EFFECTIVENESS OF DELTAMETHRIN (DM)-INCORPORATED POLYPROPYLENE BAGS TO MITIGATE STORED-PRODUCT INSECT PEST INFESTATION

By

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Rampur, Chitwan, Nepal

2011

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2016

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ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Dr. George P. Opit, my major advisor, for his extreme patience, constant help, constructive guidance, and encouragement throughout my stay at Oklahoma State University. Additionally, I thank Vestergaard Frandsen SA, Switzerland for funding my Master of Science (MS) program.

I am very grateful to Dr. Sandipa Gautam for her guidance, valuable suggestions, and support. I thank Dr. Frank Arthur for his guidance and support. Dr. Arthur was instrumental in providing me with the skills I needed when I commenced my MS degree research. Additionally, I thank other members of my Advisory Committee, Drs. Brad Kard and Bruce Noden. The contribution and perspective of Drs. Kard and Noden significantly helped me improve the ideas and analyses presented in this thesis

I sincerely thank Kandara Shakya, Friendly Yang, Lee Vong Yang, and Ashana Shakya for the constant help they extended to me in the lab and for all their moral support. I thank faculty, staff, and friends in the Department of Entomology and Plant Pathology for teaching and training me, and for their support during my MS graduate-level study.

I give thanks to my siblings, Prashanta Paudyal and Smriti Paudyal and to my uncle Dr. Krishna Hari Koirala for the moral support. Last but not least, I feel extremely grateful to my parents, Prof. Daya Ram Paudyal and Mrs. Gauri Devi Paudyal for the

Acknowledgements reflect the views of the author and are not endorsed by committee members or Oklahoma State University.

encouragement and support they have offered me during my MS graduate-level study.

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Name: SULOCHANA PAUDYAL

Date of Degree: MAY, 2016

Title of Study: EFFECTIVENESS OF DELTAMETHRIN (DM)-INCORPORATED POLYPROPYLENE `BAGS TO MITIGATE STORED-PRODUCT INSECT PEST INFESTATION

Major Field: ENTOMOLOGY AND PLANT PATHOLOGY-ENTOMOLOGY OPTION

Abstract: The ZeroFly® Storage Bag is a deltamethrin-incorporated polypropylene (PP) bag, and is a novel tool used to reduce postharvest losses caused by stored-product insect pests. Data on the effectiveness of ZeroFly bags in preventing damaging insect infestations is critical for the scale-up of these bags in the developing world. Therefore, this study investigated response by adults of *Sitophilus oryzae*, *Tribolium castaneum*, and *Rhyzopertha dominica* to different concentrations of deltamethrin, 1, 25, 50, 100, 250, 500, 1000, and 3000 ppm over two exposure periods, 24 or 48 h. Deltamethrin was highly effective, achieving 99% knockdown of insects of all species within 4 h after exposure at concentrations ≥ 25 ppm. LC_{95} values for these pests, for the 48-h exposure period, were ≈ 3,000 ppm, the concentration of deltamethrin in new ZeroFly bag fabric. Experiments were also conducted to measure the sensitivity of insects in contact with fabric of ZeroFly bags, PP bags, jute bags, malathion-treated PP bags, malathion-treated jute bags, and GrainPro® bags — data for time to knockdown, mortality, and number of progeny of *S. oryzae* and *T. castaneum* were collected. Times to knockdown of 99% of insects were ≤ 3 h; in the 72-h exposure period, mortality of *S. oryzae* and *T. castaneum* was 76.7 and 62.2%, respectively, for the ZeroFly bag fabric. Relative to other fabrics, ZeroFly bag fabric significantly suppressed progeny production by *S. oryzae* and *T. castaneum* at all exposure periods. Evaluation of miniature ZeroFly bags showed that none of the insects were able to bore (chew) through the bag. Although no insects gained entry into PP bags or laminated PP mini bags, mortality of insects in these mini bags was significantly lower than in ZeroFly mini bags. The field study conducted in the Middle Belt of Ghana showed that ZeroFly bags are more effective than PP bags in terms of reducing insect populations, insect damaged kernels, and percent weight loss. Results indicate that the ZeroFly bags can be highly effective in protecting grain for long-term storage, if insect-free grains are stored in them.

