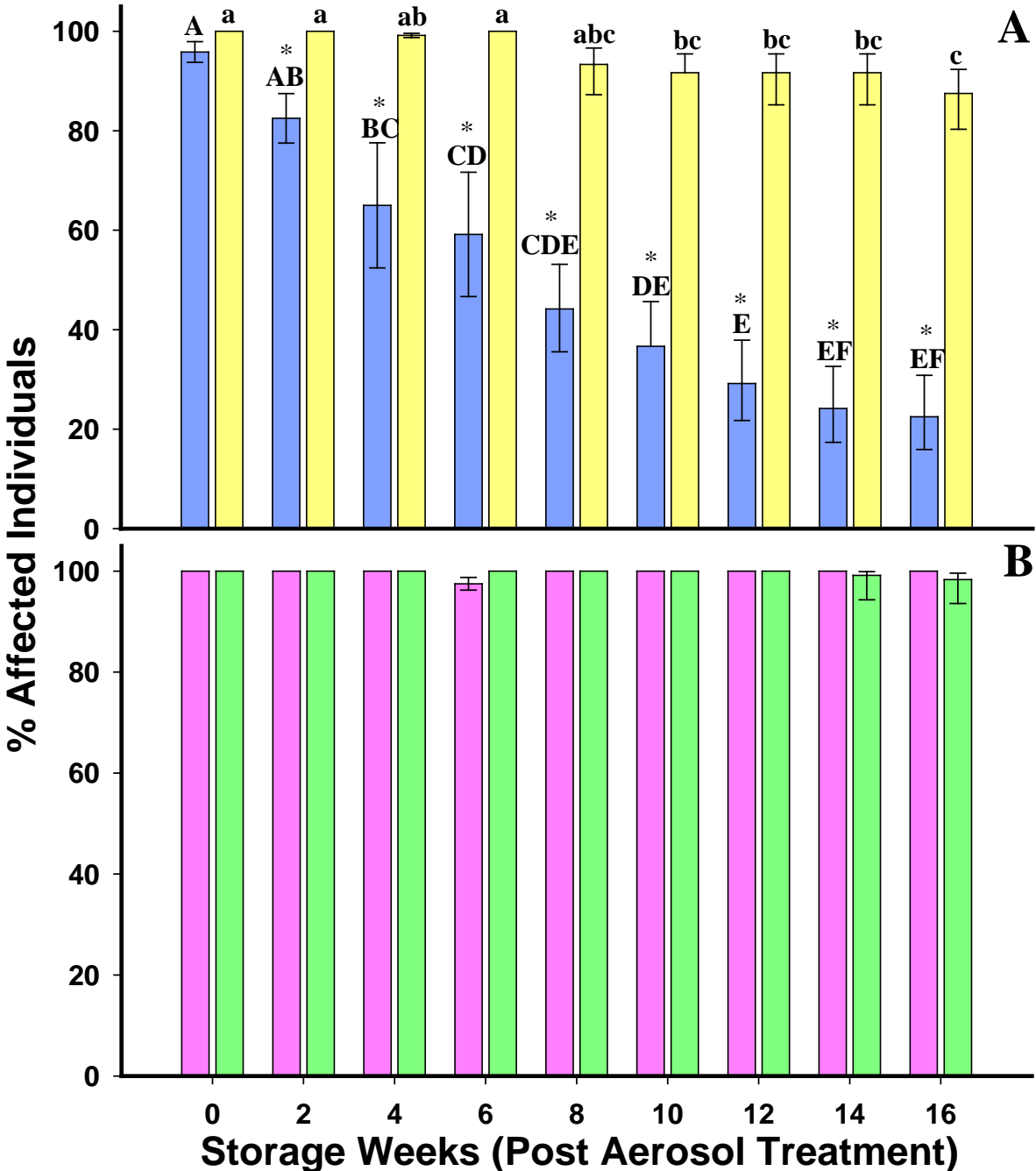
Table 3.7. Comparison of mean \pm standard error (S.E.) of the scored morphological categories corresponding to each individual treatment category, when the aerosol formulations are applied to a cotton bag surface. *T. castaneum* and *T. confusum* exposed to both of the applied Entech Fog-30[®] + Methoprene and 1% pyrethrin aerosol formulations.

Cotton Bag	<i>T. castaneum</i> Entech Fog-10 [®] + Diacon II [™]	<i>T. castaneum</i> Entech Fog-30 [®] + Diacon II [™]	<i>T. confusum</i> Entech Fog-10 [®] + Diacon II [™]	<i>T. confusum</i> Entech Fog-30 [®] + Diacon II [™]
Normal, Live Adults	0.19 \pm 0.002	0.00 \pm 0.000	31.30 \pm 0.03	13.98 \pm 0.02
Adults w/ Twisted Wing Deformity	0.01 \pm 0.001	0.09 \pm 0.001	2.04 \pm 0.01	1.76 \pm 0.01
Adults Unable to Fold Wings	0.19 \pm 0.002	0.00 \pm 0.004	3.33 \pm 0.002	1.94 \pm 0.005
Adults Unable to Shed Pupal Cuticle	0.74 \pm 0.003	0.09 \pm 0.001	4.81 \pm 0.01	1.85 \pm 0.01
Arrested Pupae-Adult Intermediates	21.76 \pm 0.03	20.74 \pm 0.04	28.61 \pm 0.03	28.89 \pm 0.01
Arrested Larvae-Pupae Intermediates	2.78 \pm 0.002	1.48 \pm 0.003	0.28 \pm 0.002	0.28 \pm 0.002
Dead Larvae	35.83 \pm 0.04	32.69 \pm 0.04	11.76 \pm 0.01	19.35 \pm 0.02
Dead Pupae	24.91 \pm 0.02	23.43 \pm 0.01	10.65 \pm 0.02	20.46 \pm 0.02
Live Larvae	11.02 \pm 0.04	15.28 \pm 0.03	4.81 \pm 0.01	7.41 \pm 0.02
Live Pupae	2.50 \pm 0.01	5.83 \pm 0.005	2.41 \pm 0.004	4.07 \pm 0.01

Figure 3.2 (A-B). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the paper bag treated surface, within each biweekly storage period. Resultant graph depicts how the concentration of the applied aerosol formulation consequently affects development within both exposed *Tribolium* spp.

The proportion of (A) *T. castaneum* and (B) *T. confusum* adversely affected following a 30-day exposure with Entech Fog-30 ® + Methoprene or 1% pyrethrin , applied directly to the wheat flour substrate. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

PaperBag



■ *T. confusum* (Entech Fog-10[®] + Diacon II[™])
 ■ *T. castaneum* (Entech Fog-10[®] + Diacon II[™])
■ *T. confusum* (Entech Fog-30[®] + Diacon II[™])
 ■ *T. castaneum* (Entech Fog-30[®] + Diacon II[™])

Figure 3.2 (C-D). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the paper bag treated surface, at both the 1% pyrethrin and the 3% pyrethrin aerosol formulations, within each biweekly storage period. Resultant graph depicts the species-specific response, at both applied aerosol concentrations. Comparison of means for differences in the percentage of *T. castaneum* and *T. confusum* adversely affected following a 30 day exposure with 3% pyrethrin or with 1% pyrethrin (A and B, respectively) applied directly to wheat flour. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

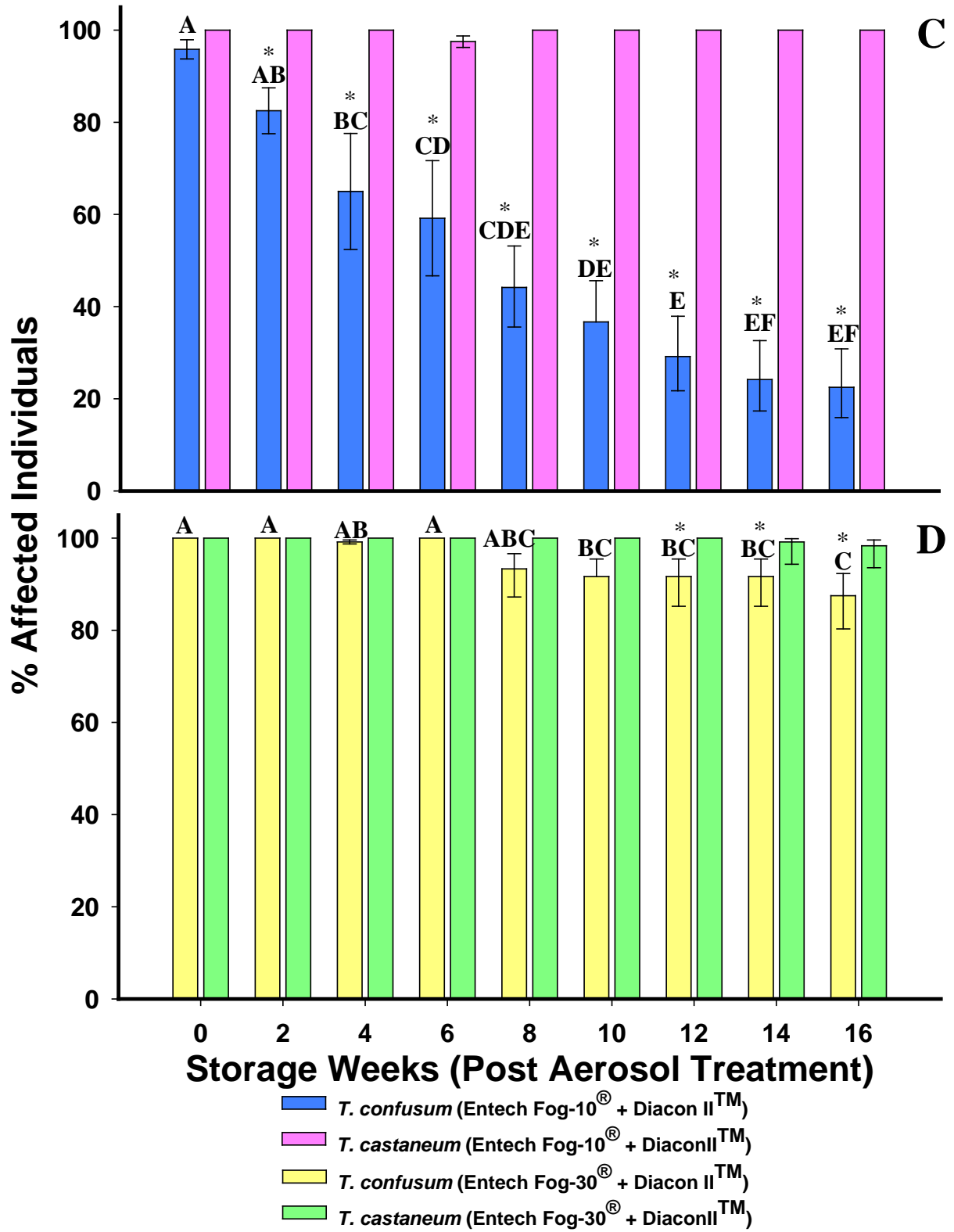


Figure 3.3 (A-B). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the cardboard treated surface, within each biweekly storage period. Resultant graph depicts how the concentration of the applied aerosol formulation consequently affects development within both exposed *Tribolium* spp. The proportion of (A) *T. castaneum* and (B) *T. confusum* adversely affected following a 30-day exposure with Entech Fog-30 ® + Methoprene or 1% pyrethrin , applied directly to the wheat flour substrate. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

Cardboard

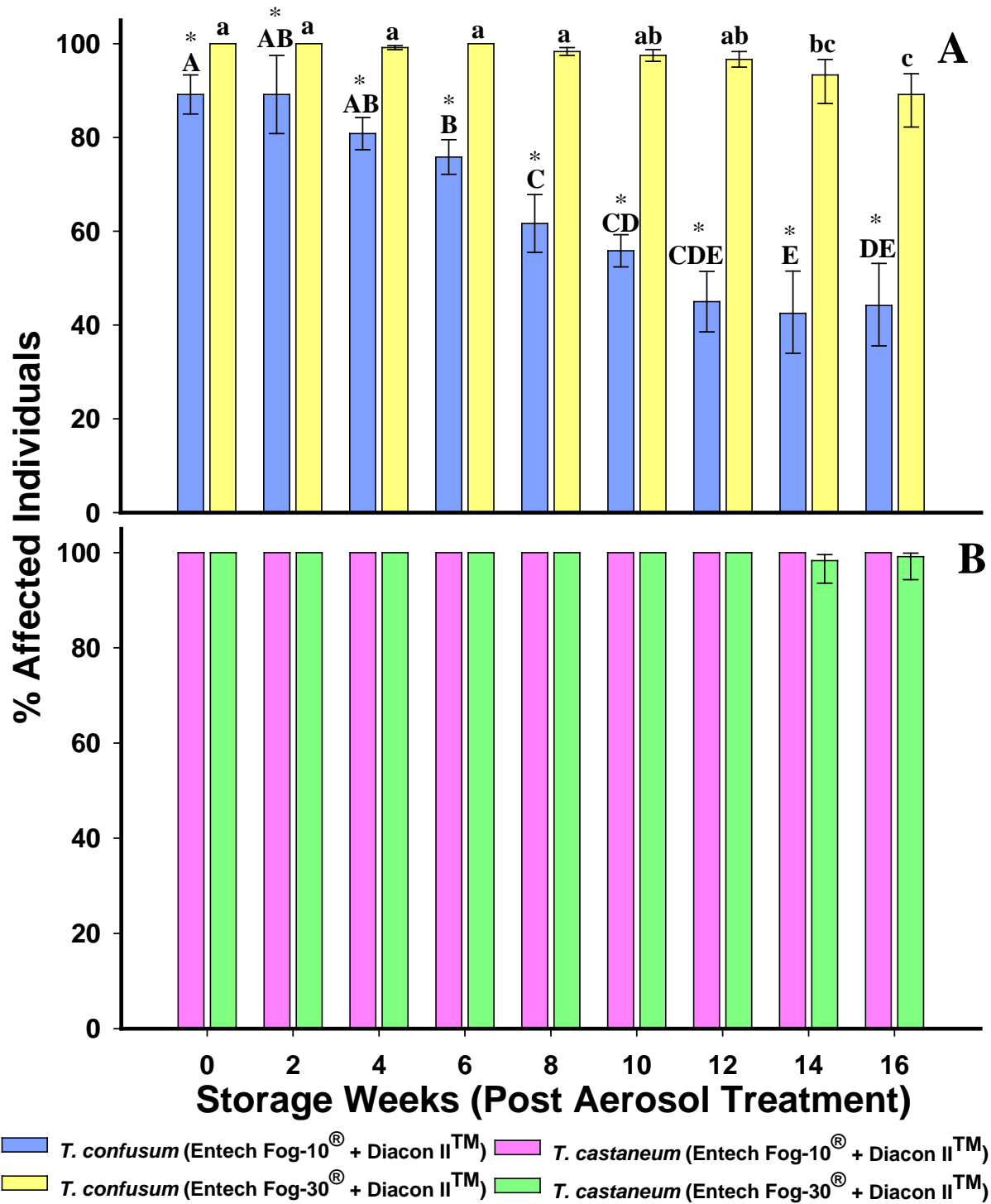


Figure 3.3 (C-D). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the cardboard treated surface, at both the 1% pyrethrin and the 3% pyrethrin aerosol formulations, within each biweekly storage period. Resultant graph depicts the species-specific response, at both applied aerosol concentrations. Comparison of means for differences in the percentage of *T. castaneum* and *T. confusum* adversely affected following a 30 day exposure with 3% pyrethrin or with 1% pyrethrin (A and B, respectively) applied directly to wheat flour. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

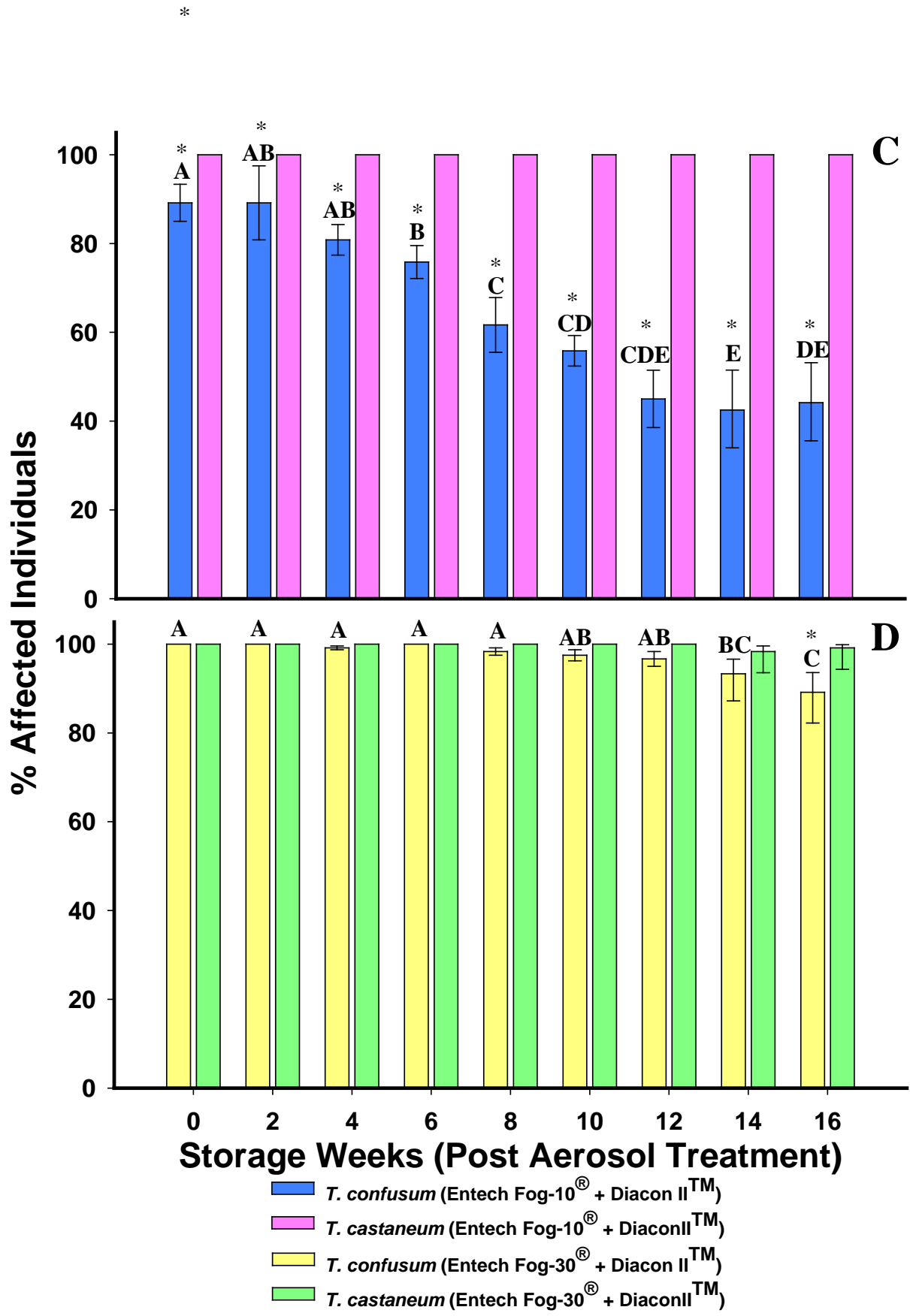


Figure 3.4 (A-B). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the plastic treated surface, within each biweekly storage period. Resultant graph depicts how the concentration of the applied aerosol formulation consequently affects development within both exposed *Tribolium* spp. The proportion of (A) *T. castaneum* and (B) *T. confusum* adversely affected following a 30-day exposure with Entech Fog-30 ® + Methoprene or 1% pyrethrin , applied directly to the wheat flour substrate. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

Plastic

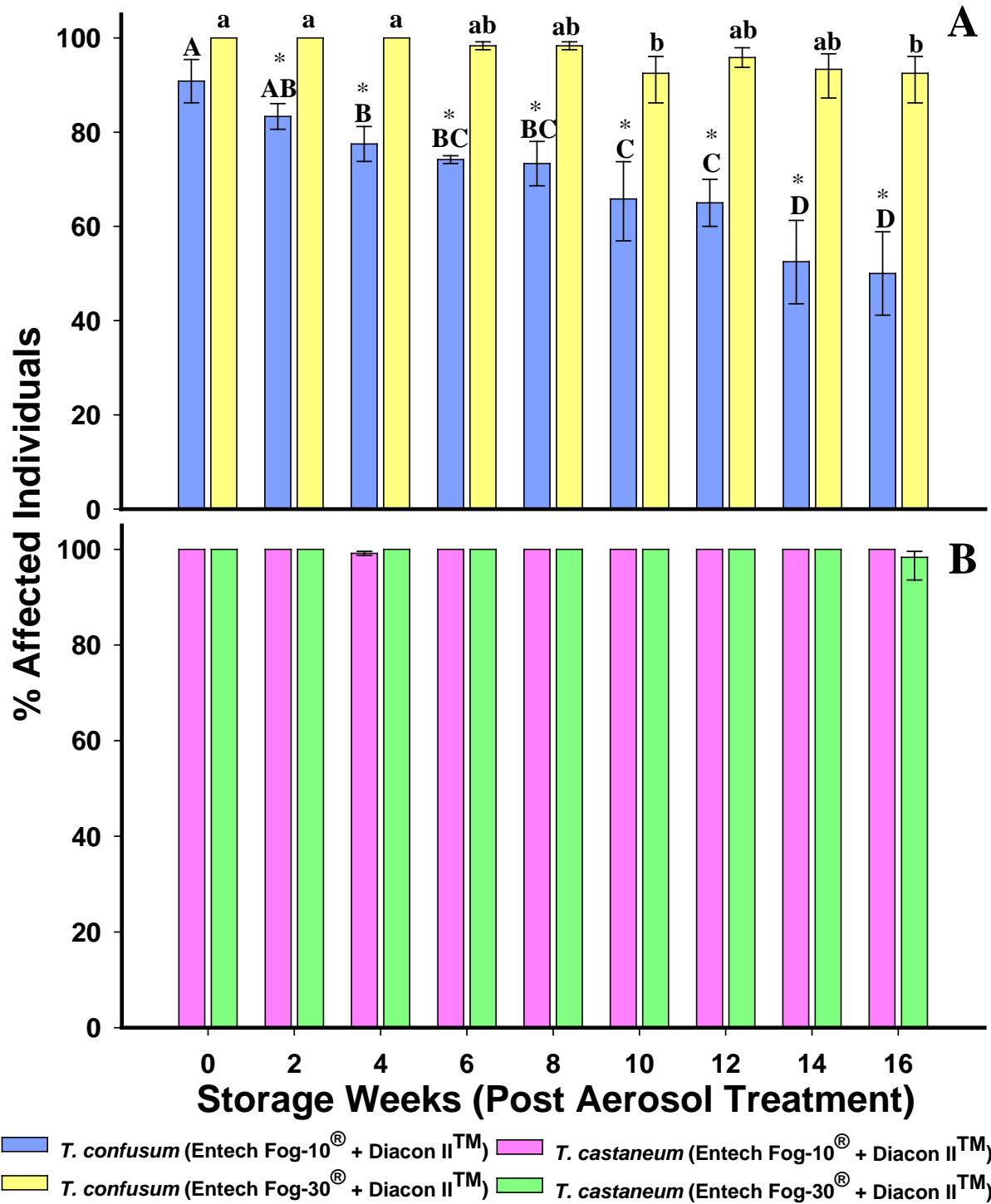


Figure 3.4 (C-D). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the plastic treated surface, at both the 1% pyrethrin and the 3% pyrethrin aerosol formulations, within each biweekly storage period. Resultant graph depicts the species-specific response, at both applied aerosol concentrations. Comparison of means for differences in the percentage of *T. castaneum* and *T. confusum* adversely affected following a 30 day exposure with 3% pyrethrin or with 1% pyrethrin (A and B, respectively) applied directly to wheat flour. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