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CHAPTER I

INTRODUCTION

Long-term extensive storage of cereal grains began during the Old Kingdom (Period in 3rd millennium BC when Egypt attained first peak of civilization), about 4.5 millennia ago (Levinson and Levinson 1994, Rees 2004). Documented grain storage by inhabitants of ancient Egypt found evidence of insect infestation in food offerings in ancient tombs, built in 4500 B.C. – 395 A.D. thereby suggesting that insect pests of stored commodities evolved together with storage (Levinson and Levinson 1994, Rees 2004). Entomologists approximate that there are 2.5–5 million insect species worldwide, but about 1 million have been recognized, described, and named (Grimaldi and Engel 2005). However, < 0.5% of insect species are regarded as pests, and only few of them cause serious damage (Sallam 2008). Postharvest losses due to insect pests have been estimated from 5 to 10% in the United States (Harein and Davis 1992, Cao et al. 2002), but this percentage is much higher and approaches 40% or more in developing countries (Kitinoja 2010, Parfitt et al. 2010, FAO and World Bank 2011). The high percentage of insect-related food losses in developing countries occurs despite many pest management approaches employed to protect stored grain from insect damage. Such high loss of food is a threat to food security.

The 1996 World Food Summit in Rome defined food security as existing "when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life" (FAO 1996). Food security is a major concern because of the rapidly increasing world human population projected to reach 9.6 billion by the end of 2050 (FAO 2013). Though food production is increasing due to improved cultivation practices, deployment appropriate technologies, fertilizer application, and use of chemical pesticides, the rate of increase is declining and cannot meet the exponentially increasing population. FAO (2013) reported that 842 million people or 12% of world population in 2011–13 were in state of chronic hunger.

Postharvest losses in sub-Saharan Africa are estimated about $4 billion a year (FAO and World Bank 2011). In Vietnam, rice losses are usually 10-25% but may reach 80% under extremely poor storage conditions (these losses relate to insect pests, extreme temperatures, mold, and handling losses) (FAO and World Bank 2011). Tefera (2012) estimated that total maize postharvest losses in Africa range from 14% to 36%. These postharvest losses of 14–36% indicate that losses in the developing world are severe; these losses are due to a combination of factors such as losses in the field, during various processing operations, in storage, and during marketing. Specifically in the developing world, food quality and food security are an increasing challenge and hence the urgent need to ensure food security.

To fulfill the nutritional requirements of a large number of people, the strategy of relying on only improved food production may not be sufficient; other techniques such as preventing losses in the postharvest system also need to be used (FAO and World Bank

2011). The techniques of preventing losses in storage have gradually been developed, researched, and scaled up (Evan 1985, USDA 1986, Arthur 1997, Tefera 2012, Gitonga et al. 2013); however, farmers in developing countries have been too slow in adopting up to date tools and techniques to minimize postharvest losses (FAO 2013, Affognon et al. 2015). The cost of new technology and traditional behavior may be some of the reasons behind limited success of scale up of known effective storage tools and techniques (Parfitt et al. 2010, Rembold et al. 2011).

Use of proper and well-managed grain storage facilities can minimize postharvest losses that are rampant in developing countries. Proper grain storage is an important aspect of food preservation and food security until the next harvest season. Farmers have to store the grain for future household needs or income (FAO 1994, FAO and World Bank 2011, Rembold et al. 2011).

Storage techniques or tools that have been employed by smallholder farmers in developing countries include hanging maize cobs from trees to keep them aerially suspended, grains solar dried on the ground, baskets or cribs made of plant material, earthenware pots, underground storage, storehouses or warehouses or use of bags (FAO 1994, Adejumo and Raji 2007). However, these traditional methods are unable to protect grains from insect pests. To date, there have been lots of attempts to improve storage methods in developing countries. Some recent storage techniques such as metal silos have resulted in a reduction in insect-related postharvest losses (Kimenju and De Groote 2010, De Groote et al. 2013). Despite the benefits of metal silos, their adoption in Kenya was limited because of the high initial cost (De Groote et al. 2013).

Storage in bags is widely practiced in the developing world and some smallholder farmers wrongly believe bags mitigate stored-product insect losses (FAO 1994, Koona et al. 2007, De Groote et al. 2013, Costa 2014). To address the challenge of postharvest losses due to insect pest infestations in bagged commodities, an innovative tool, the ZeroFly® Storage Bag, hereafter referred as ZeroFly bag, was developed by Vestergaard Frandsen SA, Switzerland, as a potentially logical and relatively affordable solution (Anankware et al. 2014, Costa 2014). However, little is known about the insecticidal activity of the ZeroFly bag and its mechanism of action that leads to the control of stored-product insect pests. Therefore, this study investigated the adulticidal effects of deltamethrin, the insecticide incorporated in ZeroFly bag fabric and evaluated the effectiveness of this bag as a barrier to adults of major stored-product insects.

OBJECTIVES

- To evaluate knockdown and adulticidal activity of deltamethrin for *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), the lesser grain borer; *Sitophilous oryzae* (L.) (Coleoptera: Curculionidae), the rice weevil; and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle, adults.
- To evaluate the effectiveness of ZeroFly bag fabric against stored-product insect pests in laboratory.
- Field evaluation of the deltamethrin-incorporated bag (ZeroFly bag) as a barrier to insect pest infestation.

CHAPTER II

REVIEW OF LITERATURE

Postharvest Losses

FAO (1992) defined postharvest loss as "a measurable quantitative and qualitative loss in a given product". After harvest, the given product is subjected to different postharvest operations such as, drying, threshing, winnowing, transportation and storing in the farm and market, where qualitative losses (loss in edibility, nutritional quality, caloric value, and consumer acceptability) and quantitative losses (weight loss) can occur (Kader 2005, Basavaraja 2007, FAO and World Bank 2011, Rembold et al. 2011, Shankar and Abrol 2012). A postharvest system is a set of all operations between harvesting and consumption of agricultural commodities which are fulfilled by different actors in order to achieve an effective and efficient food supply (Sallam 2008, Kiaya 2014). In all operations of the postharvest system, losses can be due to spillage during transportation, or deterioration caused by insects, rodents, molds, and birds (Basavaraja 2007, Kitinoja 2010, FAO and World Bank 2011, Abass et al. 2014). Abass et al. (2014) estimated that 15% of postharvest losses occur in the field, 13-20% during processing, and 15-25% during storage; while storage pest solely accounts for 16% of losses.

Inadequate postharvest infrastructure and storage technologies, lack of knowledge and skills in postharvest management by smallholder farmers in developing countries are the main cause of losses (Abass et al. 2014). According to Kitinoja (2010), farmers use poor quality storage methods such as jute sacks or baskets that get easily damaged during handling and transportation resulting in losses. Recent studies in countries like India, Ghana, Benin, and Rwanda show that postharvest losses of about 20-80% are incurred (Kitinoja 2010). Rembold et al. (2011) reported that 17.4% of the total annual production of cereal grain produced in Africa was lost during postharvest operations. In India postharvest losses of 5.19 kg/q (1 quintal = 100 kg) in rice and 4.32 kg/q in wheat, for both on farm-level and market-level have been reported (Basavaraja 2007). These losses are significant clearly indicate an urgent need for greater attention to postharvest loss mitigation.

Insects are one of the most significant causes for postharvest losses of stored commodities (Hill 1990, Pimental 2002, FAO and World Bank 2011). Insects attack stored food including cereals (rice, wheat, maize, sorghum, millet), legumes (mungbean, blackgram, soybeans), and other dried products (coffee, cardamom, pepper). Presence of live or dead insects and their excreta in stored commodities reduces palatability and acceptability by consumers (Hill 1990, Jood and Kapoor 1992a).

Types of Postharvest Losses due to Insect Pests during Storage

Quantitative Loss

Reduction in physical quantity can be referred as quantitative loss or weight loss. Insect consumption, spillage, and physical loss due to changes in temperature, moisture

content, or chemical composition are responsible for reduction in weight of a commodity (Aulakh and Regmi 2013, FAO 1992). It is estimated that insects and rodents cause loss of 40% in developing countries, however, losses can be more depending on the local area (Kitinoja 2010, Parfitt et al. 2010, FAO and World Bank 2011, Affognon et al. 2015). Assessment of insect-related losses is difficult given the variability that occurs from year to year. However, there are many studies on estimated weight losses due to different stored insect pests (Alonso-Amelot and Avila-Núñez 2011, Togola et al. 2013). Using samples from Venezuela, Alonso-Amelot and Avila-Núñez (2011) estimated that the *S. oryzae* caused 2.2 ± 0.7% to 12.5 ± 2.5% dry weight loss of wheat in a 90-d storage period. Togola et al. (2013) found that 3.29% to 10.94% of rice was infested in the southern region of Benin (West Africa) and the resulting weight loss was up to 5.47% after 6 mo of storage.