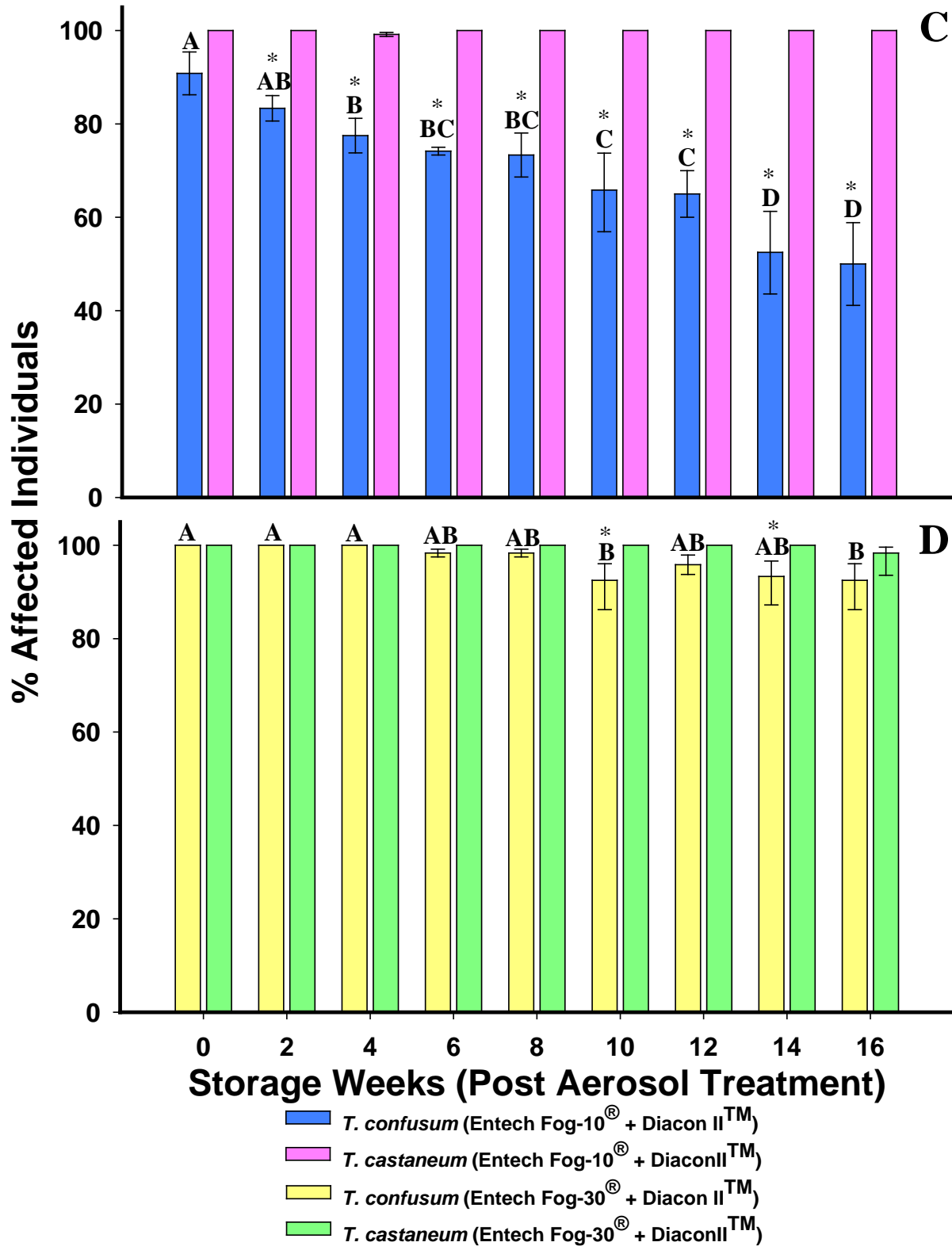


Figure 3.5 (A-B). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the pallet wrap treated surface, within each biweekly storage period. Resultant graph depicts how the concentration of the applied aerosol formulation consequently affects development within both exposed *Tribolium* spp. The proportion of (A) *T. castaneum* and (B) *T. confusum* adversely affected following a 30-day exposure with Entech Fog-30 ® + Methoprene or 1% pyrethrin , applied directly to the wheat flour substrate. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

Pallet Wrap

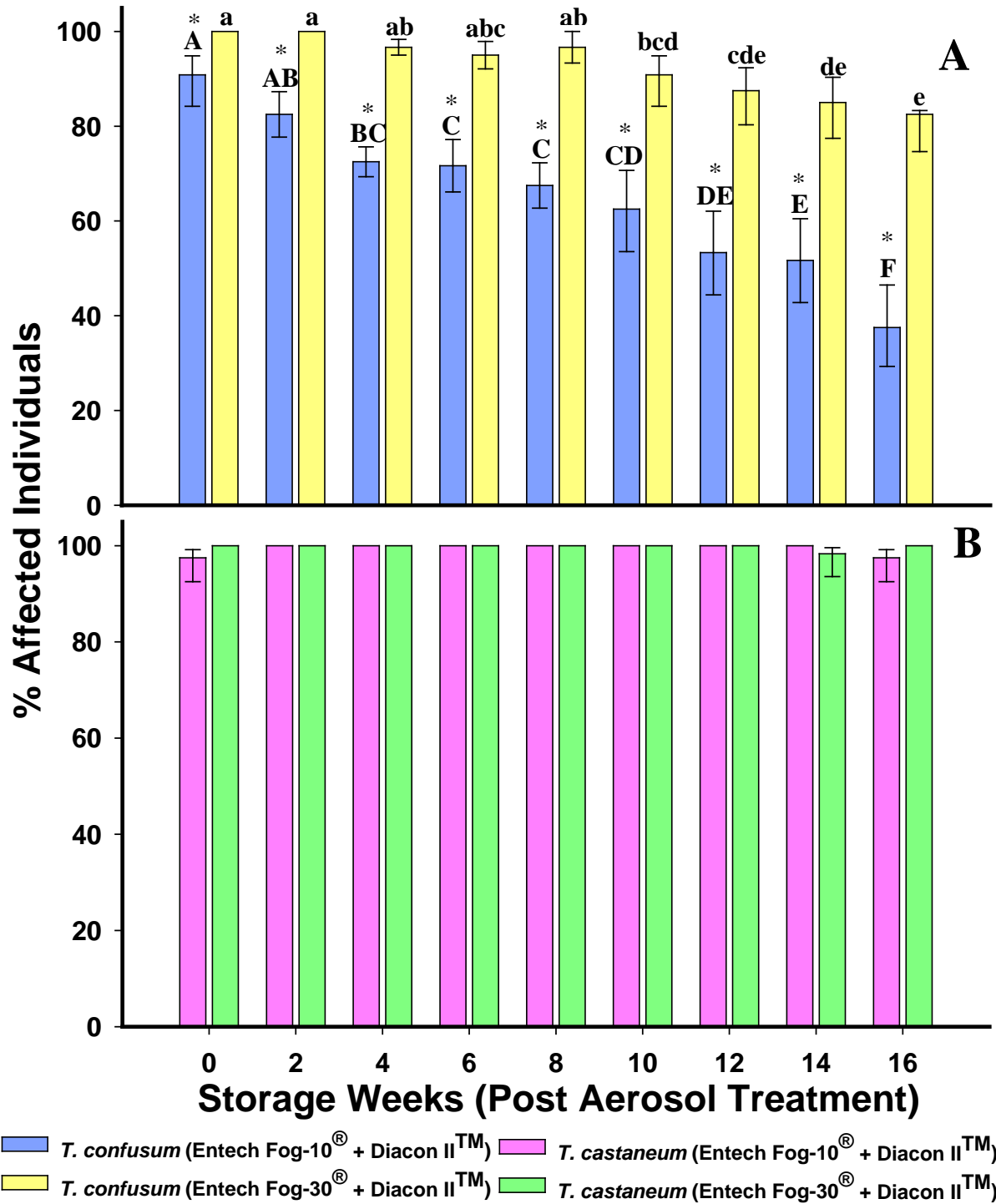


Figure 3.5 (C-D). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the pallet wrap treated surface, at both the 1% pyrethrin and the 3% pyrethrin aerosol formulations, within each biweekly storage period. Resultant graph depicts the species-specific response, at both applied aerosol concentrations. Comparison of means for differences in the percentage of *T. castaneum* and *T. confusum* adversely affected following a 30 day exposure with 3% pyrethrin or with 1% pyrethrin (A and B, respectively) applied directly to wheat flour. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

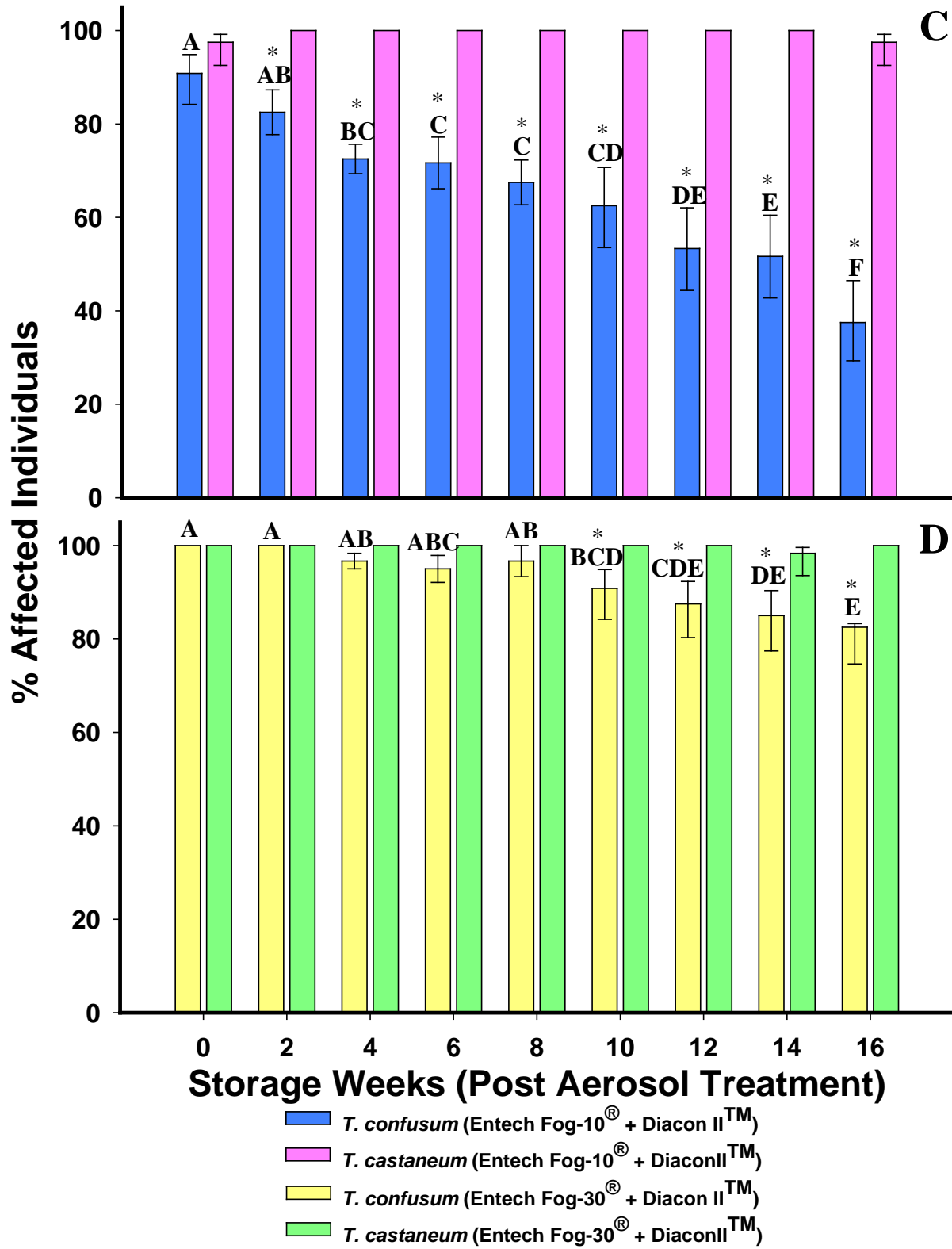
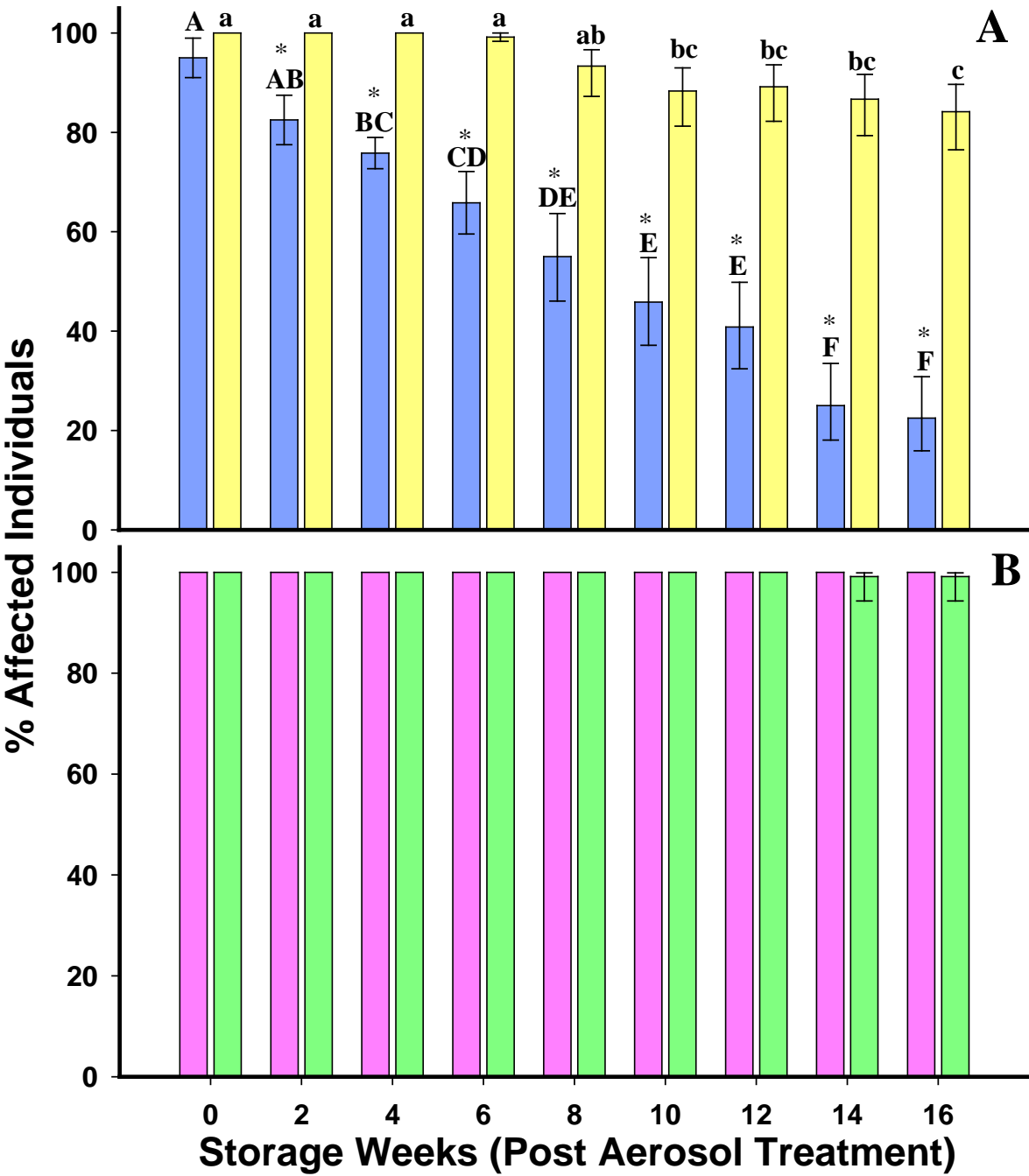


Figure 3.6 (A-B). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the commercial flour bag treated surface, within each biweekly storage period. Resultant graph depicts how the concentration of the applied aerosol formulation consequently affects development within both exposed *Tribolium* spp. The proportion of (A) *T. castaneum* and (B) *T. confusum* adversely affected following a 30-day exposure with Entech Fog-30 ® + Methoprene or 1% pyrethrin , applied directly to the wheat flour substrate. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

Flour Bag



■ *T. confusum* (Entech Fog-10[®] + Diacon II[™])
 ■ *T. castaneum* (Entech Fog-10[®] + Diacon II[™])
■ *T. confusum* (Entech Fog-30[®] + Diacon II[™])
 ■ *T. castaneum* (Entech Fog-30[®] + Diacon II[™])

Figure 3.6 (C-D). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the commercial flour bag treated surface, at both the 1% pyrethrin and the 3% pyrethrin aerosol formulations, within each biweekly storage period. Resultant graph depicts the species-specific response, at both applied aerosol concentrations. Comparison of means for differences in the percentage of *T. castaneum* and *T. confusum* adversely affected following a 30 day exposure with 3% pyrethrin or with 1% pyrethrin (A and B, respectively) applied directly to wheat flour. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

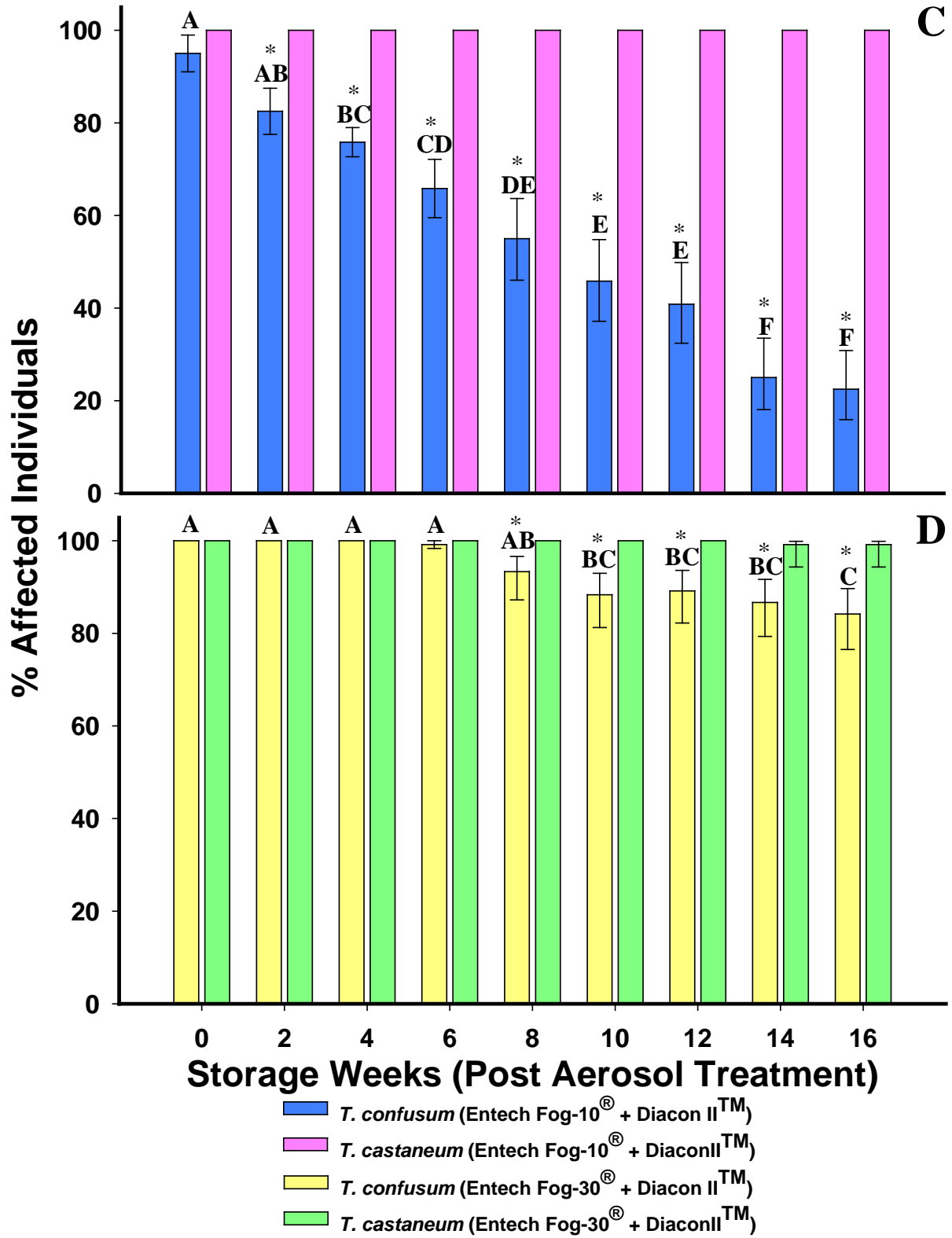


Figure 3.7 (A-B). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the pallet wrap treated surface, within each biweekly storage period. Resultant graph depicts how the concentration of the applied aerosol formulation consequently affects development within both exposed *Tribolium* spp. The proportion of (A) *T. castaneum* and (B) *T. confusum* adversely affected following a 30-day exposure with Entech Fog-30 ® + Methoprene or 1% pyrethrin , applied directly to the wheat flour substrate. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

Polyester Bag

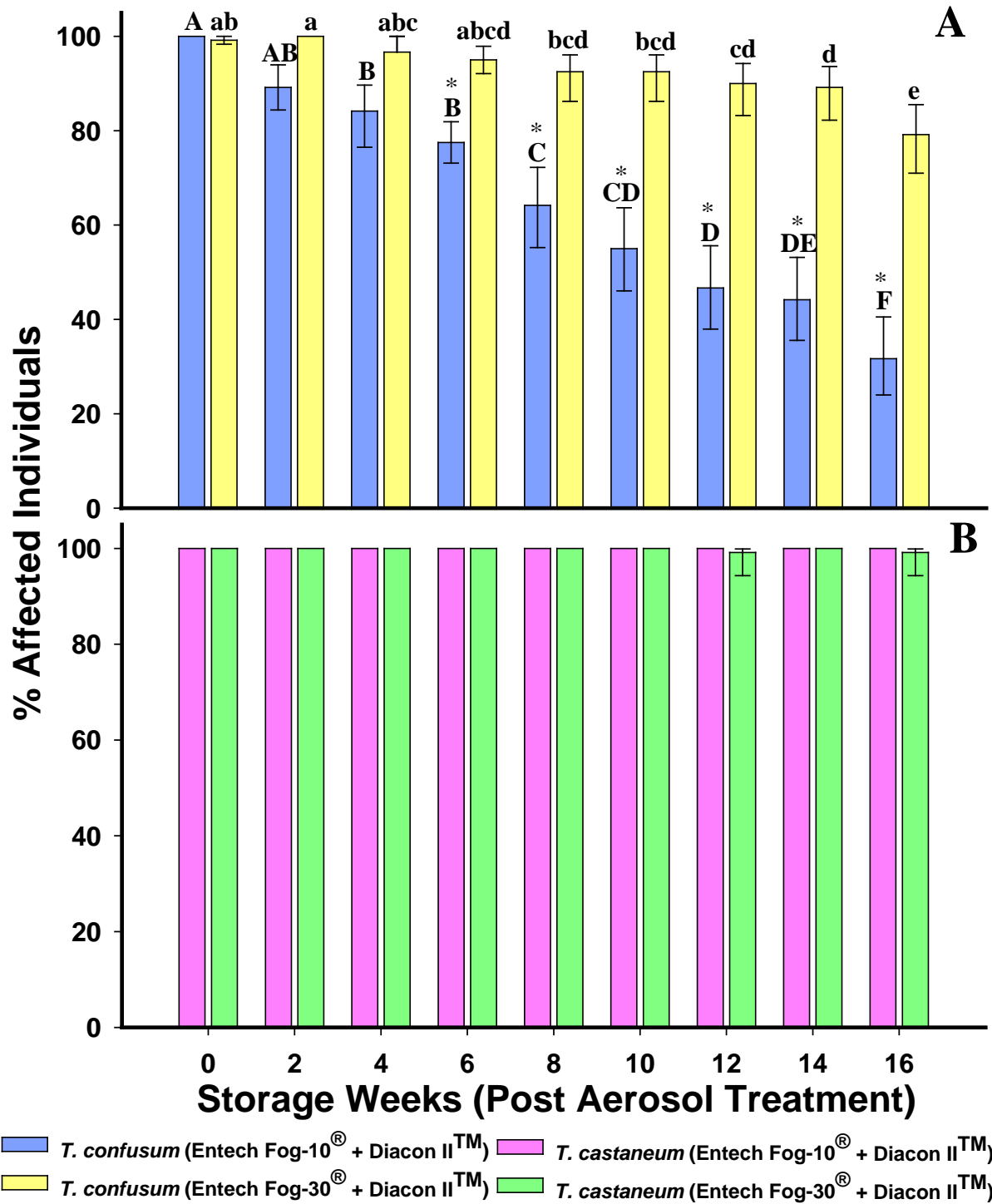


Figure 3.7 (C-D). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the pallet wrap treated surface, at both the 1% pyrethrin and the 3% pyrethrin aerosol formulations, within each biweekly storage period. Resultant graph depicts the species-specific response, at both applied aerosol concentrations. Comparison of means for differences in the percentage of *T. castaneum* and *T. confusum* adversely affected following a 30 day exposure with 3% pyrethrin or with 1% pyrethrin (A and B, respectively) applied directly to wheat flour. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

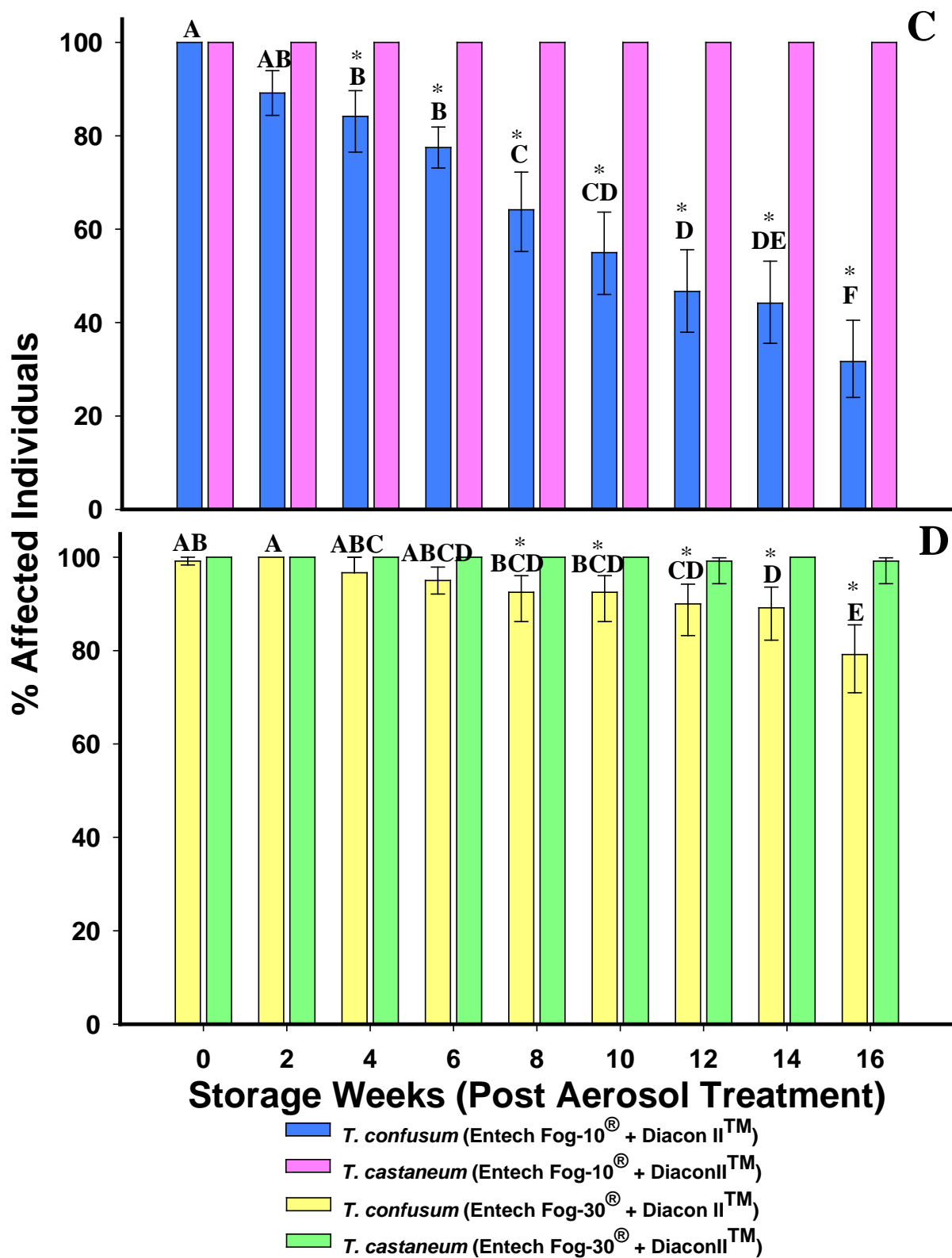


Figure 3.8 (A-B). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the cotton (muslin) bag treated surface, within each biweekly storage period. Resultant graph depicts how the concentration of the applied aerosol formulation consequently affects development within both exposed *Tribolium* spp. The proportion of (A) *T. castaneum* and (B) *T. confusum* adversely affected following a 30-day exposure with Entech Fog-30 ® + Methoprene or 1% pyrethrin , applied directly to the wheat flour substrate. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

Cotton Bag

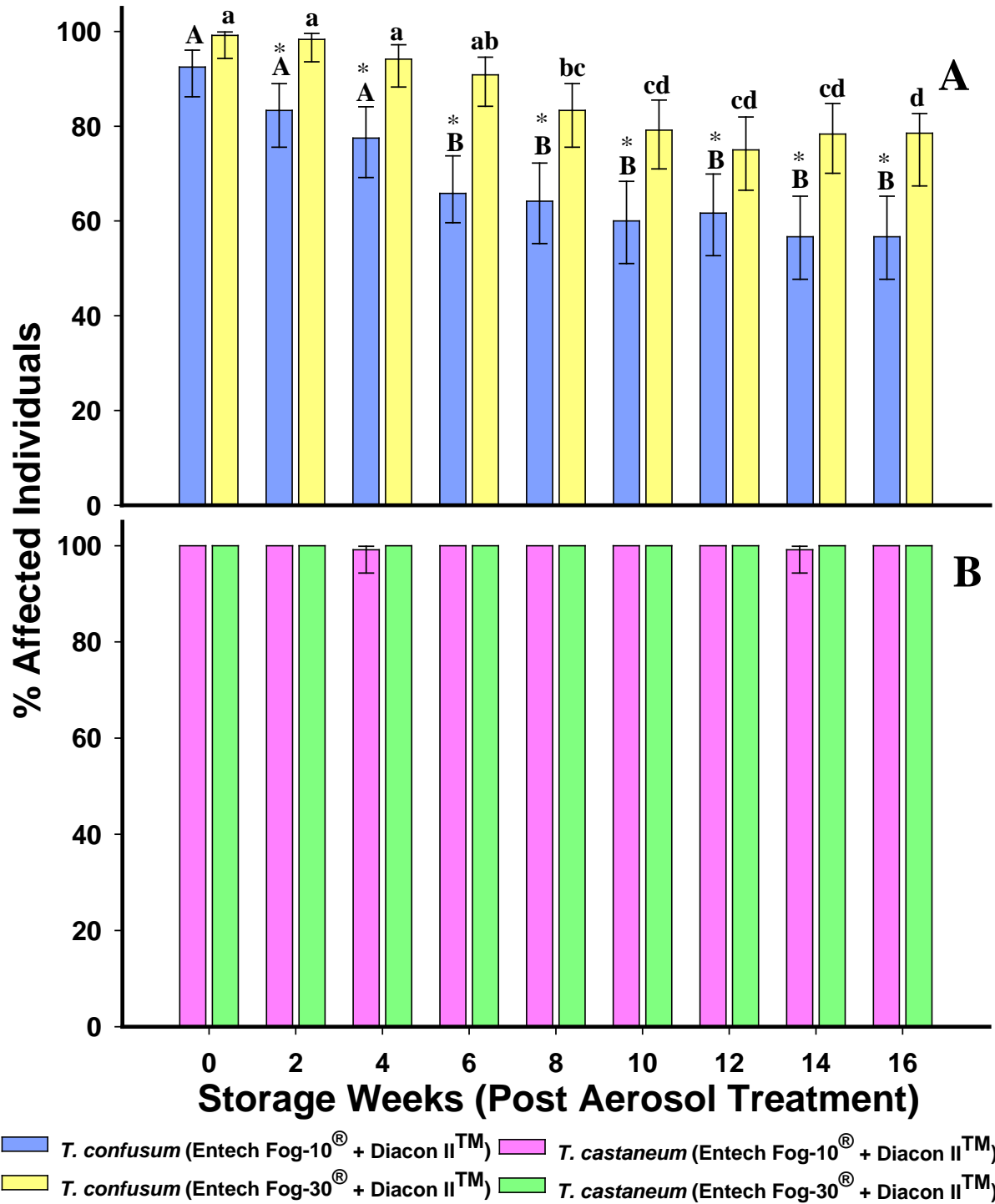


Figure 3.8 (C-D). Comparison of means \pm confidence limits of the proportion of ‘affected’ *T. castaneum* and *T. confusum* individuals, exposed to the cotton (muslin) bag treated surface, at both the 1% pyrethrin and the 3% pyrethrin aerosol formulations, within each biweekly storage period. Resultant graph depicts the species-specific response, at both applied aerosol concentrations. Comparison of means for differences in the percentage of *T. castaneum* and *T. confusum* adversely affected following a 30 day exposure with 3% pyrethrin or with 1% pyrethrin (A and B, respectively) applied directly to wheat flour. Means with different letters are significantly different by experiment-wise t-tests ($P < 0.05$ Fishers LSD Method Rejection Matrix, ProStat Software, 2001). Means (ADCD) correspond to the 1% pyrethrin aerosol formulation applied, whereas means (abcd) correspond to the applied 3% pyrethrin formulation, upon exposure to both *Tribolium* spp. Means reported with (*) represent a statistically significant difference (unpaired t-test) between the two reported means at each biweekly post aerosol treatment.

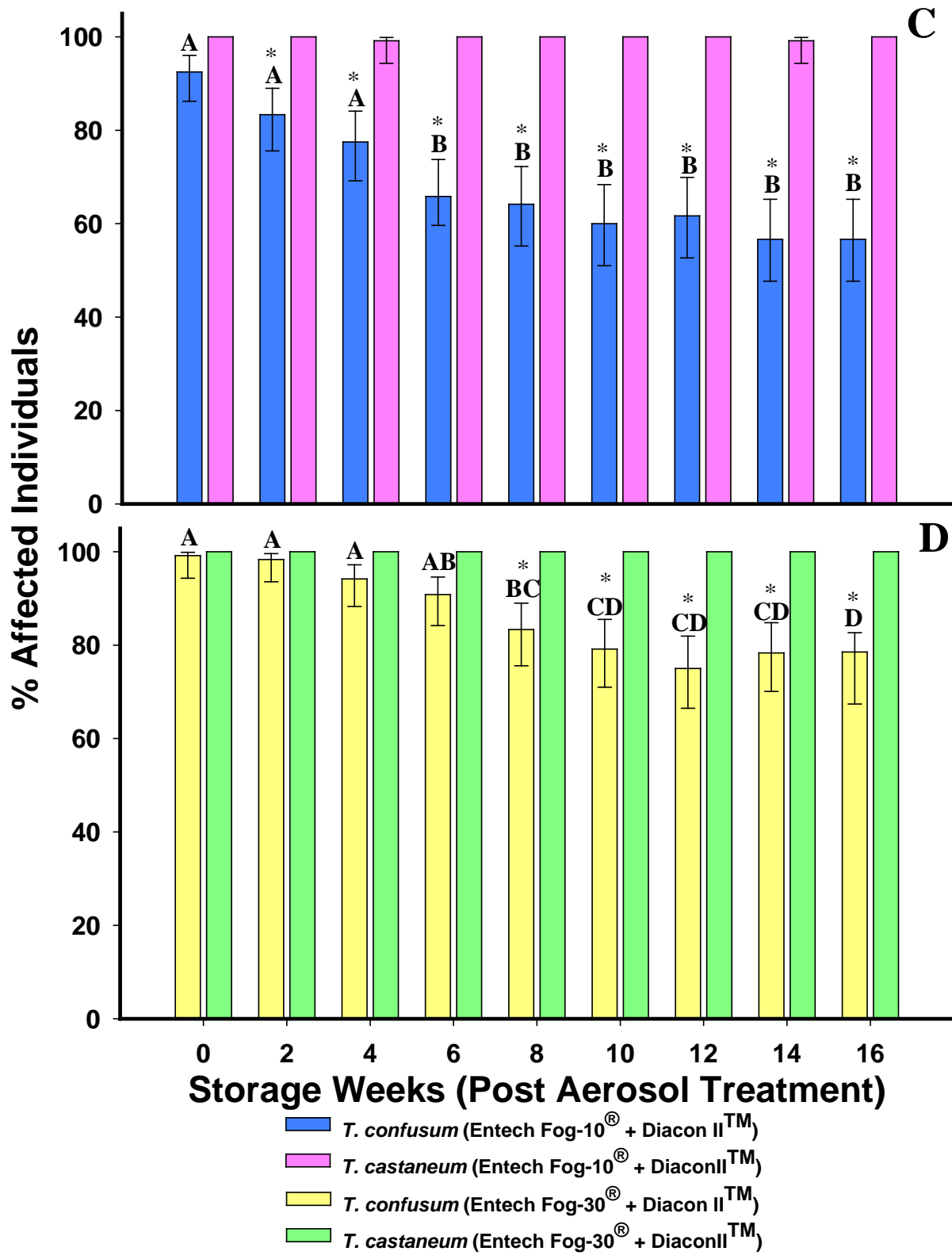


Table 3.8A. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of total individuals that emerged in each morphological category, at each aerosol concentration (1% pyrethrin and 3% pyrethrin) on treated paper bag substrate. Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	Entech Fog-10 [®] + DiaconII [™]	1.11	2.90	7.38	1.66	0.1979
	Entech Fog-30 [®] + DiaconII [™]	0.69	1.22	2.16		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	0.54	0.97	1.74	8.25	0.0041
	Entech Fog-30 [®] + DiaconII [™]	0.00	0.00	0.00		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	1.72	2.36	3.24	17.08	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	0.18	0.49	1.31		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + DiaconII [™]	1.92	2.52	3.31	10.94	0.0009
	Entech Fog-30 [®] + DiaconII [™]	0.55	0.98	1.76		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + DiaconII [™]	27.42	29.36	31.38	30.43	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	20.19	21.89	23.70		
Arrested Larvae	Entech Fog-10 [®] + DiaconII [™]	0.37	0.68	1.25	5.38	0.0204
	Entech Fog-30 [®] + DiaconII [™]	1.03	1.50	2.17		
Dead Larvae	Entech Fog-10 [®] + DiaconII [™]	10.54	11.93	13.47	88.83	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	21.37	23.17	24.94		
Dead Pupae	Entech Fog-10 [®] + DiaconII [™]	12.38	13.87	15.51	82.36	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	24.34	26.15	28.05		
Live Larvae	Entech Fog-10 [®] + DiaconII [™]	2.91	3.72	4.74	123.82	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	12.20	13.64	15.23		
Live Pupae	Entech Fog-10 [®] + DiaconII [™]	1.54	2.15	2.99	49.09	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	5.73	6.71	7.85		

Table 3.8B. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated paper bag substrate. Data collapsed over the 16-week storage period.

Morphological Category	Spp.	Lower C.L.	Mean	Upper C.L.	X ²	p
Normal, Live Adults	<i>T. confusum</i>	16.51	18.64	20.98	398.28	<0.0001
	<i>T. castaneum</i>	0.05	0.16	0.50		
Live Adults Displaying Twisted Wing Deformity	<i>T. confusum</i>	1.32	1.85	2.58	38.56	<0.0001
	<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults w/ Unfolded Wing Deformity	<i>T. confusum</i>	2.94	3.67	4.59	61.97	<0.0001
	<i>T. castaneum</i>	0.11	0.31	0.85		
Live Adults Unable to Shed Pupal Cuticle	<i>T. confusum</i>	2.90	3.61	4.48	42.89	<0.0001
	<i>T. castaneum</i>	0.37	0.68	1.25		
Arrested Pupae-Adult Intermediates	<i>T. confusum</i>	21.45	23.18	25.02	11.83	0.0006
	<i>T. castaneum</i>	25.93	27.86	29.87		
Arrested Larvae	<i>T. confusum</i>	0.25	0.48	0.92	22.83	<0.0001
	<i>T. castaneum</i>	1.58	2.12	2.83		
Dead Larvae	<i>T. confusum</i>	10.57	11.96	13.50	87.47	<0.0001
	<i>T. castaneum</i>	21.31	23.05	24.89		
Dead Pupae	<i>T. confusum</i>	13.15	14.70	16.40	65.51	<0.0001
	<i>T. castaneum</i>	23.08	24.86	26.73		
Live Larvae	<i>T. confusum</i>	3.15	4.01	5.09	93.81	<0.0001
	<i>T. castaneum</i>	11.32	12.74	14.30		
Live Pupae	<i>T. confusum</i>	1.84	2.56	3.53	22.30	<0.0001
	<i>T. castaneum</i>	4.78	5.68	6.75		

Table 3.8C. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated paper bag substrate, at each applied aerosol concentration (1% pyrethrin and 3% pyrethrin)Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Spp.	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	46.01	48.98	51.96	12.96	0.0003
		<i>T. castaneum</i>	0.01	0.09	0.65		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	4.01	5.19	6.68		
		<i>T. castaneum</i>	0.09	0.28	0.86		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	2.41	3.33	4.59	1.52	0.2184
		<i>T. castaneum</i>	0.09	0.28	0.86		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	0.56	1.02	1.83		
		<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	4.17	5.37	6.88	3.31	0.0687
		<i>T. castaneum</i>	0.56	1.02	1.83		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	1.72	2.50	3.62		
		<i>T. castaneum</i>	0.01	0.09	0.65		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	2.81	3.80	5.12	8.42	0.0037
		<i>T. castaneum</i>	1.05	1.67	2.63		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	2.49	3.43	4.69		
		<i>T. castaneum</i>	0.09	0.28	0.86		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	18.34	20.65	23.17	95.07	<0.0001
		<i>T. castaneum</i>	37.03	39.91	42.86		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	23.40	25.93	28.62		
		<i>T. castaneum</i>	16.14	18.33	20.75		
Arrested Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	0.09	0.28	0.86	0.75	0.3860
		<i>T. castaneum</i>	1.05	1.67	2.96		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	0.43	0.83	1.59		
		<i>T. castaneum</i>	1.87	2.69	3.84		
Dead Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	5.57	6.94	8.62	21.59	<0.0001
		<i>T. castaneum</i>	17.46	19.72	22.20		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	17.54	19.82	22.30		
		<i>T. castaneum</i>	24.20	26.76	29.48		
Dead Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	6.49	7.96	9.73	53.29	<0.0001
		<i>T. castaneum</i>	20.64	23.06	25.66		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	23.04	25.56	28.24		
		<i>T. castaneum</i>	24.20	26.76	29.48		
Live Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	1.13	1.76	2.74	4.17	0.0411
		<i>T. castaneum</i>	6.24	7.69	9.43		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	7.33	8.89	10.74		
		<i>T. castaneum</i>	18.07	20.37	22.88		
Live Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	0.50	0.93	1.71	24.96	<0.0001
		<i>T. castaneum</i>	3.77	4.91	6.37		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	5.49	6.85	8.52		
		<i>T. castaneum</i>	5.24	6.57	8.22		

Table 3.9A. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of total individuals that emerged in each morphological category, at each aerosol concentration (1% pyrethrin and 3% pyrethrin) on treated cardboard substrate. Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	Entech Fog-10 [®] + Diaconil [™]	0.00	0.00	0.00	0.32	0.5736
	Entech Fog-30 [®] + Diaconil [™]	0.50	0.90	1.62		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + Diaconil [™]	0.00	0.00	0.00	0.41	0.5242
	Entech Fog-30 [®] + Diaconil [™]	0.09	0.25	0.60		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + Diaconil [™]	0.28	0.75	0.02	0.04	0.8436
	Entech Fog-30 [®] + Diaconil [™]	0.32	0.66	1.35		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + Diaconil [™]	1.56	2.24	0.03	14.35	0.0002
	Entech Fog-30 [®] + Diaconil [™]	0.28	0.57	1.18		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + Diaconil [™]	27.30	29.19	0.31	30.57	<0.0001
	Entech Fog-30 [®] + Diaconil [™]	19.86	21.65	23.55		
Arrested Larvae	Entech Fog-10 [®] + Diaconil [™]	0.00	0.00	0.00	4.74	0.0294
	Entech Fog-30 [®] + Diaconil [™]	0.54	0.88	1.44		
Dead Larvae	Entech Fog-10 [®] + Diaconil [™]	14.32	15.96	0.18	18.53	<0.0001
	Entech Fog-30 [®] + Diaconil [™]	19.64	21.33	23.13		
Dead Pupae	Entech Fog-10 [®] + Diaconil [™]	15.69	17.30	0.19	35.96	<0.0001
	Entech Fog-30 [®] + Diaconil [™]	24.79	26.61	28.52		
Live Larvae	Entech Fog-10 [®] + Diaconil [™]	3.53	4.33	0.05	187.41	<0.0001
	Entech Fog-30 [®] + Diaconil [™]	16.06	17.75	19.58		
Live Pupae	Entech Fog-10 [®] + Diaconil [™]	1.53	2.06	0.03	21.36	<0.0001
	Entech Fog-30 [®] + Diaconil [™]	3.77	4.58	5.56		

Table 3.9B. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated cardboard substrate. Data collapsed over the 16-week storage period.