Qualitative Loss

Losses in grain quality may vary widely. Reduction in market price of the commodity can be assessed as quality loss (Hodges 2012, Aulakh and Regmi 2013). Change in economic and nutrient value can occur due to incidence of insect pests, mites, rodents and birds, or from handling, physical changes or chemical changes, or by contamination with mycotoxins, pesticide residues, insect fragments, or excreta of rodents and their dead bodies (Rajendran 2005, Aulakh and Regmi 2013). Insects feed on the germ (embryo) of seeds and grains, which is the region where proteins, minerals, and vitamins are present; this reduces nutritional quality (Hill 1990). Insect contamination could affect the sale of cereal grains and legume grains or cause them to be rejected by consumers; both scenarios lead to significant economic loss (Hill 1990). It is reported

that insects can cause change in chemical composition for example, increase in non-protein nitrogen, fat acidity, and decrease in thiamine level and pH of wheat flour (Venkatrao et al. 1960, Jood and Kapoor 1992).

Stored-Product Insect Problems in the Developing World

Cereal grains and grain legumes are a main part of human diet and provide major portion of energy and nutrient needs. Effective grain storage is important for the preservation of food until the next harvest season. Farmers must store the grain for future household needs or for income. Unfortunately, grain storage is risky business with farmers experiencing challenges such as stored-product insect infestation. Stored-product insects infesting various types of grains are cosmopolitan. Major insect pests, which cause damage to stored grain are beetles (Coleoptera) and moths (Lepidoptera) (Groot 2004, Chomchalow 2003). Among them, beetles are most destructive and have a worldwide occurence.

In ancient Egypt there is documentation of grain insects such as *Tribolium* spp. and *Sitophilus* spp. infesting grain and food offerings in ancient tombs (Levinson and Levinson 1994). These insects along with others still continue to cause losses in grain during storage. According to the FAO (1999), Wheat-Postharvest Compendium, major insect pests of wheat in storage in developing countries are, Khapra beetle (*Trogoderma granarium* Everts) (Coleoptera: Dermestidae), *R. dominica, S. oryzae* (L.), and *T. castaneum*. A recent study by Togola et al. (2013) reported that the major insects that damage grain in storage in parts of sub-Saharan Africa are *S. oryzae*, angoumois grain moth (*Sitotroga cerealella*) (Lepidoptera: Gelechiidae) and *R. dominica*. Raoul and Leonard (2013) also reported that *Sitophilus* spp., *R. dominica*, and *Tribolium* spp. are

major pests in parts of sub-Saharan Africa. Postharvest losses due to the above mentioned insects are one of the most important constraints to effective grain storage in the developing countries (Togola et. al. 2013, Raoul and Leonard 2013)

Types of Stored-Product Insects

Stored grain insects are classified as either primary pests (internal grain feeders) or secondary pests (external grain feeders). *S. cerealella*, *R. dominica*, and *S. oryzae* are considered primary pests because they penetrate the hard seed coat of undamaged kernels to feed on the germ and endosperm. Secondary pests such as *T. castaneum*, *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae), which feed on the broken grain or kernels with cracked seed coats, only attack damaged grain (Groot 2004). Larvae of internal feeders such as *R. dominica* and *S. oryzae* feed on and damage the endosperm (Hagstrum et al. 2012).

Major Insect Species Studied in this Thesis

***Rhyzopertha dominica* (F.)** The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is the serious pest of cereal grains worldwide and is believed to have originated from a tropical region (Jia et al. 2008, Edde 2012). *Rhyzopertha dominica* is a highly destructive pest and its larval and adult stages feed on intact whole grain kernels. *Rhyzopertha dominica* attacks wheat, maize, rice, nuts, or dry fruits (USDA 1986, Hagstrum et al. 2012, Edde 2012). Adults and larva both are voracious feeders. Females can lay up to 500 eggs loosely outside the grain kernels and upon hatching the larvae enter the grain kernels and feed (USDA 1986, Hagstrum et al. 2012). Eggs can hatch in few days after which larvae feed inside kernels where they cause significant damage to the endosperm and cause weight loss (Edde 2012, Hagstrum et al.

2012). Several studies have shown that *R. dominica* can cause weight losses of up to 17% (Gundu Rao and Wilbur 1972, Campbell and Sinha 1976). Not only does *R. dominica* cause weight loss, there is evidence that it causes quality loss (nutrient loss) as well (Girish et. al. 1975, Jood et. al. 1992b, Jood and Kapoor 1992a).

Sitophilus oryzae L.

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), is widely distributed in tropical and temperate regions and a destructive insect pest of stored products (Munro 1996, Rees 2004). It is a small beetle (3.0 - 4.6 mm) with a long snout and varies in color from reddish brown to black. *Sitophilus oryzae* is an internal feeder which mainly feeds on the endosperm (USDA 1986, Bello et al. 2001, Rees 2004, Hagstrum et al. 2012). The female bores a small hole in the grain kernel with her mandible to lay eggs in it and covers it with gelatinous fluid that seals the hole (Munro 1996, Hagstrum et al. 2012). Each female lays 300 to 400 eggs during its average life span of 4 to 5 months (USDA 1986). After the egg hatches, the larva starts to feed on the kernel internally and ultimately develops into an adult (USDA 1986 and Hagstrum et al. 2012). Developmental time from egg to adult depends on temperature and relative humidity, but on average a minimum of 30 days is required (USDA 1986).

Sitophilus oryzae is the most common pest of cereal grains and can seriously damage both quality and quantity of whole grains such as wheat, maize, barley, and rice if left untreated (Munro 1996, Rees 2004). It also feeds on beans, nuts, and other stored products. Both adults and larvae feed on cereal grains voraciously (Munro 1996). Insects can fly from one place to other which helps them to infest grains stored in other warehouses.

Tribolium castaneum (Herbst) The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a stored-product pest of economic importance worldwide; it is also considered as a major pest of the food industry (Rees 2004). *Tribolium castaneum* is a polyphagous and cosmopolitan pest which usually feeds on flour and damaged grain (Lu et al. 2010). Each female can lay two to three eggs per day; eggs are laid directly on flour or other food materials and 90% of the eggs laid are viable (Robinson 2005). Eggs hatch into small, cylindrical wormlike larvae in 5 to 12 days (UDSA 1986). *Tribolium castaneum* prefer floury (farinaceous) food materials such as flour or meal, but they can also feed on cracked, damaged or broken kernels. They cannot feed on undamaged intact kernels (USDA 1986, Subramanyam and Nelson 1999).The scent glands of adult *T. castaneum* secrete a malodorous fluid which has a highly unappealing smell (Lu et al. 2010, Velki et al. 2014).

Management of Stored-Product Insect Pests

Great effort has been expended to try and mitigate stored-product insect pest problems in developing countries. Inert dusts, including both non-silica and silica dusts (kaolin, sand) are traditionally used as grain protectants. (Subramanyam and Roesli 2000, Rajendran 2003). Inert dusts control insects by causing desiccation which results from damage of the cuticular wax layer of the cuticle by abrasion and/or adsorption (Golob 1997, Rajendran 2003). However, inert dusts act slowly and can take 20 or more days to kill insects, and these dusts affect grain bulk density, create dusty conditions during grain handling and do not work well when relative humidity is high. Dust also impede flow of grain, and dusts containing crystalline silica may cause silicosis and other respiratory diseases (Pimental 2002, Rajendran 2003). Nevertheless, there is great interest in this

technology and there are many recent studies on the efficacy of diatomaceous earths (DEs) against stored-product insect pests (Subramanyam and Roesli 2000, Wakil et al. 2010, Nwaubani et al. 2014).

Traditionally, farmers in many sub-Saharan countries use inexpensive and locally available plant materials for insect control (Otitodun et al. 2015). Research studies have shown that botanical insecticides are effective against stored-product insect pests (Nikpay 2004, Lee et al. 2004, Negahban et al. 2006, Otitodun et al. 2015). Plant materials are less harmful to human and animals because they can rapidly break down to harmless metabolites (Rajashekar et al. 2012, Hodges 1998). Despite the fact that botanical insecticides are relatively safe for humans, in some cases, they have been found to have only moderate efficacy, to lack consistent efficacy, or to leave unpleasant odor in treated grains (Phillips and Throne 2010). Other approaches to manage insect pests in stored grain include temperature management (Rajendran 2003, Ileleji et al 2007, Pimental 2002), however, smallholder farmers in developing countries cannot afford expensive aerators or other temperature management equipment.