Morphological Category	Spp.	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	<i>T. confusum</i>	9.51	11.26	0.13	223.25	<0.0001
	<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults Displaying Twisted Wing Deformity	<i>T. confusum</i>	0.89	1.35	2.02	30.54	<0.0001
	<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults w/ Unfolded Wing Deformity	<i>T. confusum</i>	2.91	3.66	4.59	85.30	<0.0001
	<i>T. castaneum</i>	0.04	0.13	0.43		
Live Adults Unable to Shed Pupal Cuticle	<i>T. confusum</i>	2.67	3.43	4.39	53.01	<0.0001
	<i>T. castaneum</i>	0.17	0.37	0.80		
Arrested Pupae-Adult Intermediates	<i>T. confusum</i>	27.77	29.67	31.64	38.08	<0.0001
	<i>T. castaneum</i>	19.49	21.26	23.14		
Arrested Larvae	<i>T. confusum</i>	0.00	0.00	0.00	41.63	<0.0001
	<i>T. castaneum</i>	1.52	2.04	2.74		
Dead Larvae	<i>T. confusum</i>	10.71	12.06	13.56	156.90	<0.0001
	<i>T. castaneum</i>	25.45	27.29	29.21		
Dead Pupae	<i>T. confusum</i>	16.11	17.74	19.51	26.95	<0.0001
	<i>T. castaneum</i>	24.21	26.02	27.91		
Live Larvae	<i>T. confusum</i>	4.50	5.45	6.58	80.02	<0.0001
	<i>T. castaneum</i>	12.85	14.50	16.33		
Live Pupae	<i>T. confusum</i>	1.83	2.42	3.20	7.27	0.0070
	<i>T. castaneum</i>	3.14	3.91	4.84		

Table 3.9C. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated cardboard substrate, at each applied aerosol concentration (1% pyrethrin and 3% pyrethrin)Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Spp.	Lower C.L.	Mean	Upper C.L.	X ²	p
Normal, Live Adults	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	32.48	35.28	0.38	17.51	<0.0001
		<i>T. castaneum</i>	0.00	0.00	0.00		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	2.03	2.87	4.05	3.25	0.0713
		<i>T. castaneum</i>	0.09	0.28	0.86		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	1.95	2.78	3.95	3.25	0.0713
		<i>T. castaneum</i>	0.00	0.00	0.00		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	0.31	0.65	1.35	1.85	0.1734
		<i>T. castaneum</i>	0.01	0.09	0.65		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	4.50	5.74	7.30	1.85	0.1734
		<i>T. castaneum</i>	0.01	0.09	0.65		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	1.57	2.31	3.40	0.00	0.9776
		<i>T. castaneum</i>	0.05	0.19	0.74		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	5.24	6.57	8.22	0.00	0.9776
		<i>T. castaneum</i>	0.37	0.74	1.47		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	1.12	1.76	2.74	117.00	<0.0001
		<i>T. castaneum</i>	0.05	0.19	0.74		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	23.40	25.93	28.62	117.00	<0.0001
		<i>T. castaneum</i>	29.95	32.69	35.54		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	30.95	33.70	36.58	8.47	0.0036
		<i>T. castaneum</i>	11.17	13.06	15.20		
Arrested Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	0.00	0.00	0.00	8.47	0.0036
		<i>T. castaneum</i>	1.72	2.50	3.62		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	0.19	0.46	1.11	39.10	<0.0001
		<i>T. castaneum</i>	1.05	1.67	2.63		
Dead Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	6.66	8.15	9.94	39.10	<0.0001
		<i>T. castaneum</i>	26.26	28.89	31.67		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	15.35	17.50	19.88	76.38	<0.0001
		<i>T. castaneum</i>	23.22	25.74	28.43		
Dead Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	9.20	10.93	12.93	76.38	<0.0001
		<i>T. castaneum</i>	23.76	26.30	29.01		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	24.92	27.50	30.24	7.67	0.0056
		<i>T. castaneum</i>	23.22	25.74	28.43		
Live Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	21.18	3.06	4.27	7.67	0.0056
		<i>T. castaneum</i>	48.29	6.11	7.71		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	79.23	9.54	11.44	0.08	0.7816
		<i>T. castaneum</i>	27.97	30.65	33.46		
Live Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	0.98	15.74	2.52	0.08	0.7816
		<i>T. castaneum</i>	1.87	2.69	3.84		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	2.73	3.70	5.01	0.08	0.7816
		<i>T. castaneum</i>	4.42	5.65	7.19		

Table 3.10A. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of total individuals that emerged in each morphological category, at each aerosol concentration (1% pyrethrin and 3% pyrethrin) on treated plastic substrate. Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	Entech Fog-10 [®] + DiaconII [™]	0.74	1.94	5.02	1.69	0.1940
	Entech Fog-30 [®] + DiaconII [™]	0.39	0.78	1.58		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	0.00	0.00	0.00	0.00	1.0000
	Entech Fog-30 [®] + DiaconII [™]	0.00	0.00	0.00		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	0.57	1.15	2.29	1.84	0.1747
	Entech Fog-30 [®] + DiaconII [™]	0.27	0.56	1.15		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + DiaconII [™]	1.69	2.42	3.45	18.28	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	0.16	0.43	11.56		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + DiaconII [™]	25.96	27.81	29.74	68.16	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	15.64	17.22	18.92		
Arrested Larvae	Entech Fog-10 [®] + DiaconII [™]	0.48	0.87	1.57	0.03	0.8601
	Entech Fog-30 [®] + DiaconII [™]	0.44	0.81	1.46		
Dead Larvae	Entech Fog-10 [®] + DiaconII [™]	16.35	18.04	19.86	58.94	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	26.41	28.28	30.24		
Dead Pupae	Entech Fog-10 [®] + DiaconII [™]	15.81	17.41	19.13	36.52	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	29.27	31.20	33.19		
Live Larvae	Entech Fog-10 [®] + DiaconII [™]	4.22	5.15	6.27	152.53	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	15.72	17.30	19.01		
Live Pupae	Entech Fog-10 [®] + DiaconII [™]	1.33	1.84	2.52	32.54	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	4.13	4.97	5.97		

Table 3.10B. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated plastic substrate. Data collapsed over the 16-week storage period.

Morphological Category	Spp.	Lower C.L.	Mean	Upper C.L.	X ²	p
Normal, Live Adults	<i>T. confusum</i>	9.04	10.64	12.48	224.85	<0.0001
	<i>T. castaneum</i>	0.04	0.13	0.43		
Live Adults Displaying Twisted Wing Deformity	<i>T. confusum</i>	0.65	1.09	1.82	25.78	<0.0001
	<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults w/ Unfolded Wing Deformity	<i>T. confusum</i>	2.63	3.39	4.35	67.91	<0.0001
	<i>T. castaneum</i>	0.07	0.19	0.49		
Live Adults Unable to Shed Pupal Cuticle	<i>T. confusum</i>	3.07	3.88	4.90	67.86	<0.0001
	<i>T. castaneum</i>	0.09	0.26	0.74		
Arrested Pupae-Adult Intermediates	<i>T. confusum</i>	23.37	25.16	27.03	20.85	<0.0001
	<i>T. castaneum</i>	17.56	19.25	21.06		
Arrested Larvae	<i>T. confusum</i>	0.12	0.28	0.62	44.47	<0.0001
	<i>T. castaneum</i>	1.91	2.49	3.24		
Dead Larvae	<i>T. confusum</i>	14.03	15.57	17.24	157.41	<0.0001
	<i>T. castaneum</i>	30.08	32.01	34.01		
Dead Pupae	<i>T. confusum</i>	17.17	18.84	20.64	12.23	0.0005
	<i>T. castaneum</i>	27.27	29.16	31.13		
Live Larvae	<i>T. confusum</i>	4.88	5.91	7.15	88.29	<0.0001
	<i>T. castaneum</i>	13.78	15.32	16.99		
Live Pupae	<i>T. confusum</i>	1.80	2.41	3.23	6.23	0.0126
	<i>T. castaneum</i>	3.05	3.81	4.74		

Table 3.10C. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated plastic substrate, at each applied aerosol concentration (1% pyrethrin and 3% pyrethrin)Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Spp.	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	27.07	29.72	32.52	6.67	0.0098
		<i>T. castaneum</i>	0.01	0.09	0.65		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	2.34	3.24	4.48		
		<i>T. castaneum</i>	0.05	0.19	0.74		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	2.26	3.15	4.37	0.00	1.0000
		<i>T. castaneum</i>	0.00	0.00	0.00		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	0.14	0.37	0.98		
		<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	5.41	6.76	8.42	1.84	0.1747
		<i>T. castaneum</i>	0.05	0.19	0.74		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	1.05	1.67	2.63		
		<i>T. castaneum</i>	0.05	0.19	0.74		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	6.16	7.59	9.33	0.43	0.5112
		<i>T. castaneum</i>	0.37	0.74	1.47		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	1.27	1.94	2.96		
		<i>T. castaneum</i>	0.01	0.09	0.65		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	24.11	26.67	29.39	37.41	<0.0001
		<i>T. castaneum</i>	26.35	28.98	31.76		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	21.26	23.70	26.33		
		<i>T. castaneum</i>	10.40	12.22	14.31		
Arrested Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	0.09	0.28	0.86	0.03	0.8601
		<i>T. castaneum</i>	1.87	2.69	3.84		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	0.09	0.28	0.86		
		<i>T. castaneum</i>	1.57	2.31	3.40		
Dead Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	8.35	10.00	11.94	31.71	<0.0001
		<i>T. castaneum</i>	27.70	30.37	33.18		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	21.00	23.43	26.05		
		<i>T. castaneum</i>	30.95	33.70	36.58		
Dead Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	9.97	11.76	13.82	72.93	<0.0001
		<i>T. castaneum</i>	22.51	25.00	27.67		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	26.17	28.80	31.57		
		<i>T. castaneum</i>	30.95	33.70	36.58		
Live Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	1.95	2.78	3.95	3.61	<0.0001
		<i>T. castaneum</i>	7.75	9.35	11.24		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	10.31	12.13	14.22		
		<i>T. castaneum</i>	21.62	24.07	26.72		
Live Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	0.77	1.30	2.18	1.53	0.2159
		<i>T. castaneum</i>	1.80	2.59	3.73		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	3.37	4.44	5.85		
		<i>T. castaneum</i>	4.34	5.56	7.09		

Table 3.11A. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of total individuals that emerged in each morphological category, at each aerosol concentration (1% pyrethrin and 3% pyrethrin) on treated pallet wrap substrate. Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	Entech Fog-10 [®] + DiaconII [™]	3.48	5.14	7.52	15.56	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	0.60	1.20	2.39		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	0.48	0.87	1.57	5.70	0.0169
	Entech Fog-30 [®] + DiaconII [™]	0.00	0.00	0.00		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	2.03	2.77	3.77	13.84	0.0002
	Entech Fog-30 [®] + DiaconII [™]	0.25	0.67	1.79		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + DiaconII [™]	2.99	3.77	4.73	29.40	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	0.70	1.09	1.70		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + DiaconII [™]	26.78	28.67	30.64	1.37	0.2417
	Entech Fog-30 [®] + DiaconII [™]	25.03	27.00	29.06		
Arrested Larvae	Entech Fog-10 [®] + DiaconII [™]	0.86	1.30	1.96	0.01	0.9290
	Entech Fog-30 [®] + DiaconII [™]	0.91	1.33	1.95		
Dead Larvae	Entech Fog-10 [®] + DiaconII [™]	14.10	15.67	17.39	14.52	0.0001
	Entech Fog-30 [®] + DiaconII [™]	18.65	20.35	22.15		
Dead Pupae	Entech Fog-10 [®] + DiaconII [™]	14.48	16.04	17.73	11.53	0.0007
	Entech Fog-30 [®] + DiaconII [™]	19.92	21.64	23.46		
Live Larvae	Entech Fog-10 [®] + DiaconII [™]	3.68	4.48	5.44	86.99	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	11.13	12.55	14.11		
Live Pupae	Entech Fog-10 [®] + DiaconII [™]	1.93	2.52	3.30	10.14	0.0014
	Entech Fog-30 [®] + DiaconII [™]	3.55	4.43	5.52		

Table 3.11B. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated pallet wrap substrate. Data collapsed over the 16-week storage period.

Morphological Category	Spp.	Lower C.L.	Mean	Upper C.L.	X ²	p
Normal, Live Adults	<i>T. confusum</i>	15.16	16.92	18.83	405.38	<0.0001
	<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults Displaying Twisted Wing Deformity	<i>T. confusum</i>	1.53	2.06	2.76	46.79	<0.0001
	<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults w/ Unfolded Wing Deformity	<i>T. confusum</i>	4.96	5.89	6.97	121.18	<0.0001
	<i>T. castaneum</i>	0.11	0.31	0.85		
Live Adults Unable to Shed Pupal Cuticle	<i>T. confusum</i>	2.99	3.77	4.73	29.40	<0.0001
	<i>T. castaneum</i>	0.70	1.09	1.70		
Arrested Pupae-Adult Intermediates	<i>T. confusum</i>	30.27	32.26	34.33	35.87	<0.0001
	<i>T. castaneum</i>	21.96	23.79	25.72		
Arrested Larvae	<i>T. confusum</i>	0.45	0.74	1.20	19.38	<0.0001
	<i>T. castaneum</i>	1.79	2.35	3.08		
Dead Larvae	<i>T. confusum</i>	10.14	11.43	12.87	168.53	<0.0001
	<i>T. castaneum</i>	25.06	26.89	28.80		
Dead Pupae	<i>T. confusum</i>	11.64	13.01	14.52	91.44	<0.0001
	<i>T. castaneum</i>	24.26	26.07	27.97		
Live Larvae	<i>T. confusum</i>	4.66	5.57	6.64	27.98	<0.0001
	<i>T. castaneum</i>	8.88	10.24	11.78		
Live Pupae	<i>T. confusum</i>	1.48	19.86	2.67	37.48	<0.0001
	<i>T. castaneum</i>	4.65	5.59	6.70		

Table 3.11C. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated pallet wrap substrate, at each applied aerosol concentration (1% pyrethrin and 3% pyrethrin)Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Spp.	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	31.67	34.44	37.33	0.80	0.3710
		<i>T. castaneum</i>	0.25	0.56	1.23		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	5.91	7.32	9.03		
		<i>T. castaneum</i>	0.05	0.19	0.74		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	1.87	2.69	3.84	2.64	0.1044
		<i>T. castaneum</i>	0.09	0.28	0.86		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	0.98	1.57	2.52		
		<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	5.91	7.31	9.03	5.37	0.0205
		<i>T. castaneum</i>	0.56	1.02	1.83		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	3.61	4.72	6.16		
		<i>T. castaneum</i>	0.01	0.09	0.65		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	5.24	6.57	8.22	0.13	0.7184
		<i>T. castaneum</i>	1.42	2.13	3.18		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	1.42	2.13	3.18		
		<i>T. castaneum</i>	0.25	0.56	1.23		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	20.73	23.15	25.76	208.72	<0.0001
		<i>T. castaneum</i>	32.12	34.91	37.80		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	40.04	42.96	45.94		
		<i>T. castaneum</i>	13.34	15.37	17.65		
Arrested Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	0.31	0.65	1.35	0.62	0.4317
		<i>T. castaneum</i>	1.80	2.59	3.73		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	0.43	0.83	1.59		
		<i>T. castaneum</i>	1.42	2.13	3.18		
Dead Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	7.42	8.98	10.84	6.86	0.0088
		<i>T. castaneum</i>	23.40	25.93	28.62		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	12.47	14.44	16.67		
		<i>T. castaneum</i>	25.28	27.87	30.62		
Dead Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	8.52	10.19	12.14	11.53	0.0007
		<i>T. castaneum</i>	21.88	24.35	27.00		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	14.39	16.48	18.82		
		<i>T. castaneum</i>	25.28	27.87	30.62		
Live Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	3.13	4.17	5.54	16.30	<0.0001
		<i>T. castaneum</i>	3.69	4.82	6.27		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	5.99	7.41	9.13		
		<i>T. castaneum</i>	18.16	20.46	22.97		
Live Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	1.20	1.85	2.85	5.69	0.0171
		<i>T. castaneum</i>	2.49	3.43	4.69		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	1.42	2.13	3.18		
		<i>T. castaneum</i>	7.42	8.98	10.84		

Table 3.12A. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of total individuals that emerged in each morphological category, at each aerosol concentration (1% pyrethrin and 3% pyrethrin) on treated flour bag substrate. Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	Entech Fog-10 [®] + DiaconII [™]	0.00	0.00	0.00	0.35	0.5543
	Entech Fog-30 [®] + DiaconII [™]	0.56	1.13	2.26		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	0.00	0.00	0.00	0.00	1.0000
	Entech Fog-30 [®] + DiaconII [™]	0.00	0.00	0.00		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	1.04	1.64	2.57	0.63	0.4622
	Entech Fog-30 [®] + DiaconII [™]	0.84	1.28	1.93		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + DiaconII [™]	2.62	3.30	4.16	23.91	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	0.64	1.04	1.67		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + DiaconII [™]	23.48	25.31	27.24	0.27	0.6001
	Entech Fog-30 [®] + DiaconII [™]	22.82	24.61	26.49		
Arrested Larvae	Entech Fog-10 [®] + DiaconII [™]	0.56	0.88	1.39	4.00	0.0454
	Entech Fog-30 [®] + DiaconII [™]	0.12	0.33	0.92		
Dead Larvae	Entech Fog-10 [®] + DiaconII [™]	15.40	17.03	18.79	32.33	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	22.50	24.28	26.15		
Dead Pupae	Entech Fog-10 [®] + DiaconII [™]	14.00	15.59	17.32	47.23	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	22.81	24.60	26.48		
Live Larvae	Entech Fog-10 [®] + DiaconII [™]	3.47	4.24	5.18	95.36	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	10.95	12.27	13.72		
Live Pupae	Entech Fog-10 [®] + DiaconII [™]	1.55	2.09	2.83	37.92	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	4.84	5.76	6.85		

Table 3.12B. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated flour bag substrate. Data collapsed over the 16-week storage period.

Morphological Category	Spp.	Lower C.L.	Mean	Upper C.L.	X ²	p
Normal, Live Adults	<i>T. confusum</i>	16.91	18.89	21.03	465.25	<0.0001
	<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults Displaying Twisted Wing Deformity	<i>T. confusum</i>	1.74	2.32	3.09	67.02	<0.0001
	<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults w/ Unfolded Wing Deformity	<i>T. confusum</i>	3.02	3.77	4.70	56.73	<0.0001
	<i>T. castaneum</i>	0.31	0.55	0.97		
Live Adults Unable to Shed Pupal Cuticle	<i>T. confusum</i>	2.50	3.18	4.03	20.41	<0.0001
	<i>T. castaneum</i>	0.67	1.09	1.73		
Arrested Pupae-Adult Intermediates	<i>T. confusum</i>	21.96	23.73	25.60	3.46	0.0628
	<i>T. castaneum</i>	24.38	26.23	28.16		
Arrested Larvae	<i>T. confusum</i>	0.09	0.25	0.70	13.04	0.0003
	<i>T. castaneum</i>	0.82	1.20	1.76		
Dead Larvae	<i>T. confusum</i>	12.74	14.19	15.77	129.83	<0.0001
	<i>T. castaneum</i>	26.60	28.47	30.41		
Dead Pupae	<i>T. confusum</i>	12.03	13.47	15.05	127.92	<0.0001
	<i>T. castaneum</i>	26.04	27.90	29.83		
Live Larvae	<i>T. confusum</i>	5.93	7.01	8.27	0.48	0.4888
	<i>T. castaneum</i>	6.50	7.60	8.87		
Live Pupae	<i>T. confusum</i>	1.85	2.47	3.28	16.44	<0.0001
	<i>T. castaneum</i>	4.04	4.91	5.96		

Table 3.12C. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated flour bag substrate, at each applied aerosol concentration (1% pyrethrin and 3% pyrethrin)Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Spp.	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	40.59	43.52	46.50	9.86	0.0017
		<i>T. castaneum</i>	0.00	0.00	0.00		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	5.24	6.57	8.22		
		<i>T. castaneum</i>	0.05	0.19	0.74		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	2.65	3.61	4.90	0.00	1.0000
		<i>T. castaneum</i>	0.00	0.00	0.00		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	0.91	14.82	2.40		
		<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	4.42	5.65	7.19	3.57	0.0589
		<i>T. castaneum</i>	0.19	0.46	1.11		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	1.72	2.50	3.62		
		<i>T. castaneum</i>	0.31	0.65	1.35		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	3.29	4.35	5.74	4.17	0.4411
		<i>T. castaneum</i>	1.72	2.50	3.62		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	1.57	2.31	3.40		
		<i>T. castaneum</i>	0.19	0.46	1.11		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	16.23	18.43	20.85	91.86	<0.0001
		<i>T. castaneum</i>	30.95	33.70	36.58		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	27.34	30.00	32.80		
		<i>T. castaneum</i>	17.63	19.91	22.40		
Arrested Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	0.31	0.65	1.35	4.00	0.0454
		<i>T. castaneum</i>	0.70	1.20	2.06		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	0.01	0.09	0.65		
		<i>T. castaneum</i>	0.70	1.20	2.06		
Dead Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	8.26	9.91	11.84	22.00	<0.0001
		<i>T. castaneum</i>	25.10	27.69	30.43		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	17.63	19.91	22.40		
		<i>T. castaneum</i>	26.62	29.26	32.04		
Dead Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	7.08	8.61	10.44	31.78	<0.0001
		<i>T. castaneum</i>	24.02	26.57	29.30		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	18.16	20.46	22.97		
		<i>T. castaneum</i>	26.62	29.26	32.04		
Live Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	2.89	3.89	5.21	0.58	0.4467
		<i>T. castaneum</i>	3.53	4.63	6.06		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	10.49	12.32	14.41		
		<i>T. castaneum</i>	10.40	12.22	14.31		
Live Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	0.84	1.39	2.29	0.46	0.4989
		<i>T. castaneum</i>	2.26	3.15	4.37		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	3.29	4.35	5.74		
		<i>T. castaneum</i>	6.16	7.59	9.33		

Table 3.13A. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of total individuals that emerged in each morphological category, at each aerosol concentration (1% pyrethrin and 3% pyrethrin) on treated polyester woven bag substrate. Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Lower C.L.	Mean	Upper C.L.	X ²	p
Normal, Live Adults	Entech Fog-10 [®] + Diaconil [™]	0.00	0.00	0.00	0.55	0.4577
	Entech Fog-30 [®] + Diaconil [™]	0.59	1.18	2.35		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + Diaconil [™]	0.34	0.70	1.42	2.22	0.1363
	Entech Fog-30 [®] + Diaconil [™]	0.10	0.28	0.78		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + Diaconil [™]	1.02	1.55	2.36	1.68	0.1944
	Entech Fog-30 [®] + Diaconil [™]	0.60	1.00	1.68		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + Diaconil [™]	1.83	2.42	3.20	13.33	0.0003
	Entech Fog-30 [®] + Diaconil [™]	0.53	0.89	1.51		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + Diaconil [™]	23.00	24.79	26.67	22.32	<0.0001
	Entech Fog-30 [®] + Diaconil [™]	17.08	18.73	20.50		
Arrested Larvae	Entech Fog-10 [®] + Diaconil [™]	0.58	0.92	1.44	0.81	0.3679
	Entech Fog-30 [®] + Diaconil [™]	0.31	0.63	1.29		
Dead Larvae	Entech Fog-10 [®] + Diaconil [™]	21.81	23.69	25.68	22.62	<0.0001
	Entech Fog-30 [®] + Diaconil [™]	28.48	30.41	32.41		
Dead Pupae	Entech Fog-10 [®] + Diaconil [™]	16.37	17.96	19.67	28.06	<0.0001
	Entech Fog-30 [®] + Diaconil [™]	28.00	29.92	31.92		
Live Larvae	Entech Fog-10 [®] + Diaconil [™]	2.85	3.57	4.46	85.43	<0.0001
	Entech Fog-30 [®] + Diaconil [™]	9.56	10.83	12.25		
Live Pupae	Entech Fog-10 [®] + Diaconil [™]	1.78	2.34	3.07	25.11	<0.0001
	Entech Fog-30 [®] + Diaconil [™]	4.37	5.24	6.28		

Table 3.13B. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated polyester woven bag substrate. Data collapsed over the 16-week storage period.