Recent hermetic bagging technologies such as PICS bag and GrainPro super bags have been deployed to solve the problem of stored-product insects. A PICS bag consists of one woven polypropylene bag surrounding two layers of high density polyethylene (HDPE) which costs about 2USD on local markets (Baoua et al. 2014, Costa 2014, Martin et al. 2015). It is effective in reducing population growth by lowering O_2 and increasing CO_2 level since double layer of thick HDPE greatly restrict the exchange of internal O_2 and CO_2 (Baoua et al. 2014, Martin et al. 2015). GrainPro technology consists of multi-layer polyethylene film which is highly impermeable to O_2, which cost 2.5-3

USD. Both of the techniques, PICS and GrainPro bags are equally effective to control insects during storage (Baoua et al. 2013). However, these techniques require sealable containers and with careful management to maintain a tight seal (Baoua et al. 2013).

Since the 19th century, synthetic insecticides have been an important part of pest management programs of stored grain. Fumigants and contact insecticides are examples of effective major synthetic insecticides used to control insect pests (Zettler and Arthur 2000). Contact insecticides such as Chloropyrifos or deltamethrin are often mixed with grains or used to treat surfaces of grain storage structures (Arthur 1994, Kljajic and Peric 2009). The choice of insecticide depends on the knowledge level of the user, type of insects targeted, stored produce, legal restrictions, and toxicity (FAO 1994, Arthur 1996). Chemical control has proved to be one of the best insect control measures for many decades (Arthur 1996, Zettler and Arthur 2000, Kljajic and Peric 2009). However, farmers in developing countries have limited access to newer reduced risk insecticides. These farmers rarely sufficiently trained on proper handling and use of insecticides, which may affect human health and/or negatively affect the environment (ecosystems) (USDA 1986, Kaminski and Christiaensen 2014). Some insect control technologies currently used by smallholder farmers have often proved to be unaffordable, unsuitable for smallholder farmers, and to negatively affect the environment. However, eliminating these insect control technologies entirely could have serious economic consequences. Therefore, a novel technology such as insecticide incorporated storage bags could be practical, i.e. effective and affordable, for smallholder farmers and has potential to significantly minimize the insect infestations during storage.

The Concept of Insecticide-Treated Fabric

Storage bags such as jute and polypropylene bags have been extensively used to store the different commodities. Unfortunately, storing grains in jute or polypropylene bags often does not prevent stored-product insect pest infestation. The need for a bag that effectively mitigates infestation of grain stored leads to the development of the insecticide-incorporated storage bag. The concept of insecticide-incorporated textile has been successful in mosquito nets for mosquito control to mitigate malaria (Barlow et al. 2001). This success led to the concept of the insecticide-incorporated storage bags for controlling stored-product insect pests.

Parkin (1948) investigated the effectiveness of 1 or 5% dichloro-diphenyl-trichloroethane (DDT)-impregnated sacks to mitigate infestation by *Calandra granaria* (L.) (Coleoptera: Curculionidae), *T. castaneum*, *Ptinus tectus* Boield (Coleoptera: Ptinidae), and *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae). That study showed that no live insects were found in sacks impregnated with 5% DDT. According to Parkin (1948), it is much less toxic to impregnate the bag than to apply DDT directly to the grains. Atkins and Greer (1953), investigated the efficacy of DDT and BHC (Benzene hexachloride) impregnated jute fabric against *Ephestia kuehniella* (Lepidoptera: Pyralidae), *P. tectus*, and *T. confusum* (Coleoptera: Tenebrionidae) and showed that DDT impregnation was successful in minimizing the insect infestation at and above 1% (w/w). Their data support conclusions by Parkin (1948). In a study where methyl bromide, acrylonitrile, carbon tetrachloride, and ethylene dibromide (EdBr) were impregnated in the bag by Muthu and Pingale (1955), and reported that 10cc (10 ml) of EdBr per bag was enough to kill *S. oryzae*, *R. dominica*, and *T. castaneum* present in the bagged grain. Because of safety and environmental concerns associated with DDT and EdBr use, these

insecticides have since been banned for use on grain and food. Botanicals and Actellic Super also have been incorporated in storage bag fabric and bags made from such fabric have been shown to effectively control stored-grain insects for 3 to 4 mo (Koona et al. 2007, Groote et al. 2013).

One recent innovative tool, ZeroFly® Storage Bag (hereafter referred to as the ZeroFly bag), is based on the concept of insecticide incorporated fabric, that has been developed to mitigate insect pest infestation of bagged grains. The ZeroFly bag, developed by Vestergaard Frandsen SA, Switzerland, could be an extremely effective method for mitigating insect pests for long-term storage of grain (Anankware et al. 2014). The ZeroFly bag is a deltamethrin-incorporated bag that is marketed for the control of insect pests of stored grain.

The ZeroFly bag

The ZeroFly bag is a woven polypropylene bag made of deltamethrin-incorporated fabric and marketed for storage of commodities such as cereal grains, pulses and oilseeds (Anankware et al. 2014). Deltamethrin is a broad-spectrum pyrethroid insecticide approved by the Food and Agriculture Organization (FAO) and World Health Organization (WHO). It is registered in U.S.A. for use in stored grain (Dotson et al. 2010) and also registered in many parts of the world for stored grain protection (Vayias et al. 2010). Deltamethrin is an effective insecticide in the ZeroFly bag fabric and provides a powerful killing action against stored product insects before they can infest the grain or seed stored in the bag. Anankware et al. (2014) showed that the ZeroFly bag caused 100% mortality of *S. zeamais* after 48 hours of exposure to ZeroFly bag fabric. However,

little data is available on the effectiveness of the ZeroFly bag against other stored-product insect pests.

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CHAPTER III

Contact Toxicity of Deltamethrin against *Tribolium castaneum* (Coleoptera: Tenebrionidae), *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) Adult

ABSTRACT This study was conducted in the context of evaluating deltamethrin-incorporated ZeroFly® Storage Bags for efficacy against stored-product insect pests. We evaluated response to deltamethrin concentrations by adults of three stored-product insects, namely, *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), and *Rhyzopertha dominica* (F.). In insect toxicological studies, knockdown is the state of intoxication and partial paralysis as a result of exposure to an insecticide. Deltamethrin concentrations of 1, 25, 50, 100, 250, 500, 1000, and 3000 ppm were sprayed on glass Petri dishes and tested over two exposure periods, 24 or 48 h. After drying, twenty adults of either of the species were introduced to determine the contact toxicity of deltamethrin. Assessments for knockdown were made at 15-minute intervals after the initial exposure and at 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h after being transferred to clean Petri dishes with diet. Mortality was assessed 72 h after transfer to clean diet. Deltamethrin was highly effective against all three species tested and achieved 99% knockdown of insects of all species within 4 h after exposure at concentrations ≥ 25 ppm. Although some insects recovered from initial knockdown at concentrations ≤ 1000 ppm, nearly all the insects were killed at 3000 ppm when exposed to 48 h. LC_{95} values for all species tested, for the 48-h exposure period, were ≈ 3,000 ppm, the concentration of deltamethrin present in new ZeroFly bag fabric.

KEY WORDS knockdown, bagged grain, *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.)

Introduction

Insect pests are a major cause of postharvest losses and are responsible for 5−10% of the food destroyed or contaminated after harvest in the United States (Harein and Davis 1998, Cao et al. 2002). Postharvest losses experienced by smallholder farmers due to insect pests in developing countries are much larger and range from 30−80%, and are a major threat to food security (Giga et al. 1991, FAO and World Bank 2011, Kitinoja et al. 2011, Affognon et al. 2015). Insects feeding on kernels cause weight loss, contaminate food with feces, exuviae, webbing, insect cadavers, and open avenues for microfloral contamination thereby causing nutritional and quality losses that make food unfit for consumption (Harris and Lindbland 1976, Boxall et al. 1979, Bhargava et al. 2007, FAO and World Bank 2011, Tefera et al. 2011). The amount of grain lost to insect pests by smallholder farmers during storage is greater for farmers who do not have access to modern grain storage techniques and facilities (FAO and World Bank 2011, Hodges et al. 2014, Affognon et al. 2015). Development of proper storage techniques that are economically and socially practical to smallholder farmers can reduce the amount of insect-related loss during storage.

Farmers in developing countries predominantly use traditional storage techniques which include open platforms, woven baskets, pots, mud rhombus, maize cribs, bamboo storage structures, straw roofed storage structures, underground storage, bag storage, and storehouses (warehouses) (FAO 1994, Adejumo and Raji 2007). Because most of these structures are made of locally available materials, small-scale farmers find them economically feasible to construct. Currently, bag storage is the most preferred storage technique in developing countries (FAO 1994, Koona et al. 2007, De Groote et al. 2013).