Morphological Category	Spp.	Lower C.L.	Mean	Upper C.L.	X ²	p
Normal, Live Adults	<i>T. confusum</i>	14.89	16.64	18.55	438.04	<0.0001
	<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults Displaying Twisted Wing Deformity	<i>T. confusum</i>	1.01	1.47	2.14	26.11	<0.0001
	<i>T. castaneum</i>	0.04	0.13	0.43		
Live Adults w/ Unfolded Wing Deformity	<i>T. confusum</i>	2.69	3.39	4.25	54.60	<0.0001
	<i>T. castaneum</i>	0.24	0.45	0.85		
Live Adults Unable to Shed Pupal Cuticle	<i>T. confusum</i>	2.18	2.81	3.62	24.55	<0.0001
	<i>T. castaneum</i>	0.45	0.77	1.31		
Arrested Pupae-Adult Intermediates	<i>T. confusum</i>	22.10	23.86	25.71	11.43	0.0007
	<i>T. castaneum</i>	17.83	19.52	21.32		
Arrested Larvae	<i>T. confusum</i>	0.16	0.35	0.76	18.95	<0.0001
	<i>T. castaneum</i>	1.19	1.66	2.31		
Dead Larvae	<i>T. confusum</i>	16.54	18.14	19.87	209.39	<0.0001
	<i>T. castaneum</i>	35.94	37.96	40.03		
Dead Pupae	<i>T. confusum</i>	16.45	18.04	19.75	26.62	<0.0001
	<i>T. castaneum</i>	27.89	29.82	31.81		
Live Larvae	<i>T. confusum</i>	5.16	6.11	7.22	0.18	0.6701
	<i>T. castaneum</i>	5.34	6.46	7.79		
Live Pupae	<i>T. confusum</i>	2.27	2.92	3.75	4.93	0.0264
	<i>T. castaneum</i>	3.41	4.22	5.21		

Table 3.13C. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated polyester woven bag substrate, at each applied aerosol concentration (1% pyrethrin and 3% pyrethrin)Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Spp.	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	31.40	34.17	37.05	8.14	0.0043
		<i>T. castaneum</i>	0.00	0.00	0.00		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	57.39	7.13	8.83		
		<i>T. castaneum</i>	0.05	0.19	0.74		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	1.80	2.59	3.73	0.12	0.7272
		<i>T. castaneum</i>	0.05	0.19	0.74		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	0.43	0.83	1.59		
		<i>T. castaneum</i>	0.01	0.09	0.65		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	3.21	4.26	5.64	0.01	0.9190
		<i>T. castaneum</i>	0.25	0.56	1.23		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	1.87	2.69	3.84		
		<i>T. castaneum</i>	0.14	0.37	0.98		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	2.73	3.70	5.01	2.30	0.1292
		<i>T. castaneum</i>	0.98	1.57	2.52		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	1.42	2.13	3.18		
		<i>T. castaneum</i>	0.14	0.37	0.98		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	18.69	21.02	23.55	83.40	<0.0001
		<i>T. castaneum</i>	26.35	28.98	31.76		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	24.38	26.94	29.67		
		<i>T. castaneum</i>	10.74	12.59	14.71		
Arrested Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	0.31	0.65	1.35	4.85	0.0277
		<i>T. castaneum</i>	0.77	1.30	2.18		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	0.05	0.19	0.74		
		<i>T. castaneum</i>	1.42	2.13	3.18		
Dead Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	11.35	13.24	15.40	31.81	<0.0001
		<i>T. castaneum</i>	35.84	38.70	41.65		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	21.88	24.35	27.00		
		<i>T. castaneum</i>	34.39	37.22	40.15		
Dead Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	11.69	13.61	15.79	12.69	0.0004
		<i>T. castaneum</i>	20.91	23.33	25.95		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	21.08	23.52	26.14		
		<i>T. castaneum</i>	34.39	37.22	40.15		
Live Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	3.61	4.72	6.16	22.77	<0.0001
		<i>T. castaneum</i>	1.87	2.69	3.84		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	6.41	7.87	9.63		
		<i>T. castaneum</i>	12.73	14.72	16.96		
Live Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	1.34	2.04	3.07	8.33	0.5673
		<i>T. castaneum</i>	1.87	2.69	3.84		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	3.13	4.17	5.54		
		<i>T. castaneum</i>	5.24	6.57	8.22		

Table 3.14A. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of total individuals that emerged in each morphological category, at each aerosol concentration (1% pyrethrin and 3% pyrethrin) on treated cotton bag substrate. Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Lower C.L.	Mean	Upper C.L.	X ²	p
Normal, Live Adults	Entech Fog-10 [®] + DiaconII [™]	1.43	2.83	5.51	5.32	0.0211
	Entech Fog-30 [®] + DiaconII [™]	0.00	0.00	0.00		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	0.16	0.44	1.18	0.01	0.9179
	Entech Fog-30 [®] + DiaconII [™]	0.15	0.41	1.10		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	0.39	0.79	1.61	0.02	0.8755
	Entech Fog-30 [®] + DiaconII [™]	0.50	0.85	1.45		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + DiaconII [™]	1.32	1.91	2.75	13.36	0.0003
	Entech Fog-30 [®] + DiaconII [™]	0.15	0.42	1.13		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + DiaconII [™]	23.24	25.03	26.91	0.11	0.7387
	Entech Fog-30 [®] + DiaconII [™]	22.80	24.59	25.46		
Arrested Larvae	Entech Fog-10 [®] + DiaconII [™]	0.49	0.88	1.59	0.53	0.4657
	Entech Fog-30 [®] + DiaconII [™]	0.35	0.64	1.19		
Dead Larvae	Entech Fog-10 [®] + DiaconII [™]	19.62	21.43	23.37	8.78	0.0030
	Entech Fog-30 [®] + DiaconII [™]	23.62	25.45	27.36		
Dead Pupae	Entech Fog-10 [®] + DiaconII [™]	15.01	16.58	18.29	18.87	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	24.28	26.11	28.04		
Live Larvae	Entech Fog-10 [®] + DiaconII [™]	6.27	5.98	8.57	14.11	0.0002
	Entech Fog-30 [®] + DiaconII [™]	9.45	13.00	12.15		
Live Pupae	Entech Fog-10 [®] + DiaconII [™]	1.88	2.45	3.20	18.16	<0.0001
	Entech Fog-30 [®] + DiaconII [™]	4.04	4.88	5.88		

Table 3.14B. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated cotton bag substrate. Data collapsed over the 16-week storage period.

Morphological Category	Spp.	Lower C.L.	Mean	Upper C.L.	χ^2	p
Normal, Live Adults	<i>T. confusum</i>	19.64	21.39	23.25	647.94	<0.0001
	<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults Displaying Twisted Wing Deformity	<i>T. confusum</i>	1.40	1.89	2.56	43.59	<0.0001
	<i>T. castaneum</i>	0.02	0.09	0.37		
Live Adults w/ Unfolded Wing Deformity	<i>T. confusum</i>	1.95	2.55	3.32	45.65	<0.0001
	<i>T. castaneum</i>	0.11	0.26	0.61		
Live Adults Unable to Shed Pupal Cuticle	<i>T. confusum</i>	2.32	3.00	3.86	50.19	<0.0001
	<i>T. castaneum</i>	0.09	0.26	0.74		
Arrested Pupae-Adult Intermediates	<i>T. confusum</i>	26.88	28.75	32.70	32.53	<0.0001
	<i>T. castaneum</i>	19.57	21.25	23.02		
Arrested Larvae	<i>T. confusum</i>	0.12	0.28	0.62	31.96	<0.0001
	<i>T. castaneum</i>	1.50	2.03	2.74		
Dead Larvae	<i>T. confusum</i>	13.70	15.17	16.77	212.65	<0.0001
	<i>T. castaneum</i>	32.27	34.24	36.27		
Dead Pupae	<i>T. confusum</i>	13.42	14.90	16.51	57.91	<0.0001
	<i>T. castaneum</i>	26.76	28.64	30.59		
Live Larvae	<i>T. confusum</i>	5.05	5.98	7.08	62.25	<0.0001
	<i>T. castaneum</i>	11.64	13.00	14.50		
Live Pupae	<i>T. confusum</i>	2.47	3.14	3.98	1.47	0.2253
	<i>T. castaneum</i>	3.07	3.83	4.77		

Table 3.14C. Type 3 statistics from the SAS GENMOD procedure, used to analyze the number of *T. castaneum* and *T. confusum* that emerged in each morphological category on treated cotton bag substrate, at each applied aerosol concentration (1% pyrethrin and 3% pyrethrin). Data collapsed over the 16-week storage period.

Morphological Category	Applied Aerosol Concentration	Spp.	Lower C.L.	Mean	Upper C.L.	X ²	p
Normal, Live Adults	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	28.60	31.30	34.13	1.22	0.2696
		<i>T. castaneum</i>	0.05	0.19	0.74		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	12.04	13.98	16.18		
		<i>T. castaneum</i>	0.00	0.00	0.00		
Live Adults Displaying Twisted Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	1.34	2.04	3.07	0.01	0.9179
		<i>T. castaneum</i>	0.01	0.09	0.65		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	1.12	1.76	2.74		
		<i>T. castaneum</i>	0.01	0.09	0.65		
Live Adults w/ Unfolded Wing Deformity	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	2.41	3.33	4.59	2.01	0.1558
		<i>T. castaneum</i>	0.05	0.19	0.74		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	12.71	1.94	2.96		
		<i>T. castaneum</i>	0.14	0.37	0.98		
Live Adults Unable to Shed Pupal Cuticle	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	3.69	4.81	6.26	1.28	0.2580
		<i>T. castaneum</i>	0.38	0.74	1.47		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	1.20	1.85	2.85		
		<i>T. castaneum</i>	0.01	0.09	0.65		
Arrested Pupae-Adult Intermediates	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	25.99	28.61	31.38	0.28	0.5997
		<i>T. castaneum</i>	19.40	21.76	24.32		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	26.26	28.89	31.67		
		<i>T. castaneum</i>	18.43	20.74	23.26		
Arrested Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	0.09	0.28	0.86	0.53	0.4657
		<i>T. castaneum</i>	1.95	2.78	3.95		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	0.09	0.28	0.86		
		<i>T. castaneum</i>	0.91	1.48	2.40		
Dead Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	9.97	11.76	13.82	23.29	<0.0001
		<i>T. castaneum</i>	33.03	35.83	38.74		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	17.10	19.35	21.82		
		<i>T. castaneum</i>	29.95	32.69	35.54		
Dead Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	8.94	10.65	12.63	28.89	<0.0001
		<i>T. castaneum</i>	22.42	24.91	27.58		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	18.16	20.46	22.97		
		<i>T. castaneum</i>	29.95	32.69	35.54		
Live Larvae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	3.69	4.82	2.65	0.14	0.7126
		<i>T. castaneum</i>	9.29	11.02	13.03		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	59.89	7.41	9.13		
		<i>T. castaneum</i>	13.25	15.28	17.55		
Live Pupae	Entech Fog-10 [®] + DiaconII [™]	<i>T. confusum</i>	1.64	2.41	3.51	0.97	0.3240
		<i>T. castaneum</i>	1.72	25.00	3.62		
	Entech Fog-30 [®] + DiaconII [™]	<i>T. confusum</i>	3.05	4.07	5.43		
		<i>T. castaneum</i>	4.58	5.83	7.40		

Table 3.15

Percentage (mean ± SEM) of ‘affected’ *T. castaneum* and *T. confusum*, at each biweekly post-aerosol application storage period, upon exposure to 1% pyrethrin and 3% pyrethrin formulations, on each treated packaging material surface.

			Wk 0	Wk 2	Wk 4	Wk 6	Wk 8	Wk 10	Wk 12	Wk 14	Wk 16	
Entech Fog-10®+ Diaconil™	<i>T. confusum</i>	Paper Bag	95.8 ± 2.1 a	82.5 ± 5.0 ab	65.0 ± 12.6 abc	59.2 ± 12.5 ab	44.2 ± 10.4 ab	36.7 ± 9.5 a	29.2 ± 10.4 a	24.2 ± 7.2 abc	22.5 ± 2.5 a	
		Cardboard	89.2 ± 4.2 abc	87.5 ± 5.5 a	80.8 ± 3.4 a	75.8 ± 3.7 a	61.7 ± 0.6 ab	55.8 ± 3.4 a	45.0 ± 6.5 a	42.5 ± 3.2 a	44.2 ± 2.1 a	
		Plastic	90.8 ± 5.0 d	83.3 ± 2.7 bc	77.5 ± 3.7 bc	74.2 ± 0.8 ab	73.3 ± 4.7 ab	65.8 ± 6.0 a	65.0 ± 5.0 a	52.5 ± 4.4 ab	50.0 ± 1.4 a	
		Pallet Wrap	90.8 ± 2.1 ab	82.5 ± 4.8 c	72.5 ± 3.2 c	71.7 ± 5.5 b	67.5 ± 4.8 b	62.5 ± 3.4 a	53.3 ± 1.4 a	51.7 ± 2.2 ab	37.5 ± 3.4 a	
		FlourBag	95.0 ± 4.0 bc	82.5 ± 5.0 bc	75.8 ± 3.2 d	65.8 ± 6.3 b	55.0 ± 8.4 b	45.8 ± 3.7 a	40.8 ± 4.2 a	25.0 ± 8.8 bcd	22.5 ± 2.5 b	
		Poly. Bag	100.0 ± 0.0 bc	89.2 ± 4.8 c	84.2 ± 5.7 ab	77.5 ± 4.4 c	64.2 ± 7.6 c	55.0 ± 5.5 b	46.7 ± 3.0 b	44.2 ± 3.7 d	31.7 ± 3.2 a	
		Cotton Bag	92.5 ± 3.7 c	83.3 ± 4.1 bc	77.5 ± 5.5 abc	65.8 ± 4.4 ab	64.2 ± 1.6 ab	60.0 ± 4.1 b	61.7 ± 3.5 b	56.7 ± 8.3 cd	56.7 ± 3.6 a	
		<i>T. castaneum</i>	Paper Bag	100.0 ± 0.0 a	100.0 ± 0.0 a	99.2 ± 0.8 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	Cardboard	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	Plastic	100.0 ± 0.0 a	100.0 ± 0.0 a	99.2 ± 0.8 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	Pallet Wrap	97.5 ± 2.5 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	97.5 ± 1.6 a	
	FlourBag	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	
	Poly. Bag	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	
	Cotton Bag	100.0 ± 0.0 a	100.0 ± 0.0 a	99.2 ± 0.8 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	99.2 ± 0.8 a	100.0 ± 0.0 a	
	Entech Fog-30®+ Diaconil™	<i>T. confusum</i>	Paper Bag	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	97.5 ± 1.6 a	93.3 ± 3.0 a	91.7 ± 4.0 a	91.7 ± 2.2 a	91.7 ± 3.2 a	87.5 ± 1.6 a
			Cardboard	100.0 ± 0.0 a	100.0 ± 0.0 a	99.2 ± 0.8 a	100.0 ± 0.0 a	98.3 ± 1.0 a	97.5 ± 1.6 a	96.7 ± 2.4 a	93.3 ± 2.4 a	89.2 ± 2.5 a
Plastic			100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	98.3 ± 1.0 a	98.3 ± 1.7 a	92.5 ± 2.1 a	95.8 ± 4.2 a	93.3 ± 2.4 a	92.5 ± 2.5 a	
Pallet Wrap			100.0 ± 0.0 a	100.0 ± 0.0 a	96.7 ± 3.3 a	95.0 ± 2.9 a	96.7 ± 3.3 a	90.8 ± 3.2 a	87.5 ± 2.5 a	85.0 ± 2.2 a	82.5 ± 3.2 a	
FlourBag			100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	99.2 ± 0.8 a	93.3 ± 3.6 a	88.3 ± 3.5 a	89.2 ± 5.0 a	86.7 ± 2.7 a	84.2 ± 3.9 a	
Poly. Bag			99.2 ± 0.8 a	100.0 ± 0.0 a	96.7 ± 2.4 a	95.0 ± 2.9 a	92.5 ± 2.1 a	92.5 ± 2.5 a	90.0 ± 3.0 a	89.2 ± 3.4 a	79.2 ± 3.4 b	
Cotton Bag			99.2 ± 0.8 a	98.3 ± 1.0 a	99.2 ± 2.5 a	90.8 ± 5.3 a	83.3 ± 3.6 b	79.2 ± 3.7 b	75.0 ± 2.9 b	78.3 ± 1.0 b	78.5 ± 1.6 b	
<i>T. castaneum</i>			Paper Bag	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	99.2 ± 0.8 a	98.3 ± 1.7 a
Cardboard		100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	98.3 ± 1.0 a	99.2 ± 0.8 a	
Plastic		100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	98.3 ± 1.7 a	
Pallet Wrap		100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	98.3 ± 1.0 a	100.0 ± 0.0 a	
FlourBag		100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	99.2 ± 0.8 a	99.2 ± 0.8 a	
Poly. Bag		100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	99.2 ± 0.8 a	100.0 ± 0.0 a	99.2 ± 0.8 a	
Cotton Bag		100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	

^a Means within columns corresponding to each treatment, followed by different letters are significantly different by experiment-wise t-tests (P<0.05 Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

Appendix Tables and Figures

Appendix Table 3.1A. Percentage (mean \pm SE) of adult morphological characteristics: normal adult development; individuals displaying the twisted wing deformity, individuals unable to properly fold their wings beneath the elytra/wings; and adults unable to shed the pupae cuticle, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated paper bag surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Normal Adult Development				Adults w/ Twisted Wing Deformity				Adults w/ Unfolded Wing Deformity				Adults Unable to Shed Pupal Cuticle											
	Entech Fog-30 [®] + Diacon II [™]				Entech Fog-30 [®] + Diacon II [™]				Entech Fog-30 [®] + Diacon II [™]				Entech Fog-30 [®] + Diacon II [™]											
	<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>									
Ctrl	88.33	±	3.47	91.67	±	3.97	b	±	b	b	±	b	b	±	b	b	±	b						
0	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	0.83	±	0.83	2.50	±	2.50			
2	b	±	b	b	±	b	b	±	b	b	±	b	2.50	±	2.50	b	±	b	5.83	±	5.83			
4	b	±	b	b	±	b	b	±	b	b	±	b	0.83	±	0.83	b	±	b	0.83	±	0.83			
6	b	±	b	2.50	±	1.60	b	±	b	b	±	0.83	b	±	b	1.67	±	1.67	b	±	b	3.33	±	1.36
8	b	±	b	6.67	±	3.04	b	±	b	b	±	0.83	0.83	±	0.83	1.67	±	1.67	b	±	b	1.67	±	1.67
10	b	±	b	8.33	±	3.97	b	±	b	b	±	0.83	b	±	b	0.83	±	0.83	0.83	±	0.83	0.83	±	0.83
12	b	±	b	8.33	±	2.15	b	±	b	b	±	b	b	±	b	2.50	±	1.60	0.83	±	0.83	4.17	±	4.17
14	0.83	±	0.83	8.33	±	3.19	b	±	b	3.33	±	1.36	b	±	b	8.33	±	3.47	b	±	b	6.67	±	2.36
16	1.67	±	1.67	12.50	±	1.60	b	±	b	3.33	±	1.92	b	±	b	4.17	±	1.60	b	±	b	5.00	±	2.15
Storage Wks.	Entech Fog-10 [®] + Diacon II [™]				Entech Fog-10 [®] + Diacon II [™]				Entech Fog-10 [®] + Diacon II [™]				Entech Fog-10 [®] + Diacon II [™]											
	<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>									
	Ctrl	93.33	±	2.72	95.83	±	2.10	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b		
0	b	±	b	4.17	±	2.10	b	±	b	0.83	±	0.83	b	±	b	13.33	±	2.36	b	±	b	0.83	±	0.83
2	b	±	b	17.50	±	4.98	b	±	b	1.67	±	1.67	b	±	b	5.83	±	2.85	2.50	±	1.60	7.50	±	3.15
4	0.83	±	b	35.00	±	12.58	b	±	b	2.50	±	1.60	b	±	b	7.50	±	3.44	0.83	±	0.83	6.67	±	0.00
6	b	±	0.00	40.83	±	12.50	b	±	b	3.33	±	1.36	2.50	±	2.50	5.00	±	2.15	0.83	±	0.83	3.33	±	1.92
8	b	±	b	55.83	±	10.40	b	±	b	1.67	±	1.67	0.83	±	0.83	5.00	±	0.96	1.67	±	0.96	1.67	±	0.96
10	b	±	b	63.33	±	9.53	b	±	b	6.67	±	2.36	0.83	±	0.83	3.33	±	1.36	1.67	±	0.96	3.33	±	1.92
12	b	±	b	70.83	±	10.40	b	±	b	3.33	±	2.36	1.67	±	1.67	4.17	±	2.50	1.67	±	1.67	5.83	±	2.85
14	b	±	b	75.83	±	7.25	0.83	±	0.83	2.50	±	1.60	2.50	±	1.60	1.67	±	0.96	2.50	±	1.60	2.50	±	2.50
16	b	±	b	77.50	±	2.50	1.67	±	0.96	7.50	±	3.70	0.83	±	0.83	2.50	±	1.60	3.33	±	3.33	2.50	±	1.60

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.1B. Percentage (mean ± SE) of pupae morphological characteristics: pupae-adult intermediates, pupae not able to undergo further development, and dead pupae, as a percentage of the total number of individuals (four-week old larvae) initially to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated paper bag surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Pupae-Adult Intermediates						Pupae (Alive)						Dead Pupae					
	Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
Ctrl	b	±	b	b	±	b	b	±	b	b	±	b	4.17	±	2.10	2.50	±	1.60
0	5.83	±	2.85	17.50	±	4.17	8.33	±	1.67	5.83	±	2.10	20.83	±	4.59	36.67	±	6.94
2	10.83	±	3.70	12.50	±	4.98	5.83	±	2.10	8.33	±	0.96	19.17	±	3.15	34.17	±	3.70
4	10.00	±	5.77	20.00	±	4.91	9.17	±	4.17	10.83	±	2.85	21.67	±	5.00	35.00	±	3.97
6	17.50	±	4.79	22.50	±	3.70	7.50	±	2.50	7.50	±	2.50	25.00	±	3.19	26.67	±	4.30
8	24.17	±	0.83	24.17	±	3.70	9.17	±	2.50	6.67	±	2.72	25.83	±	5.16	23.33	±	4.08
10	25.83	±	5.51	31.67	±	2.15	4.17	±	2.50	11.67	±	4.19	28.33	±	2.15	15.00	±	2.89
12	22.50	±	6.44	34.17	±	3.15	6.67	±	1.36	8.33	±	2.89	25.00	±	0.96	20.00	±	2.36
14	21.67	±	5.53	35.00	±	1.67	5.83	±	2.85	0.83	±	0.83	27.50	±	2.50	15.00	±	4.81
16	26.67	±	2.36	35.83	±	2.85	2.50	±	0.83	1.67	±	0.96	28.33	±	1.67	24.17	±	5.16
Storage Wks.	Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
	Ctrl	b	±	b	b	±	b	b	±	b	b	±	b	2.50	±	1.60	b	±
0	16.67	±	3.04	36.67	±	3.60	10.00	±	2.36	0.83	±	0.83	36.67	±	6.94	16.67	±	3.60
2	36.67	±	5.61	34.17	±	4.59	4.17	±	2.10	5.00	±	3.19	34.17	±	3.70	9.17	±	3.70
4	33.33	±	4.51	27.50	±	2.50	1.67	±	0.96	0.83	±	0.83	35.00	±	3.97	10.83	±	4.38
6	44.17	±	4.98	22.50	±	6.44	5.00	±	3.97	b	±	b	26.67	±	4.30	13.33	±	3.33
8	40.00	±	4.91	23.33	±	5.77	5.00	±	2.89	0.83	±	0.83	23.33	±	4.08	7.50	±	3.70
10	43.33	±	4.91	14.17	±	3.70	5.83	±	2.85	0.83	±	0.83	15.00	±	2.89	4.17	±	3.15
12	50.00	±	4.91	9.17	±	3.44	5.00	±	2.15	b	±	b	20.00	±	2.36	3.33	±	1.92
14	46.67	±	5.93	10.00	±	4.30	4.17	±	1.60	b	±	b	15.00	±	4.81	5.00	±	2.15
16	48.33	±	7.52	8.33	±	3.47	3.33	±	1.92	b	±	b	24.17	±	5.16	1.67	±	0.96

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.1C. Percentage (mean \pm SE) of larvae morphological characteristics: individuals remaining in the larvae stage, those that died while in the larvae stage and those that were arrested between larvae and pupae development, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated paper bag surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Larvae (Alive)						Dead Larvae						Arrested Larvae					
	Entech Fog-30 [®] + Diacon II [™]						Entech Fog-30 [®] + Diacon II [™]						Entech Fog-30 [®] + Diacon II [™]					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
Ctrl	b	±	b	b	±	b	7.50	±	3.70	5.83	±	2.50	b	±	b	b	±	b
0	40.00	±	3.04	15.83	±	2.85	24.17	±	6.14	21.67	±	5.53	b	±	b	b	±	b
2	29.17	±	4.98	12.50	±	4.59	34.17	±	10.13	24.17	±	3.15	0.83	±	0.83	0.00	±	0.00
4	32.50	±	5.99	11.67	±	2.15	25.83	±	7.62	20.00	±	3.60	0.83	±	0.83	0.83	±	0.83
6	20.83	±	4.79	8.33	±	4.19	20.00	±	7.58	25.83	±	2.10	9.17	±	3.44	0.83	±	0.83
8	15.83	±	4.38	9.17	±	5.16	23.33	±	3.04	23.33	±	3.60	0.83	±	0.83	2.50	±	1.60
10	13.33	±	5.27	9.17	±	5.16	26.67	±	1.36	20.00	±	2.72	0.83	±	0.83	1.67	±	0.96
12	13.33	±	4.71	6.67	±	1.92	26.67	±	3.60	15.83	±	2.50	5.00	±	2.89	b	±	b
14	10.00	±	3.60	5.83	±	3.44	30.83	±	0.83	16.67	±	3.04	3.33	±	1.36	b	±	b
16	8.33	±	4.81	0.83	±	0.83	29.17	±	1.60	10.83	±	0.83	3.33	±	1.36	1.67	±	0.96
Storage Wks.	Entech Fog-10 [®] + Diacon II [™]						Entech Fog-10 [®] + Diacon II [™]						Entech Fog-10 [®] + Diacon II [™]					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
	Ctrl	0.83	±	0.83	0.83	±	0.83	5.00	±	2.50	3.33	±	1.36	b	±	b	b	±
0	17.50	±	2.85	7.50	±	2.50	30.83	±	5.53	17.50	±	2.10	0.83	±	0.83	1.67	±	0.96
2	10.83	±	6.29	4.17	±	2.10	27.50	±	3.15	15.00	±	2.89	2.50	±	1.60	b	±	b
4	9.17	±	4.59	1.67	±	1.67	24.17	±	3.60	7.50	±	4.38	2.50	±	1.60	b	±	b
6	8.33	±	5.18	2.50	±	1.60	8.33	±	2.10	9.17	±	2.10	1.67	±	1.67	b	±	b
8	9.17	±	3.94	0.00	±	0.00	19.17	±	3.60	4.17	±	2.50	0.83	±	0.83	b	±	b
10	10.00	±	2.36	0.00	±	0.00	14.17	±	2.72	3.33	±	1.92	2.50	±	0.83	0.83	±	0.83
12	0.83	±	0.83	0.00	±	0.00	14.17	±	2.50	3.33	±	3.33	1.67	±	0.96	b	±	b
14	1.67	±	1.67	0.00	±	0.00	21.67	±	3.04	2.50	±	1.60	0.83	±	0.83	b	±	b
16	1.67	±	1.67	0.00	±	0.00	17.50	±	0.83	0.00	±	0.00	1.67	±	1.67	b	±	b

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.2A. Percentage (mean ± SE) of adult morphological characteristics: normal adult development; individuals displaying the twisted wing deformity, individuals unable to properly fold their wings beneath the elytra/wings; and adults unable to shed the pupae cuticle, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated cardboard surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Normal Adult Development				Adults w/ Twisted Wing Deformity				Adults w/ Unfolded Wing Deformity				Adults Unable to Shed Pupal Cuticle											
	Entech Fog-30® + Diacon II™								Entech Fog-30® + Diacon II™								Entech Fog-30® + Diacon II™							
	<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>									
Ctrl	90.83	±	1.60	91.67	±	1.67	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	5.00	±	3.97
0	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b
2	b	±	b	b	±	b	b	±	b	0.83	±	0.83	b	±	b	1.67	±	0.96	b	±	b	b	±	b
4	b	±	b	0.83	±	0.83	b	±	b	b	±	b	b	±	b	0.83	±	0.83	b	±	b	0.83	±	0.83
6	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b
8	b	±	b	1.67	±	0.96	b	±	b	b	±	b	b	±	b	1.67	±	1.67	b	±	b	1.67	±	0.96
10	b	±	b	2.50	±	1.60	b	±	b	b	±	b	b	±	b	3.33	±	2.36	b	±	b	1.67	±	0.96
12	b	±	b	3.33	±	2.36	b	±	b	1.67	±	1.67	b	±	b	4.17	±	2.10	b	±	b	0.83	±	0.83
14	1.67	±	0.96	6.67	±	2.36	b	±	b	1.67	±	0.96	b	±	b	3.33	±	3.33	b	±	b	1.67	±	0.96
16	0.83	±	0.83	10.83	±	2.50	0.83	±	0.83	1.67	±	0.96	1.67	±	1.67	5.83	±	1.60	0.83	±	0.83	4.17	±	2.50
Storage Wks.	Entech Fog-10® + Diacon II™				Entech Fog-10® + Diacon II™				Entech Fog-10® + Diacon II™				Entech Fog-10® + Diacon II™											
	<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>									
	Ctrl	97.50	±	1.60	98.33	±	0.96	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±
0	0.00	±	0.00	10.83	±	4.17	0.00	±	0.00	0.83	±	0.83	0.00	±	0.00	2.50	±	1.60	0.00	±	0.00	0.83	±	0.83
2	0.00	±	0.00	12.50	±	5.51	0.00	±	0.00	0.83	±	0.83	0.00	±	0.00	5.00	±	3.97	0.83	±	0.83	5.00	±	3.19
4	0.00	±	0.00	19.17	±	3.44	0.00	±	0.00	2.50	±	2.50	0.00	±	0.00	5.00	±	2.15	0.00	±	0.00	11.67	±	5.53
6	0.00	±	0.00	24.17	±	3.70	0.00	±	0.00	1.67	±	0.96	0.00	±	0.00	13.33	±	2.36	0.83	±	0.83	7.50	±	4.38
8	0.00	±	0.00	38.33	±	6.16	0.00	±	0.00	4.17	±	2.50	0.00	±	0.00	5.00	±	0.96	0.83	±	0.83	8.33	±	2.15
10	0.00	±	0.00	44.17	±	3.44	0.00	±	0.00	4.17	±	1.60	0.00	±	0.00	2.50	±	0.83	0.00	±	0.00	6.67	±	2.72
12	0.00	±	0.00	55.00	±	6.45	0.00	±	0.00	1.67	±	0.96	0.00	±	0.00	5.00	±	2.89	0.83	±	0.83	10.00	±	2.36
14	0.00	±	0.00	57.50	±	3.15	0.00	±	0.00	1.67	±	1.67	0.00	±	0.00	9.17	±	2.85	0.83	±	0.83	4.17	±	2.10
16	0.00	±	0.00	55.83	±	2.10	0.00	±	0.00	7.50	±	1.60	0.83	±	0.83	4.17	±	0.83	2.50	±	1.60	5.00	±	2.15

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.2B. Percentage (mean \pm SE) of pupae morphological characteristics: pupae-adult intermediates, pupae not able to undergo further development, and dead pupae, as a percentage of the total number of individuals (four-week old larvae) initially to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated cardboard surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Pupae-Adult Intermediates						Pupae (Alive)						Dead Pupae					
	Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
Ctrl	b	±	b	b	±	b	b	±	b	b	±	b	3.33	±	1.36	4.17	±	2.10
0	1.67	±	0.96	8.33	±	4.41	8.33	±	0.96	9.17	±	4.17	13.33	±	3.60	35.00	±	7.01
2	4.17	±	3.15	20.00	±	4.71	3.33	±	2.36	7.50	±	2.85	14.17	±	4.38	32.50	±	4.59
4	6.67	±	4.51	25.00	±	7.26	9.17	±	2.85	2.50	±	2.50	20.00	±	6.38	39.17	±	3.44
6	10.00	±	2.36	33.33	±	5.27	6.67	±	1.36	5.00	±	2.15	20.00	±	5.77	35.00	±	2.15
8	15.00	±	4.81	43.33	±	5.61	5.83	±	2.85	1.67	±	0.96	32.50	±	4.59	30.83	±	7.25
10	16.67	±	2.36	45.83	±	1.60	4.17	±	2.10	2.50	±	1.60	25.83	±	0.83	22.50	±	2.85
12	14.17	±	3.70	43.33	±	4.30	5.00	±	1.67	4.17	±	2.50	29.17	±	5.83	19.17	±	3.15
14	23.33	±	4.08	45.83	±	6.44	4.17	±	0.83	0.00	±	0.00	24.17	±	5.99	19.17	±	5.51
16	25.83	±	2.50	38.33	±	5.85	4.17	±	2.10	0.83	±	0.83	23.33	±	2.72	14.17	±	3.44
Storage Wks.	Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
	Ctrl	b	±	b	b	±	b	b	±	b	b	±	b	4.17	±	2.10	b	±
0	23.33	±	3.04	36.67	±	11.14	2.50	±	1.60	1.67	±	1.67	35.00	±	7.01	20.00	±	8.92
2	25.00	±	7.26	38.33	±	4.41	1.67	±	1.67	2.50	±	1.60	32.50	±	4.59	20.83	±	3.15
4	29.17	±	6.72	30.83	±	2.85	0.83	±	0.83	2.50	±	1.60	39.17	±	3.44	15.00	±	3.47
6	31.67	±	9.67	26.67	±	2.36	5.83	±	1.60	1.67	±	1.67	35.00	±	2.15	10.83	±	3.70
8	34.17	±	4.38	21.67	±	5.53	3.33	±	1.36	2.50	±	1.60	30.83	±	7.25	9.17	±	2.50
10	34.17	±	3.94	26.67	±	3.60	b	±	b	0.83	±	0.83	22.50	±	2.85	8.33	±	2.15
12	40.00	±	6.24	19.17	±	1.60	3.33	±	1.92	1.67	±	0.96	19.17	±	3.15	3.33	±	1.92
14	38.33	±	8.44	18.33	±	5.00	3.33	±	1.36	0.83	±	0.83	19.17	±	5.51	4.17	±	1.60
16	38.33	±	8.44	15.00	±	0.96	3.33	±	1.36	0.00	±	0.00	14.17	±	3.44	6.67	±	0.00

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.2C. Percentage (mean ± SE) of larvae morphological characteristics: individuals remaining in the larvae stage, those that died while in the larvae stage and those that were arrested between larvae and pupae development, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene

and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated cardboard surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Larvae (Alive)				Dead Larvae				Arrested Larvae									
	Entech Fog-30® + Diacon II™				Entech Fog-30® + Diacon II™				Entech Fog-30® + Diacon II™									
	<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>							
Ctrl	b	±	b	0.83	±	0.83	5.83	±	2.10	3.33	±	1.36	b	±	b	b	±	b
0	55.00	±	5.69	25.00	±	3.97	21.67	±	3.19	17.50	±	4.98	b	±	b	b	±	b
2	59.17	±	10.03	19.17	±	2.50	19.17	±	3.70	17.50	±	2.85	b	±	b	0.83	±	0.83
4	44.17	±	13.84	13.33	±	6.38	20.00	±	3.60	17.50	±	2.85	b	±	b	b	±	b
6	37.50	±	7.25	8.33	±	2.15	23.33	±	3.85	17.50	±	2.10	2.50	±	1.60	0.83	±	0.83
8	18.33	±	2.15	3.33	±	1.36	25.83	±	2.10	14.17	±	2.50	1.67	±	0.96	1.67	±	0.96
10	21.67	±	2.15	5.83	±	3.70	28.33	±	2.15	15.83	±	4.17	3.33	±	1.36	b	±	b
12	20.00	±	4.71	0.83	±	0.83	29.17	±	0.83	22.50	±	6.44	2.50	±	1.60	b	±	b
14	10.00	±	4.30	2.50	±	2.50	34.17	±	3.70	19.17	±	4.79	2.50	±	0.83	b	±	b
16	10.00	±	5.27	7.50	±	1.60	30.00	±	2.36	15.83	±	5.99	2.50	±	1.60	0.83	±	0.83
Storage Wks.	Entech Fog-10® + Diacon II™				Entech Fog-10® + Diacon II™				Entech Fog-10® + Diacon II™									
	<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>							
	b	±	b	b	±	b	2.50	±	1.36	1.67	±	0.96	b	±	b	b	±	b
0	25.00	±	0.96	5.83	±	2.50	23.33	±	4.98	20.83	±	4.79	1.67	±	0.96	b	±	b
2	4.17	±	4.17	4.17	±	2.10	39.17	±	2.85	10.83	±	4.79	4.17	±	2.10	b	±	b
4	5.00	±	1.67	1.67	±	1.67	34.17	±	2.85	11.67	±	5.18	4.17	±	2.10	b	±	b
6	2.50	±	1.60	7.50	±	5.34	30.00	±	2.10	6.67	±	1.92	2.50	±	0.83	b	±	b
8	1.67	±	0.96	4.17	±	3.15	26.67	±	2.50	6.67	±	2.36	3.33	±	1.36	b	±	b
10	7.50	±	3.70	2.50	±	1.60	27.50	±	4.17	4.17	±	2.50	4.17	±	2.10	b	±	b
12	4.17	±	1.60	0.00	±	0.00	24.17	±	6.44	4.17	±	2.10	1.67	±	0.96	b	±	b
14	3.33	±	2.36	1.67	±	0.96	30.83	±	4.79	2.50	±	0.83	0.83	±	0.83	b	±	b
16	1.67	±	1.67	0.00	±	0.00	24.17	±	5.99	5.83	±	2.85	b	±	b	b	±	b

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.3A. Percentage (mean \pm SE) of adult morphological characteristics: normal adult development; individuals displaying the twisted wing deformity, individuals unable to properly fold their wings beneath the elytra/wings; and adults unable to shed the pupae cuticle, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated plastic surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Normal Adult Development				Adults w/ Twisted Wing Deformity				Adults w/ Unfolded Wing Deformity				Adults Unable to Shed Pupal Cuticle																			
	Entech Fog-30 [®] + Diacon II [™]								Entech Fog-30 [®] + Diacon II [™]								Entech Fog-30 [®] + Diacon II [™]								Entech Fog-30 [®] + Diacon II [™]							
	<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>													
Ctrl	89.17	±	3.70	87.50	±	4.98	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b											
0	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	2.50	±	2.50											
2	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	0.83	±	0.83											
4	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	0.00	±	0.00											
6	b	±	b	1.67	±	0.96	b	±	b	b	±	b	b	±	b	0.83	±	0.83	b	±	b											
8	b	±	b	1.67	±	1.67	b	±	b	b	±	b	b	±	b	1.67	±	1.67	b	±	b											
10	b	±	b	7.50	±	2.10	b	±	b	b	±	b	b	±	b	1.67	±	1.67	b	±	b											
12	b	±	b	4.17	±	4.17	b	±	b	1.67	±	0.96	0.83	±	0.83	4.17	±	1.60	b	±	b											
14	b	±	b	6.67	±	2.36	b	±	b	0.83	±	0.83	b	±	b	3.33	±	1.36	b	±	b											
16	1.67	±	1.67	7.50	±	2.50	b	±	b	0.83	±	0.83	0.83	±	0.83	3.33	±	1.36	b	±	b											
5.00	±	5.00	5.00	±	5.00	5.00	±	5.00	5.00	±	5.00	5.00	±	5.00	5.00	±	5.00	5.00	±	5.00	5.00	±	5.00									

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.3B. Percentage (mean ± SE) of pupae morphological characteristics: pupae-adult intermediates, pupae not able to undergo further development, and dead pupae, as a percentage of the total number of individuals (four-week old

larvae) initially to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated plastic surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Pupae-Adult Intermediates						Pupae (Alive)						Dead Pupae					
	Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
Ctrl	b	±	b	b	±	b	0.83	±	0.83	b	±	b	5.83	±	2.85	5.83	±	3.94
0	1.67	±	0.96	10.83	±	4.98	3.33	±	2.36	2.50	±	2.50	21.67	±	3.19	41.67	±	9.67
2	4.17	±	3.15	20.83	±	4.38	5.83	±	2.85	5.00	±	3.97	18.33	±	5.85	34.17	±	4.59
4	10.00	±	3.60	20.83	±	3.70	7.50	±	1.60	8.33	±	0.96	15.83	±	2.10	30.00	±	2.72
6	10.83	±	4.59	23.33	±	2.36	6.67	±	2.36	9.17	±	0.83	19.17	±	3.44	30.83	±	4.79
8	11.67	±	5.00	25.00	±	1.67	5.83	±	3.44	5.00	±	0.96	24.17	±	2.50	25.00	±	6.31
10	13.33	±	3.60	25.00	±	4.19	2.50	±	1.60	3.33	±	1.92	25.00	±	3.97	24.17	±	3.94
12	20.83	±	5.34	22.50	±	2.50	11.67	±	4.19	3.33	±	1.36	24.17	±	2.10	27.50	±	2.50
14	18.33	±	4.41	29.17	±	5.51	4.17	±	0.83	2.50	±	1.60	20.83	±	2.50	22.50	±	5.34
16	19.17	±	3.94	35.83	±	3.70	2.50	±	1.60	0.83	±	0.83	25.83	±	1.60	23.33	±	3.33
Storage Wks.	Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
Ctrl	b	±	b	b	±	b	b	±	b	b	±	b	5.83	±	3.94	0.83	±	0.83
0	5.00	±	3.97	35.83	±	4.98	1.67	±	1.67	b	±	b	41.67	±	9.67	20.00	±	6.53
2	25.83	±	1.60	27.50	±	5.83	4.17	±	2.10	1.67	±	0.96	34.17	±	4.59	12.50	±	4.38
4	27.50	±	2.10	29.17	±	5.51	2.50	±	0.83	0.83	±	0.83	30.00	±	2.72	14.17	±	2.85
6	34.17	±	4.98	32.50	±	7.74	1.67	±	0.96	b	±	b	30.83	±	4.79	10.83	±	4.59
8	32.50	±	6.72	24.17	±	5.83	1.67	±	1.67	3.33	±	b	25.00	±	6.31	11.67	±	2.15
10	32.50	±	5.67	25.00	±	6.16	5.00	±	1.67	3.33	±	1.36	24.17	±	3.94	8.33	±	3.19
12	31.67	±	7.01	26.67	±	2.72	5.00	±	2.15	1.67	±	0.96	27.50	±	2.50	9.17	±	4.59
14	34.17	±	5.83	17.50	±	2.85	b	±	b	0.83	±	0.83	22.50	±	5.34	10.83	±	3.44
16	37.50	±	5.99	21.67	±	1.67	1.67	±	0.96	b	±	b	23.33	±	3.33	8.33	±	0.96

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.3C. Percentage (mean ± SE) of larvae morphological characteristics: individuals remaining in the larvae stage, those that died while in the larvae stage and those that were arrested between larvae and pupae development, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated plastic surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Larvae (Alive)					Dead Larvae					Arrested Larvae							
	Entech Fog-30® + Diacon II™					Entech Fog-30® + Diacon II™					Entech Fog-30® + Diacon II™							
	<i>T. castaneum</i>		<i>T. confusum</i>			<i>T. castaneum</i>		<i>T. confusum</i>			<i>T. castaneum</i>		<i>T. confusum</i>					
Ctrl	b	±	b	b	±	b	4.17	±	1.60	6.67	±	2.36	b	±	b	b	±	b
0	42.50	±	4.17	20.00	±	5.77	28.33	±	2.15	22.50	±	3.70	2.50	±	1.60	b	±	b
2	40.83	±	3.70	17.50	±	3.70	29.17	±	5.34	21.67	±	5.53	1.67	±	0.96	b	±	b
4	35.00	±	2.15	18.33	±	1.67	29.17	±	4.59	22.50	±	3.94	2.50	±	2.50	b	±	b
6	26.67	±	2.36	13.33	±	2.36	33.33	±	5.93	19.17	±	3.94	3.33	±	1.36	0.83	±	0.83
8	20.00	±	4.51	15.00	±	4.81	34.17	±	2.50	26.67	±	1.36	3.33	±	1.36	b	±	b
10	16.67	±	5.93	15.00	±	4.81	39.17	±	4.38	21.67	±	2.89	3.33	±	1.92	0.83	±	0.83
12	10.00	±	4.91	6.67	±	2.36	32.50	±	6.29	25.00	±	2.89	b	±	b	0.83	±	0.83
14	14.17	±	5.51	3.33	±	1.92	40.00	±	6.09	28.33	±	5.18	2.50	±	0.83	b	±	b
16	10.83	±	5.51	0.00	±	0.00	37.50	±	4.79	23.33	±	1.36	1.67	±	0.96	b	±	b
Storage Wks.	Entech Fog-10® + Diacon II™					Entech Fog-10® + Diacon II™					Entech Fog-10® + Diacon II™							
	<i>T. castaneum</i>		<i>T. confusum</i>			<i>T. castaneum</i>		<i>T. confusum</i>			<i>T. castaneum</i>		<i>T. confusum</i>					
Ctrl	0.83	±	0.83	0.00	±	0.00	0.83	±	2.36	1.67	±	0.96	b	±	b	b	±	b
0	28.33	±	5.18	8.33	±	5.53	31.67	±	3.70	14.17	±	3.44	5.00	±	1.67	0.83	±	0.83
2	10.00	±	5.77	6.67	±	1.36	32.50	±	5.53	20.00	±	5.27	7.50	±	1.60	b	±	b
4	14.17	±	5.16	2.50	±	1.60	30.00	±	3.94	11.67	±	2.89	1.67	±	0.96	b	±	b
6	9.17	±	3.70	1.67	±	0.96	27.50	±	3.94	9.17	±	4.17	0.83	±	0.83	b	±	0.00
8	2.50	±	1.60	3.33	±	1.92	33.33	±	1.36	10.00	±	3.60	2.50	±	1.60	0.83	±	0.83
10	3.33	±	1.36	0.83	±	0.83	27.50	±	2.89	10.00	±	1.36	1.67	±	0.96	b	±	b
12	5.83	±	2.85	0.83	±	0.83	30.83	±	2.89	5.83	±	3.44	2.50	±	1.60	b	±	b
14	5.00	±	2.15	0.83	±	0.83	33.33	±	5.18	5.00	±	2.15	0.83	±	0.83	b	±	b
16	5.83	±	2.85	0.00	±	0.00	26.67	±	1.36	4.17	±	2.10	1.67	±	1.67	0.83	±	0.83

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.4A. Percentage (mean \pm SE) of adult morphological characteristics: normal adult development; individuals displaying the twisted wing deformity, individuals unable to properly fold their wings beneath the elytra/wings; and adults unable to shed the pupae cuticle, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated pallet wrap surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Normal Adult Development			Adults w/ Twisted Wing Deformity			Adults w/ Unfolded Wing Deformity			Adults Unable to Shed Pupal Cuticle		
	Entech Fog-30 [®] + Diacon II [™]			Entech Fog-30 [®] + Diacon II [™]			Entech Fog-30 [®] + Diacon II [™]			Entech Fog-30 [®] + Diacon II [™]		
	<i>T. castaneum</i>	<i>T. confusum</i>		<i>T. castaneum</i>	<i>T. confusum</i>		<i>T. castaneum</i>	<i>T. confusum</i>		<i>T. castaneum</i>	<i>T. confusum</i>	
Ctrl	80.83 ± 3.70	87.50 ± 2.85		b ± b	b ± b		b ± b	b ± b		b ± b	b ± b	
0	b ± b	b ± b		b ± b	b ± b		b ± b	1.67 ± 0.96		b ± b	0.83 ± 0.83	
2	b ± b	b ± b		b ± b	0.83 ± 0.83		b ± b	2.50 ± 1.60		b ± b	0.83 ± 0.83	
4	b ± b	3.33 ± 3.33		b ± b	1.67 ± 0.96		b ± b	3.33 ± 2.36		b ± b	1.67 ± 1.67	
6	b ± b	5.00 ± 2.89		b ± b	0.83 ± 0.83		b ± b	5.83 ± 0.83		b ± b	3.33 ± 1.36	
8	b ± b	3.33 ± 3.33		b ± b	b ± b		b ± b	4.17 ± 2.10		0.83 ± 0.83	0.83 ± 0.83	
10	b ± b	9.17 ± 3.15		b ± b	b ± b		b ± b	3.33 ± 2.36		b ± b	1.67 ± 0.96	
12	b ± b	12.50 ± 2.50		b ± b	3.33 ± 1.36		b ± b	10.83 ± 3.70		0.83 ± 0.83	1.67 ± 0.96	
14	1.67 ± 0.96	15.00 ± 2.15		b ± b	2.50 ± 1.60		0.83 ± 0.83	3.33 ± 1.36		b ± b	2.50 ± 1.60	
16	b ± b	17.50 ± 3.15		b ± b	5.00 ± 0.96		b ± b	7.50 ± 1.60		3.33 ± 2.36	5.83 ± 1.60	
Storage Wks.	Entech Fog-10 [®] + Diacon II [™]			Entech Fog-10 [®] + Diacon II [™]			Entech Fog-10 [®] + Diacon II [™]			Entech Fog-10 [®] + Diacon II [™]		
	<i>T. castaneum</i>	<i>T. confusum</i>		<i>T. castaneum</i>	<i>T. confusum</i>		<i>T. castaneum</i>	<i>T. confusum</i>		<i>T. castaneum</i>	<i>T. confusum</i>	
	Ctrl	92.50 ± 3.70	88.33 ± 3.97		b ± b	b ± b		b ± b	b ± b		b ± b	b ± b
0	2.50 ± 2.50	9.17 ± 2.10		b ± b	0.83 ± 0.83		1.67 ± 1.67	4.17 ± 1.60		b ± b	1.67 ± 0.96	
2	b ± b	17.50 ± 4.79		b ± b	3.33 ± 1.36		b ± b	5.00 ± 1.67		1.67 ± 0.96	5.83 ± 2.10	
4	b ± b	27.50 ± 3.15		b ± b	1.67 ± 0.96		b ± b	5.00 ± 3.97		0.83 ± 0.83	8.33 ± 3.47	
6	b ± b	28.33 ± 5.53		b ± b	1.67 ± 0.96		0.83 ± 0.83	5.00 ± 2.15		1.67 ± 0.96	8.33 ± 4.19	
8	b ± b	32.50 ± 4.79		b ± b	1.67 ± 0.96		b ± b	10.00 ± 1.36		3.33 ± 1.36	10.00 ± 5.61	
10	b ± b	37.50 ± 3.44		b ± b	5.00 ± 2.15		b ± b	14.17 ± 2.10		5.00 ± 3.97	5.00 ± 1.67	
12	b ± b	46.67 ± 1.36		0.83 ± 0.83	3.33 ± 1.36		0.83 ± 0.83	5.83 ± 1.60		b ± b	3.33 ± 1.36	
14	b ± b	48.33 ± 2.15		0.83 ± 0.83	4.17 ± 2.50		1.67 ± 0.96	10.00 ± 3.60		1.67 ± 0.96	9.17 ± 2.85	
16	2.50 ± 1.60	62.50 ± 3.44		0.83 ± 0.83	2.50 ± 0.83		4.17 ± 3.15	6.67 ± 1.36		5.00 ± 1.67	7.50 ± 1.60	

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.4B. Percentage (mean ± SE) of pupae morphological characteristics: pupae-adult intermediates, pupae not able to undergo further development, and dead pupae, as a percentage of the total number of individuals (four-week old

larvae) initially to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated pallet wrap surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Pupae-Adult Intermediates						Pupae (Alive)						Dead Pupae					
	Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
Ctrl	b	±	b	b	±	b	b	±	b	b	±	b	8.33	±	2.89	5.83	±	2.85
0	3.33	±	3.33	31.67	±	3.97	16.67	±	1.36	0.83	±	0.83	13.33	±	2.72	28.33	±	2.89
2	10.83	±	2.85	35.83	±	3.44	16.67	±	4.91	0.00	±	0.00	17.50	±	6.29	23.33	±	3.04
4	8.33	±	2.89	33.33	±	4.08	14.17	±	4.79	4.17	±	2.10	20.83	±	2.85	20.83	±	3.70
6	14.17	±	5.16	40.00	±	4.51	5.83	±	0.83	b	±	b	23.33	±	9.33	25.83	±	3.44
8	12.50	±	3.70	50.83	±	6.72	9.17	±	3.44	2.50	±	2.50	31.67	±	2.89	15.83	±	2.10
10	14.17	±	4.17	49.17	±	6.72	5.83	±	1.60	7.50	±	3.70	33.33	±	3.04	7.50	±	3.44
12	16.67	±	5.61	50.00	±	6.53	2.50	±	1.60	4.17	±	2.10	30.83	±	2.85	9.17	±	2.50
14	28.33	±	0.96	48.33	±	2.89	5.00	±	0.96	b	±	b	26.67	±	1.92	10.83	±	2.10
16	30.00	±	2.36	47.50	±	5.83	5.00	±	1.67	b	±	b	21.67	±	2.89	6.67	±	2.72
Storage Wks.	Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
	Ctrl	b	±	b	b	±	b	b	±	b	b	±	b	5.83	±	2.85	5.83	±
0	20.00	±	5.27	34.17	±	8.96	4.17	±	3.15	5.00	±	2.15	28.33	±	2.89	11.67	±	2.15
2	27.50	±	6.99	27.50	±	5.99	2.50	±	1.60	b	±	b	23.33	±	3.04	14.17	±	1.60
4	33.33	±	6.24	19.17	±	2.50	4.17	±	2.50	2.50	±	0.83	20.83	±	3.70	11.67	±	4.41
6	34.17	±	4.38	27.50	±	2.10	3.33	±	1.36	3.33	±	1.92	25.83	±	3.44	14.17	±	2.50
8	37.50	±	2.50	19.17	±	0.83	5.00	±	2.89	2.50	±	1.60	15.83	±	2.10	10.83	±	3.44
10	34.17	±	4.98	22.50	±	3.15	2.50	±	1.60	1.67	±	0.96	7.50	±	3.44	9.17	±	3.70
12	40.00	±	8.05	24.17	±	1.60	5.00	±	2.15	0.83	±	0.83	9.17	±	2.50	10.83	±	5.51
14	42.50	±	6.14	19.17	±	4.17	1.67	±	0.96	0.00	±	0.00	10.83	±	2.10	6.67	±	2.36
16	45.00	±	6.45	15.00	±	5.69	2.50	±	1.60	0.83	±	0.83	6.67	±	2.72	2.50	±	1.60

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.4C. Percentage (mean \pm SE) of larvae morphological characteristics: individuals remaining in the larvae stage, those that died while in the larvae stage and those that were arrested between larvae and pupae development, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated pallet wrap surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Larvae (Alive)				Dead Larvae				Arrested Larvae									
	Entech Fog-30 [®] + Diacon II [™]				Entech Fog-30 [®] + Diacon II [™]				Entech Fog-30 [®] + Diacon II [™]									
	<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>							
Ctrl	1.67	±	1.67	0.00	±	0.00	9.17	±	2.85	6.67	±	3.60	b	±	b	b	±	b
0	40.00	±	5.93	17.50	±	5.83	25.00	±	4.81	16.67	±	3.04	1.67	±	0.96	2.50	±	2.50
2	31.67	±	10.76	13.33	±	1.36	23.33	±	6.38	21.67	±	2.15	b	±	b	1.67	±	1.67
4	30.83	±	6.72	10.83	±	4.17	25.00	±	5.18	20.83	±	4.38	0.83	±	0.83	b	±	b
6	30.83	±	9.75	3.33	±	2.36	23.33	±	5.27	15.00	±	3.97	2.50	±	1.60	0.83	±	0.83
8	17.50	±	5.67	4.17	±	1.60	26.67	±	7.07	16.67	±	1.92	1.67	±	0.96	1.67	±	1.67
10	14.17	±	2.50	10.83	±	4.38	30.83	±	1.60	10.83	±	3.15	1.67	±	0.96	0.00	±	0.00
12	9.17	±	6.99	0.00	±	0.00	33.33	±	1.36	8.33	±	2.89	6.67	±	1.92	0.00	±	0.00
14	5.83	±	1.60	4.17	±	3.15	30.83	±	2.85	12.50	±	2.10	0.83	±	0.83	0.83	±	0.83
16	4.17	±	3.15	2.50	±	2.50	32.50	±	2.10	7.50	±	2.85	3.33	±	0.00	0.00	±	0.00
Storage Wks.	Entech Fog-10 [®] + Diacon II [™]				Entech Fog-10 [®] + Diacon II [™]				Entech Fog-10 [®] + Diacon II [™]									
	<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>							
	Ctrl	0.83	±	0.83	0.00	±	0.00	3.33	±	3.60	5.83	±	1.60	b	±	b	b	±
0	19.17	±	6.29	14.17	±	9.85	24.17	±	3.04	15.00	±	5.00	8.33	±	1.67	4.17	±	4.17
2	6.67	±	3.60	7.50	±	6.44	29.17	±	2.15	17.50	±	2.10	3.33	±	2.36	1.67	±	1.67
4	5.83	±	2.50	8.33	±	7.26	29.17	±	4.38	15.83	±	3.44	1.67	±	0.96	0.00	±	0.00
6	1.67	±	1.67	4.17	±	4.17	30.00	±	3.97	7.50	±	2.50	3.33	±	1.36	0.00	±	0.00
8	0.83	±	0.83	1.67	±	0.96	24.17	±	1.92	11.67	±	2.15	1.67	±	0.96	0.00	±	0.00
10	1.67	±	1.67	0.00	±	0.00	30.83	±	3.15	5.00	±	2.15	0.83	±	0.83	0.00	±	0.00
12	1.67	±	0.96	0.83	±	0.83	26.67	±	2.89	4.17	±	0.83	1.67	±	0.96	0.00	±	0.00
14	2.50	±	0.83	0.83	±	0.83	24.17	±	2.10	1.67	±	0.96	0.83	±	0.83	0.00	±	0.00
16	3.33	±	2.36	0.00	±	0.00	15.00	±	2.85	2.50	±	2.50	1.67	±	1.67	0.00	±	0.00

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.5A. Percentage (mean ± SE) of adult morphological characteristics: normal adult development; individuals displaying the twisted wing deformity, individuals unable to properly fold their wings beneath the elytra/wings; and adults

unable to shed the pupae cuticle, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated flour bag surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Normal Adult Development						Adults w/ Twisted Wing Deformity						Adults w/ Unfolded Wing Deformity						Adults Unable to Shed Pupal Cuticle																													
	Entech Fog-30® + Diacon II™												Entech Fog-30® + Diacon II™												Entech Fog-30® + Diacon II™												Entech Fog-30® + Diacon II™											
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>																				
Ctrl	87.50	±	5.83	92.50	±	2.85	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	6.67	±	5.61															
0	b	±	b	b	±	b	b	±	b	b	±	b	0.83	±	0.83	b	±	b	0.83	±	0.83	b	±	b	b	±	b	b	±	b	b	±	b															
2	b	±	b	b	±	b	b	±	b	0.83	±	0.83	b	±	b	1.67	±	1.67	b	±	b	1.67	±	1.67	b	±	b	b	±	b	b	±	b															
4	b	±	b	b	±	b	b	±	b	4.17	±	3.15	b	±	b	3.33	±	2.36	b	±	b	3.33	±	2.36	b	±	b	b	±	b	b	±	b															
6	b	±	b	0.83	±	0.83	b	±	b	0.83	±	0.83	b	±	b	3.33	±	0.00	b	±	b	3.33	±	0.00	b	±	b	b	±	b	2.50	±	1.60															
8	b	±	b	6.67	±	3.60	b	±	b	2.50	±	1.60	1.67	±	1.67	5.00	±	2.15	b	±	b	5.00	±	2.15	b	±	b	b	±	b	1.67	±	0.96															
10	b	±	b	11.67	±	3.47	b	±	b	0.83	±	0.83	0.83	±	0.83	2.50	±	1.60	0.83	±	0.83	2.50	±	1.60	0.83	±	0.83	b	±	b	b	±	b															
12	b	±	b	10.83	±	4.98	b	±	b	0.00	±	0.00	0.83	±	0.83	1.67	±	0.96	0.83	±	0.83	1.67	±	0.96	b	±	b	b	±	b	3.33	±	2.36															
14	0.83	±	0.83	13.33	±	2.72	b	±	b	4.17	±	2.10	1.67	±	1.67	2.50	±	2.50	0.83	±	0.83	2.50	±	2.50	0.83	±	0.83	b	±	b	4.17	±	1.60															
16	0.83	±	0.83	15.83	±	3.94	b	±	b	0.00	±	0.00	0.83	±	0.83	1.67	±	0.96	0.83	±	0.83	1.67	±	0.96	2.50	±	1.60	2.50	±	1.60	2.50	±	1.60															
Storage Wks.	Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™																													
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>																										
	Ctrl	92.50	±	3.70	95.00	±	0.96	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b														
0	b	±	b	5.00	±	3.97	b	±	b	1.67	±	0.96	b	±	b	3.33	±	3.33	b	±	b	3.33	±	3.33	b	±	b	b	±	b	b	±	b															
2	b	±	b	17.50	±	4.98	b	±	b	2.50	±	1.60	b	±	b	8.33	±	3.19	b	±	b	8.33	±	3.19	3.33	±	2.36	5.83	±	2.85	3.33	±	2.85															
4	b	±	b	24.17	±	3.15	b	±	b	3.33	±	1.36	b	±	b	5.83	±	2.10	b	±	b	5.83	±	2.10	b	±	b	0.00	±	0.00	5.83	±	0.83															
6	b	±	b	34.17	±	6.29	b	±	b	4.17	±	2.10	b	±	b	6.67	±	3.04	b	±	b	6.67	±	3.04	3.33	±	1.36	8.33	±	3.47	3.33	±	3.47															
8	b	±	b	45.00	±	8.44	b	±	b	3.33	±	1.36	b	±	b	6.67	±	3.04	b	±	b	6.67	±	3.04	6.67	±	3.60	4.17	±	1.60	6.67	±	1.60															
10	b	±	b	54.17	±	3.70	b	±	b	3.33	±	2.36	b	±	b	8.33	±	2.15	b	±	b	8.33	±	2.15	1.67	±	0.96	5.00	±	2.15	1.67	±	2.15															
12	b	±	b	59.17	±	4.17	b	±	b	3.33	±	1.92	1.67	±	1.67	5.00	±	2.15	1.67	±	1.67	5.00	±	2.15	0.83	±	0.83	3.33	±	2.36	0.83	±	2.36															
14	b	±	b	75.00	±	8.77	b	±	b	5.00	±	2.89	1.67	±	0.96	3.33	±	1.92	1.67	±	0.96	3.33	±	1.92	1.67	±	0.96	2.50	±	1.60	1.67	±	1.60															
16	b	±	b	77.50	±	2.50	b	±	b	5.83	±	2.85	0.83	±	0.83	3.33	±	1.36	0.83	±	0.83	3.33	±	1.36	5.00	±	3.19	4.17	±	0.83	5.00	±	0.83															

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.5B. Percentage (mean \pm SE) of pupae morphological characteristics: pupae-adult intermediates, pupae not able to undergo further development, and dead pupae, as a percentage of the total number of individuals (four-week old larvae) initially to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated flour bag surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Pupae-Adult Intermediates						Pupae (Alive)						Dead Pupae					
	Entech Fog-30 [®] + Diacon II [™]						Entech Fog-30 [®] + Diacon II [™]						Entech Fog-30 [®] + Diacon II [™]					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
Ctrl	b	±	b	b	±	b	b	±	b	b	±	b	7.50	±	4.38	2.50	±	1.60
0	2.50	±	2.50	19.17	±	11.09	11.67	±	5.69	0.83	±	0.83	25.83	±	3.44	25.00	±	6.74
2	12.50	±	5.99	35.00	±	4.19	8.33	±	2.15	1.67	±	1.67	20.00	±	3.60	19.17	±	4.17
4	15.00	±	5.18	30.83	±	3.15	11.67	±	2.89	9.17	±	5.67	20.83	±	3.94	19.17	±	3.44
6	15.83	±	3.44	30.00	±	2.36	8.33	±	0.96	10.83	±	1.60	29.17	±	6.72	18.33	±	2.15
8	21.67	±	2.15	30.83	±	6.99	8.33	±	4.41	4.17	±	2.10	28.33	±	4.41	20.83	±	2.10
10	25.00	±	3.47	30.00	±	2.36	5.83	±	3.94	7.50	±	2.10	32.50	±	1.60	18.33	±	4.41
12	27.50	±	1.60	30.00	±	2.36	6.67	±	1.92	3.33	±	1.36	33.33	±	6.09	19.17	±	2.50
14	26.67	±	4.91	30.00	±	4.08	0.83	±	0.83	0.00	±	0.00	33.33	±	1.36	20.00	±	4.71
16	32.50	±	3.44	34.17	±	2.50	6.67	±	3.60	1.67	±	0.96	33.33	±	4.30	24.17	±	4.59
Storage Wks.	Entech Fog-10 [®] + Diacon II [™]						Entech Fog-10 [®] + Diacon II [™]						Entech Fog-10 [®] + Diacon II [™]					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
	Ctrl	b	±	b	b	±	b	b	±	b	b	±	b	2.50	±	1.60	1.67	±
0	11.67	±	5.69	32.50	±	7.74	8.33	±	3.97	5.83	±	3.94	25.00	±	6.74	9.17	±	2.85
2	21.67	±	5.69	20.00	±	1.92	5.00	±	3.97	3.33	±	3.33	19.17	±	4.17	13.33	±	1.36
4	30.83	±	5.51	24.17	±	1.60	1.67	±	0.96	0.00	±	0.00	19.17	±	3.44	10.83	±	2.85
6	36.67	±	4.71	24.17	±	3.70	3.33	±	1.36	0.00	±	0.00	18.33	±	2.15	10.83	±	2.50
8	35.00	±	5.18	18.33	±	3.19	3.33	±	2.36	0.83	±	0.83	20.83	±	2.10	10.00	±	3.04
10	41.67	±	3.97	15.00	±	2.89	2.50	±	1.60	1.67	±	0.96	18.33	±	4.41	10.00	±	3.33
12	40.00	±	5.27	17.50	±	3.44	0.83	±	0.83	0.00	±	0.00	19.17	±	2.50	7.50	±	2.50
14	42.50	±	6.85	8.33	±	3.19	2.50	±	1.60	0.83	±	0.83	20.00	±	4.71	3.33	±	1.36
16	43.33	±	5.77	5.83	±	1.60	0.83	±	0.83	0.00	±	0.00	24.17	±	4.59	2.50	±	1.60

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.5C. Percentage (mean ± SE) of larvae morphological characteristics: individuals remaining in the larvae stage, those that died while in the larvae stage and those that were arrested between larvae and pupae development, as a

percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated flour bag surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Larvae (Alive)				Dead Larvae				Arrested Larvae									
	Entech Fog-30® + Diacon II™				Entech Fog-30® + Diacon II™				Entech Fog-30® + Diacon II™									
	<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>							
Ctrl	b	±	b	b	±	b	5.00	±	2.15	5.00	±	2.15	b	±	b	b	±	b
0	23.33	±	4.30	24.17	±	2.85	35.83	±	5.16	23.33	±	4.71	0.83	±	0.83	b	±	b
2	18.33	±	6.45	17.50	±	3.94	40.83	±	8.21	24.17	±	3.44	b	±	b	b	±	b
4	11.67	±	0.96	13.33	±	3.60	39.17	±	8.32	20.00	±	3.04	1.67	±	0.96	b	±	b
6	11.67	±	4.81	15.00	±	3.47	32.50	±	4.59	18.33	±	2.89	2.50	±	1.60	b	±	b
8	13.33	±	5.27	11.67	±	6.16	25.83	±	8.21	16.67	±	3.04	0.83	±	0.83	b	±	b
10	10.83	±	4.98	14.17	±	4.79	24.17	±	3.94	15.00	±	3.97	b	±	b	b	±	b
12	8.33	±	3.97	13.33	±	6.09	22.50	±	2.85	17.50	±	4.79	0.83	±	0.83	0.83	±	0.83
14	10.00	±	5.93	1.67	±	0.96	24.17	±	3.70	24.17	±	1.60	1.67	±	0.96	b	±	b
16	2.50	±	2.50	0.00	±	0.00	18.33	±	3.47	20.00	±	4.51	2.50	±	1.60	b	±	b
Storage Wks.	Entech Fog-10® + Diacon II™				Entech Fog-10® + Diacon II™				Entech Fog-10® + Diacon II™									
	<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>							
	b	±	b	0.83	±	0.83	3.33	±	2.15	2.50	±	0.83	b	±	b	b	±	b
0	13.33	±	5.27	16.67	±	8.50	41.67	±	4.71	22.50	±	5.16	1.67	±	0.96	3.33	±	1.36
2	8.33	±	4.41	7.50	±	3.70	33.33	±	3.44	20.83	±	5.99	1.67	±	1.67	0.83	±	0.83
4	12.50	±	5.83	9.17	±	3.70	30.83	±	3.04	15.83	±	1.60	0.83	±	0.83	0.83	±	0.83
6	b	±	b	b	±	b	27.50	±	2.89	10.83	±	3.70	2.50	±	1.60	0.83	±	0.83
8	0.83	±	0.83	0.83	±	0.83	29.17	±	3.04	10.83	±	6.44	b	±	b	b	±	b
10	0.83	±	0.83	b	±	b	28.33	±	3.97	2.50	±	2.50	b	±	b	b	±	b
12	0.00	±	0.00	b	±	b	21.67	±	4.79	4.17	±	2.10	0.83	±	0.83	b	±	b
14	3.33	±	2.36	b	±	b	20.00	±	1.60	1.67	±	0.96	0.83	±	0.83	b	±	b
16	2.50	±	1.60	0.83	±	0.83	16.67	±	4.51	0.00	±	0.00	2.50	±	1.60	b	±	b

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.6A. Percentage (mean \pm SE) of adult morphological characteristics: normal adult development; individuals displaying the twisted wing deformity, individuals unable to properly fold their wings beneath the elytra/wings; and adults unable to shed the pupae cuticle, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated polyester bag surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Normal Adult Development			Adults w/ Twisted Wing Deformity			Adults w/ Unfolded Wing Deformity			Adults Unable to Shed Pupal Cuticle		
	Entech Fog-30 [®] + Diacon II [™]			Entech Fog-30 [®] + Diacon II [™]			Entech Fog-30 [®] + Diacon II [™]			Entech Fog-30 [®] + Diacon II [™]		
	<i>T. castaneum</i>	<i>T. confusum</i>		<i>T. castaneum</i>	<i>T. confusum</i>		<i>T. castaneum</i>	<i>T. confusum</i>		<i>T. castaneum</i>	<i>T. confusum</i>	
Ctrl	77.50 ± 3.15	90.00 ± 2.36		b ± b	b ± b		b ± b	b ± b		b ± b	b ± b	
0	b ± b	b ± b		b ± b	b ± b		b ± b	b ± b		b ± b	1.67 ± 1.67	
2	b ± b	b ± b		b ± b	b ± b		b ± b	b ± b		b ± b	1.67 ± 0.96	
4	b ± b	2.50 ± 1.60		b ± b	b ± b		b ± b	1.67 ± 0.96		b ± b	0.83 ± 0.83	
6	b ± b	5.00 ± 2.89		0.83 ± 0.83	0.83 ± 0.83		0.83 ± 0.83	2.50 ± 1.60		0.83 ± 0.83	0.00 ± 0.00	
8	b ± b	7.50 ± 2.10		b ± b	1.67 ± 0.96		b ± b	4.17 ± 3.15		b ± b	1.67 ± 1.67	
10	b ± b	7.50 ± 2.50		b ± b	0.83 ± 0.83		b ± b	2.50 ± 1.60		b ± b	1.67 ± 0.96	
12	0.83 ± 0.83	10.00 ± 3.04		b ± b	1.67 ± 0.96		b ± b	2.50 ± 1.60		b ± b	6.67 ± 1.36	
14	b ± b	10.83 ± 3.44		b ± b	1.67 ± 1.67		0.83 ± 0.83	5.00 ± 0.96		b ± b	2.50 ± 0.83	
16	0.83 ± 0.83	20.83 ± 3.44		b ± b	0.83 ± 0.83		1.67 ± 1.67	5.83 ± 1.60		2.50 ± 1.60	2.50 ± 0.83	
Storage Wks.	Entech Fog-10 [®] + Diacon II [™]			Entech Fog-10 [®] + Diacon II [™]			Entech Fog-10 [®] + Diacon II [™]			Entech Fog-10 [®] + Diacon II [™]		
	<i>T. castaneum</i>	<i>T. confusum</i>		<i>T. castaneum</i>	<i>T. confusum</i>		<i>T. castaneum</i>	<i>T. confusum</i>		<i>T. castaneum</i>	<i>T. confusum</i>	
	Ctrl	91.67 ± 1.67	91.67 ± 3.47		b ± b	b ± b		b ± b	b ± b		b ± b	b ± b
0	b ± b	b ± b		b ± b	0.83 ± 0.83		b ± b	3.33 ± 2.36		b ± b	0.83 ± 0.83	
2	b ± b	10.83 ± 4.79		b ± b	0.83 ± 0.83		b ± 0.00	3.33 ± 1.92		1.67 ± 1.67	7.50 ± 3.44	
4	b ± b	15.83 ± 5.67		b ± b	b ± b		1.67 ± 1.67	4.17 ± 0.83		b ± b	5.00 ± 3.97	
6	b ± b	22.50 ± 4.38		b ± b	1.67 ± 1.67		b ± b	5.83 ± 1.60		3.33 ± 2.36	3.33 ± 1.36	
8	b ± b	35.83 ± 7.62		b ± b	2.50 ± 0.83		b ± b	5.83 ± 1.60		0.83 ± 0.83	2.50 ± 1.60	
10	b ± b	45.00 ± 5.53		b ± b	5.00 ± 3.97		b ± b	2.50 ± 1.60		3.33 ± 1.92	5.00 ± 1.67	
12	b ± b	53.33 ± 3.04		b ± b	4.17 ± 1.60		b ± b	4.17 ± 3.15		0.83 ± 0.83	3.33 ± 1.36	
14	b ± b	55.83 ± 3.70		0.83 ± 0.83	4.17 ± 1.60		0.83 ± 0.83	4.17 ± 0.83		1.67 ± 0.96	1.67 ± 0.96	
16	b ± b	68.33 ± 3.19		b ± b	4.17 ± 2.10		2.50 ± 1.60	5.00 ± 0.96		2.50 ± 1.60	4.17 ± 2.50	

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.6B. Percentage (mean ± SE) of pupae morphological characteristics: pupae-adult intermediates, pupae not able to undergo further development, and dead pupae, as a percentage of the total number of individuals (four-week old

larvae) initially to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated polyester bag surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Pupae-Adult Intermediates						Pupae (Alive)						Dead Pupae					
	Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
Ctrl	b	±	b	b	±	b	b	±	b	b	±	b	10.00	±	1.92	0.83	±	0.83
0	1.67	±	1.67	8.33	±	4.41	13.33	±	4.30	1.67	±	0.96	27.50	±	1.60	46.67	±	8.05
2	5.00	±	2.15	17.50	±	2.50	10.00	±	4.08	4.17	±	2.10	25.00	±	2.89	32.50	±	3.15
4	10.00	±	4.08	20.00	±	2.36	5.83	±	2.85	6.67	±	4.08	21.67	±	4.81	25.00	±	6.74
6	10.83	±	4.17	20.83	±	5.67	0.83	±	0.83	9.17	±	2.50	17.50	±	7.62	19.17	±	2.50
8	9.17	±	3.44	38.33	±	5.85	6.67	±	2.72	1.67	±	1.67	28.33	±	5.69	18.33	±	4.19
10	17.50	±	2.50	33.33	±	1.36	10.00	±	2.36	4.17	±	1.60	24.17	±	5.83	18.33	±	3.97
12	16.67	±	3.60	34.17	±	5.16	5.00	±	0.96	4.17	±	2.50	25.83	±	2.10	16.67	±	3.60
14	21.67	±	2.89	36.67	±	3.04	1.67	±	0.96	4.17	±	3.15	30.83	±	2.50	17.50	±	2.85
16	20.83	±	4.38	33.33	±	4.51	5.83	±	2.50	1.67	±	0.96	30.83	±	3.94	17.50	±	1.60
Storage Wks.	Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
	Ctrl	b	±	b	b	±	b	b	±	b	b	±	0.00	0.83	±	0.83	3.33	±
0	1.67	±	0.96	15.83	±	9.85	1.67	±	0.96	3.33	±	2.36	46.67	±	8.05	18.33	±	4.81
2	20.00	±	2.72	26.67	±	5.93	2.50	±	1.60	1.67	±	0.96	32.50	±	3.15	15.83	±	2.50
4	24.17	±	2.85	34.17	±	6.85	b	±	b	1.67	±	1.67	25.00	±	6.74	10.83	±	5.51
6	25.83	±	4.79	21.67	±	2.89	6.67	±	3.60	1.67	±	0.96	19.17	±	2.50	21.67	±	2.89
8	30.83	±	5.67	21.67	±	2.15	2.50	±	1.60	6.67	±	3.04	18.33	±	4.19	15.00	±	2.15
10	37.50	±	5.16	21.67	±	5.18	5.00	±	2.15	0.83	±	0.83	18.33	±	3.97	13.33	±	1.36
12	40.00	±	3.60	17.50	±	3.70	0.83	±	0.83	0.83	±	0.83	16.67	±	3.60	13.33	±	2.36
14	37.50	±	1.60	21.67	±	3.97	1.67	±	1.67	1.67	±	0.96	17.50	±	2.85	6.67	±	3.60
16	43.33	±	2.36	8.33	±	0.96	3.33	±	2.36	0.00	±	0.00	17.50	±	1.60	7.50	±	2.10

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.6C. Percentage (mean \pm SE) of larvae morphological characteristics: individuals remaining in the larvae stage, those that died while in the larvae stage and those that were arrested between larvae and pupae development, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated polyester bag surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Larvae (Alive)						Dead Larvae						Arrested Larvae					
	Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
Ctrl	b	±	b	b	±	b	12.50	±	4.38	9.17	±	2.10	b	±	b	b	±	b
0	20.83	±	2.50	11.67	±	3.97	34.17	±	1.60	29.17	±	4.17	2.50	±	1.60	b	±	b
2	16.67	±	2.72	14.17	±	2.10	40.83	±	5.67	30.00	±	3.04	2.50	±	0.83	b	±	b
4	18.33	±	6.45	10.83	±	0.83	42.50	±	6.72	31.67	±	4.41	1.67	±	1.67	b	±	b
6	26.67	±	7.58	10.00	±	1.36	40.00	±	8.05	31.67	±	5.18	1.67	±	0.96	0.83	±	0.83
8	15.00	±	5.00	5.00	±	0.96	37.50	±	6.85	20.83	±	1.60	3.33	±	1.36	0.83	±	0.83
10	12.50	±	4.17	10.83	±	1.60	32.50	±	3.70	20.83	±	6.85	3.33	±	0.00	b	±	b
12	10.83	±	2.10	5.83	±	3.70	40.00	±	4.30	18.33	±	2.15	0.83	±	0.83	b	±	b
14	6.67	±	3.04	2.50	±	2.50	35.83	±	6.44	19.17	±	2.10	2.50	±	0.83	b	±	b
16	5.00	±	1.67	0.00	±	0.00	31.67	±	6.87	17.50	±	3.15	0.83	±	0.83	b	±	b
Storage Wks.	Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
	Ctrl	2.50	±	1.60	b	±	b	5.83	±	2.10	5.00	±	2.15	b	±	b	b	±
0	11.67	±	5.85	29.17	±	10.13	67.50	±	4.17	26.67	±	5.27	0.83	±	0.83	1.67	±	0.96
2	1.67	±	0.96	6.67	±	6.67	52.50	±	3.04	26.67	±	5.61	0.83	±	0.83	b	±	b
4	1.67	±	1.67	5.00	±	3.97	48.33	±	4.41	20.00	±	5.93	2.50	±	1.60	3.33	±	2.36
6	1.67	±	0.96	0.83	±	0.83	39.17	±	5.18	20.83	±	4.17	0.83	±	0.83	b	±	b
8	2.50	±	2.50	b	±	b	37.50	±	1.60	9.17	±	5.99	0.83	±	0.83	0.83	±	0.83
10	3.33	±	3.33	0.83	±	0.83	29.17	±	6.85	5.83	±	2.50	b	±	b	b	±	b
12	b	±	b	b	±	b	27.50	±	2.15	3.33	±	1.36	0.83	±	0.83	b	±	b
14	1.67	±	1.67	b	±	b	25.83	±	2.10	4.17	±	1.60	2.50	±	0.83	b	±	b
16	b	±	b	b	±	b	20.83	±	3.15	2.50	±	1.60	2.50	±	0.83	b	±	b

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.7A. Percentage (mean ± SE) of adult morphological characteristics: normal adult development; individuals displaying the twisted wing deformity, individuals unable to properly fold their wings beneath the elytra/wings; and adults

unable to shed the pupae cuticle, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated cotton bag surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Normal Adult Development				Adults w/ Twisted Wing Deformity				Adults w/ Unfolded Wing Deformity				Adults Unable to Shed Pupal Cuticle														
	Entech Fog-30® + Diacon II™								Entech Fog-30® + Diacon II™																		
	<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>												
Ctrl	90.00	±	3.60	88.33	±	2.89	b	±	b	b	±	b	b	±	b	b	±	b									
0	b	±	b	0.83	±	0.83	b	±	b	2.50	±	0.83	b	±	b	5.00	±	5.00	0.83	±	0.83	5.83	±	4.79			
2	b	±	b	1.67	±	0.96	b	±	b	0.83	±	0.83	b	±	b	b	±	b	b	±	b	1.67	±	1.67			
4	b	±	b	5.83	±	2.50	b	±	b	2.50	±	1.60	b	±	b	1.67	±	1.67	b	±	b	1.67	±	0.96			
6	b	±	b	9.17	±	5.34	b	±	b	0.83	±	0.83	b	±	b	1.67	±	0.96	b	±	b	2.50	±	1.60			
8	b	±	b	16.67	±	3.60	b	±	b	1.67	±	0.96	b	±	b	1.67	±	0.96	b	±	b	b	±	b			
10	b	±	b	20.83	±	3.70	b	±	b	b	±	b	0.83	±	0.83	0.83	±	0.83	b	±	b	b	±	b			
12	b	±	b	25.00	±	2.89	b	±	b	2.50	±	0.83	0.83	±	0.83	0.83	±	0.83	b	±	b	3.33	±	1.36			
14	b	±	b	21.67	±	0.96	b	±	b	2.50	±	1.60	b	±	b	1.67	±	0.96	b	±	b	0.83	±	0.83			
16	b	±	b	24.17	±	1.60	0.83	±	0.83	2.50	±	1.60	1.67	±	1.67	4.17	±	2.10	b	±	b	0.83	±	0.83			
Storage Wks.	Entech Fog-10® + Diacon II™				Entech Fog-10® + Diacon II™				Entech Fog-10® + Diacon II™				Entech Fog-10® + Diacon II™														
	<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>		<i>T. castaneum</i>		<i>T. confusum</i>												
	Ctrl	90.00	±	4.30	89.17	±	3.70	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b	b	±	b		
0	b	±	b	7.50	±	3.70	b	±	b	b	±	b	b	±	b	2.50	±	1.60	b	±	b	3.33	±	2.36	b	±	b
2	b	±	b	16.67	±	4.08	b	±	b	0.83	±	0.83	b	±	b	3.33	±	1.92	b	±	b	5.00	±	1.67	b	±	b
4	b	±	b	22.50	±	5.51	b	±	b	0.83	±	0.83	b	±	b	4.17	±	1.60	b	±	b	5.00	±	2.89	b	±	b
6	b	±	b	34.17	±	4.38	b	±	b	3.33	±	2.36	b	±	b	5.83	±	2.10	b	±	b	3.33	±	1.36	b	±	b
8	b	±	b	35.83	±	1.60	b	±	b	2.50	±	0.83	0.83	±	0.83	1.67	±	0.96	1.67	±	0.96	3.33	±	1.36	1.67	±	0.96
10	b	±	b	40.00	±	4.08	b	±	b	b	±	b	b	±	b	4.17	±	1.60	b	±	b	9.17	±	2.85	b	±	b
12	b	±	b	38.33	±	3.47	b	±	b	5.83	±	2.50	b	±	b	2.50	±	1.60	0.83	±	0.83	3.33	±	1.36	0.83	±	0.83
14	0.83	±	0.83	43.33	±	8.28	b	±	b	3.33	±	3.33	0.83	±	0.83	3.33	±	1.36	3.33	±	2.36	5.00	±	0.96	3.33	±	2.36
16	b	±	b	43.33	±	3.60	0.83	±	0.83	1.67	±	0.96	b	±	b	2.50	±	0.83	0.83	±	0.83	5.83	±	2.10	0.83	±	0.83

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.7B. Percentage (mean \pm SE) of pupae morphological characteristics: pupae-adult intermediates, pupae not able to undergo further development, and dead pupae, as a percentage of the total number of individuals (four-week old larvae) initially to the Entech-Fog 10® + methoprene and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated cotton bag surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Pupae-Adult Intermediates						Pupae (Alive)						Dead Pupae					
	Entech Fog-30 [®] + Diacon II [™]						Entech Fog-30 [®] + Diacon II [™]						Entech Fog-30 [®] + Diacon II [™]					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
Ctrl	b	±	b	b	±	b	b	±	b	b	±	b	5.00	±	1.67	3.33	±	1.92
0	3.33	±	2.36	18.33	±	5.18	9.17	±	1.60	2.50	±	1.60	22.50	±	1.60	30.83	±	1.60
2	11.67	±	5.18	25.00	±	3.47	5.00	±	2.15	5.00	±	0.96	25.83	±	3.44	22.50	±	8.43
4	20.00	±	4.51	30.00	±	3.04	10.83	±	1.60	5.00	±	3.97	19.17	±	1.60	20.83	±	3.70
6	17.50	±	4.79	35.00	±	1.67	5.00	±	0.96	5.00	±	1.67	23.33	±	4.91	19.17	±	2.10
8	23.33	±	7.82	36.67	±	5.77	5.83	±	0.83	5.83	±	2.85	22.50	±	2.50	17.50	±	2.85
10	20.83	±	6.58	30.83	±	3.44	5.00	±	2.89	5.00	±	2.15	26.67	±	2.36	15.00	±	3.19
12	27.50	±	5.51	25.83	±	3.70	2.50	±	1.60	5.83	±	1.60	26.67	±	2.36	19.17	±	1.60
14	27.50	±	1.60	30.83	±	5.83	3.33	±	1.92	1.67	±	0.96	22.50	±	2.85	17.50	±	1.60
16	35.00	±	2.89	27.50	±	4.38	5.83	±	2.10	0.83	±	0.83	21.67	±	5.18	21.67	±	2.89
Storage Wks.	Entech Fog-10 [®] + Diacon II [™]						Entech Fog-10 [®] + Diacon II [™]						Entech Fog-10 [®] + Diacon II [™]					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
	Ctrl	b	±	b	b	±	b	b	±	b	b	±	b	3.33	±	1.92	4.17	±
0	11.67	±	5.69	28.33	±	9.86	5.83	±	3.94	5.00	±	2.89	30.83	±	1.60	7.50	±	2.10
2	20.00	±	3.60	29.17	±	4.38	5.00	±	3.19	1.67	±	1.67	22.50	±	8.43	13.33	±	6.80
4	20.00	±	3.60	30.83	±	2.85	2.50	±	2.50	2.50	±	0.83	20.83	±	3.70	15.83	±	2.10
6	20.00	±	4.91	29.17	±	3.70	0.83	±	0.83	2.50	±	1.60	19.17	±	2.10	7.50	±	3.44
8	23.33	±	4.51	30.83	±	2.50	1.67	±	0.96	0.83	±	0.83	17.50	±	2.85	12.50	±	4.38
10	25.00	±	4.81	27.50	±	4.59	4.17	±	0.83	2.50	±	1.60	15.00	±	3.19	9.17	±	2.10
12	26.67	±	3.60	23.33	±	6.24	0.83	±	0.83	4.17	±	2.10	19.17	±	1.60	10.83	±	2.10
14	26.67	±	3.04	28.33	±	3.97	0.83	±	0.83	1.67	±	0.96	17.50	±	1.60	7.50	±	2.10
16	22.50	±	7.12	30.00	±	4.30	0.83	±	0.83	0.83	±	0.83	21.67	±	2.89	11.67	±	3.47

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Appendix Table 3.7C. Percentage (mean ± SE) of larvae morphological characteristics: individuals remaining in the larvae stage, those that died while in the larvae stage and those that were arrested between larvae and pupae development, as a percentage of the total number of individuals (four-week old larvae) initially exposed to the Entech-Fog 10[®] + methoprene

and the Entech-30® Fog + methoprene aerosol insecticide concentration rates, on a treated cotton bag surface, for the two exposed *Tribolium* species, at each post-application storage interval.

Storage Wks.	Larvae (Alive)						Dead Larvae						Arrested Larvae					
	Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™						Entech Fog-30® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
Ctrl	b	±	b	b	±	b	5.00	±	2.15	8.33	±	2.15	b	±	b	b	±	b
0	26.67	±	3.04	7.50	±	4.79	37.50	±	2.10	26.67	±	6.24	b	±	b	b	±	b
2	20.00	±	4.30	10.83	±	3.70	36.67	±	3.60	31.67	±	3.97	0.83	±	0.83	0.83	±	0.83
4	16.67	±	1.36	8.33	±	3.97	28.33	±	3.97	24.17	±	2.50	5.00	±	0.96	b	±	b
6	16.67	±	4.91	8.33	±	3.97	35.00	±	2.89	18.33	±	5.53	2.50	±	1.60	b	±	b
8	17.50	±	5.83	10.00	±	3.60	30.00	±	5.27	10.00	±	5.27	0.83	±	0.83	b	±	b
10	13.33	±	3.60	9.17	±	3.15	32.50	±	7.25	17.50	±	4.17	0.83	±	0.83	0.83	±	0.83
12	6.67	±	3.60	6.67	±	2.36	35.00	±	8.33	10.83	±	3.44	0.83	±	0.83	b	±	b
14	12.50	±	4.59	4.17	±	2.10	31.67	±	7.52	19.17	±	3.15	2.50	±	1.60	b	±	b
16	7.50	±	4.38	1.67	±	1.67	27.50	±	6.99	15.83	±	1.60	b	±	b	0.83	±	0.83
Storage Wks.	Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™						Entech Fog-10® + Diacon II™					
	<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>			<i>T. castaneum</i>			<i>T. confusum</i>		
Ctrl	0.83	±	0.83	b	±	b	6.67	±	2.15	6.67	±	2.36	b	±	b	b	±	b
0	30.83	±	7.62	24.17	±	3.94	26.67	±	6.24	20.83	±	3.15	4.17	±	1.60	0.83	±	0.83
2	23.33	±	5.61	6.67	±	4.08	30.00	±	3.97	21.67	±	1.67	0.83	±	0.83	1.67	±	1.67
4	13.33	±	7.20	4.17	±	3.15	32.50	±	2.50	14.17	±	0.83	7.50	±	2.50	b	±	b
6	15.83	±	9.85	5.00	±	2.89	39.17	±	5.53	9.17	±	3.70	2.50	±	1.60	b	±	b
8	7.50	±	5.34	0.00	±	0.00	34.17	±	5.27	12.50	±	3.94	1.67	±	0.96	b	±	b
10	4.17	±	0.83	0.83	±	0.83	35.00	±	4.17	6.67	±	1.36	3.33	±	1.36	b	±	b
12	b	±	b	1.67	±	0.96	45.00	±	3.44	10.00	±	3.60	1.67	±	0.96	b	±	b
14	b	±	b	0.83	±	0.83	36.67	±	3.15	6.67	±	1.36	2.50	±	1.60	b	±	b
16	4.17	±	4.17	b	±	b	43.33	±	1.60	4.17	±	1.60	0.83	±	0.83	b	±	b

^a Means for percentage emergence for each of the 16 storage periods, across the scored adult morphologies, reported for each species (rows) that are followed by same letter are not significantly different ($P \geq 0.05$, Fishers LSD Method Rejection Matrix, ProStat Software, 2001).

^b No individuals emerged during these treatment intervals.

Summary and Conclusions

Tribolium spp., *T. castaneum* (Herbst) and *T. confusum* (DuVal) can be major pests in anthropogenic structures used for the processing and storage of grain-based products. These species are economically important and result in millions of dollars in lost product, every year. These *Tribolium* can be problematic at all stages, however, the larvae and the adult stages are the two stages that feed on the infested commodity. Often when an infestation arises, regardless of the entry point of the insects, along the transportation line, from processing until the products are moved onto a store shelf and purchased, the consumer is likely to lose confidence in that product and holds the company with which the product is licensed to, responsible for the damaged/infested food stuff. Management requires a quick and targeted method with which to suppress these pest populations, when they do become established. This can be accomplished through effective sanitation, as the flour beetles can feed and survive on even the smallest amount of available flour, and the use of aerosol insecticides that are deemed safe for use in these facilities where food is produced or stored, for human consumption purposes, as well as environmentally safe. A highly efficient, broad spectrum, dual synergized pyrethrin component is utilized in this study as well as an insect growth regulator, methoprene. This insecticidal combination will offer a means of suppressing these insect pest populations by two independent modes of action.

In Chapter 1, this aerosol combination, at two application rates, was applied to a flour substrate, after which, *Tribolium* spp. development and subsequent emergence was assessed. *T. castaneum* appear to be the more susceptible species, compared to *T. confusum*. Results demonstrated a lack of correlation between the normal adult emergence and each biweekly post-aerosol treatment storage interval. Bioassay result analysis did demonstrate that *T. castaneum* can be effectively controlled at both applied aerosol formulations, 1% pyrethrin and the 3% pyrethrin, throughout the 4 month (or 16-week) storage experiment, such that these *T. castaneum* normal, live adults were detected at less than 3% and ~11%, respectively. However, the less susceptible *T. confusum* bioassays showed that when exposed to the 3% pyrethrin

aerosol treated flour substrate, the percentage of *T. confusum* normal adult emergence was reported at ~30%, whereas, whereas upon exposure to the 1% pyrethrin treatment, *T. confusum* reach almost 47% normal adult emergence, by week 16. Bioassay results further demonstrated that within *T. castaneum*, the greatest vulnerability lie between the larvae and pupae stages, giving rise to a increased percentage of individuals demonstrating the larvae-pupae intermediate deformity. Whereas, within *T. confusum* this vulnerability lies between the pupae and adult stages, causing elevated percentages of the pupae-adult intermediate deformity. These results complement previous publications also demonstrating the efficacy of this particular methoprene plus dual-synergized pyrethrin aerosol mixture, as discussed in Chapter 1.

The surface of a substrate, onto which an aerosol insecticidal application is applied, plays relative importance in determining the persistence and efficacy of the applied aerosol. In Chapter 2, we utilized seven individual packaging material surfaces. The results each surface bioassay indicate a somewhat varied response between the two exposed *Tribolium* species, in regards to how both are influenced (i.e., developmental rates, proportions of normal adults, proportion of individuals displaying morphogenic abnormalities, etc.) by the two applied aerosol concentrations, and how the two aerosol formulations differentially affect the two exposed *Tribolium* species. Statistical analysis of these surface bioassay tests in which *T. confusum* individuals were exposed to 1% pyrethrin aerosol, demonstrated that the percentages of normal, live adult emergence detected was greatest for the paper bag surface (~50%) and lowest for the plastic surface (~30%); whereas, when exposed to the 3% pyrethrin aerosol, the greatest percentage of emergence was observed in the cotton bag treated surface bioassays (~14%) and the lowest in the cardboard (<3%). However, results of *T. castaneum* bioassays show that regardless of the applied aerosol application rate, this normal adult emergence was not observed over 1%, regardless of the treated surface. Overall, these results suggest that the surface chemistry of a surface likely plays a role in the observed insecticide efficacy.

There is clearly an additive effect observed in the greater a.i. synergized pyrethrin aerosol mixture. Though both combinations of aerosols contain the same amount of methoprene (1% of the overall concentration), results within both the treated flour and the treated surface bioassay tests demonstration that the greater the pyrethrin content within the mixed aerosol formulation,

the greater the achieved control. Further research should be done utilizing each component of the aerosol mixture independently, such that each are applied to each treated surface, and exposed to both of *Tribolium* species, to determine the exact role that each component plays in *Tribolium* development and subsequent emergence into the normal adult stage. Additional studies should also be performed that can better assess the additive effect that was observed. These tests would provide a clearer, more detailed understanding to the effects of aerosolized insecticide applications on these stored product pests, as well as others.

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