However, postharvest losses in bagged commodities is prevalent; for maize stored in traditional polypropylene bags for 90 d, losses in Uganda and Burkina Faso were estimated at approximately 54% and 60%, respectively (Costa 2014). The ZeroFly® Storage Bag (hereafter referred to as the ZeroFly bag) is a potentially logical and relatively affordable solution that can be used to address the challenge of postharvest losses due to insect infestations in bagged commodities (Anankware et al. 2014, Costa 2014). However, there is lack of published data that detail the toxicity of deltamethrin present in the ZeroFly bag fabric.

Deltamethrin is a broad spectrum synthetic pyrethroid insecticide that is widely used to control stored-product insect pests (Arthur 1997, Kljajic and Peric 2009). It is registered in the United States for use in stored grain (Dotson et al. 2010); it is also registered in many parts of the world for stored grain protection (Vayias et al. 2010). Deltamethrin is a synthetic version of naturally occurring pyrethrins extracted from pyrethrum of dried *Chrysanthemum* flowers (Joy 1993, Bhanu et al. 2011). It is less dangerous than other pesticides due to its low toxicity to vertebrates, (does not bio-accumulate, and can be metabolized by plants and animals) (WHO 1990 and Dotson et al. 2010). It kills insects by paralyzing their nervous system, giving a quick knockdown effect, loss of co-ordination, and eventually death (Velki et al. 2014).

Several studies have shown that deltamethrin is an effective grain protectant (Evans 1985, Arthur 1997) and can also be used as a surface treatment singly or in combination with other insecticides to control insect pests (Arthur 1997, Arthur et al. 2009, Sehgal et al. 2013). Deltamethrin is the most common pyrethroid for treating mosquito nets; it provides an excito-repellent effect when applied to nets (Barlow et al.

2001). The concept of insecticide-incorporated fabric has been successfully used in mosquito nets to control malaria (Barlow et al. 2001, Lengeler 2009). The use of deltamethrin-incorporated mosquito nets gave rise to the concept of insecticide-incorporated storage bags for the control of stored-product pests (Anankware et al. 2014).

There are few published studies documenting the contact toxicity of deltamethrin against stored-product insects and its efficacy when incorporated in polypropylene bags. Recent experiments conducted by Anankware et al. (2014) reported that 100% of *Sitophilus zeamais* (Mot) (Coleoptera: Curculionidae) were knocked down after 12 h of exposure to 1,000 ppm of deltamethrin. In this study, we determined dose-response relationships of deltamethrin for adults of three stored-product insect pests, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle; *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), the rice weevil; and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), the lesser grain borer, in order to elucidate the effects of deltamethrin i.e. active ingredient incorporated in ZeroFly bag on the species.

Materials and Methods

Insects. Adults of *T. castaneum*, *S. oryzae*, and *R. dominica* used for the experiment were obtained from laboratory cultures maintained at the Department of Entomology and Plant Pathology, Oklahoma State University. *T. castaneum* is an external infesting species and was reared on 95% all-purpose wheat flour (Great Value $_{TM}$, Walmart Inc.) and 5% Brewer's yeast (wt:wt) at a temperature and relative humidity (RH) of 28 ± 1°C and 65 ± 5%, respectively. *R. dominica* and *S. oryzae* are internal infesting species and were reared on 95% whole-wheat kernels of hard red winter wheat (Jagger) and 5% Brewer's yeast (wt:wt) at 28 ± 1°C and 65 ± 5%. One- to three-wk-old

adults of each species were used for the experiment. Voucher specimens of *T. castaneum*, *S. oryzae*, and *R. dominica* that were used in this study were deposited in the K. C. Emerson Entomology Museum at Oklahoma State University under lot numbers 136, 137, and 126, respectively.

Deltamethrin Concentrations. A type II semi-synthetic pyrethrin insecticide, technical grade deltamethrin (98% AI, Sigma Aldrich, Saint Louis, MO) was used. To determine contact toxicity of deltamethrin against *R. dominica, S. oryzae*, or *T. castaneum,* eight concentrations of deltamethrin, 1, 25, 50, 100, 250, 500, 1,000, and 3,000 ppm (0.00004717, 0.000122, 0.000242, 0.000491, 0.001212, 0.002406, 0.004811, and 0.0144 mg/cm^2, respectively) were tested. These concentrations were obtained by dissolving 98% deltamethrin in absolute ethanol (Ethanol 100%, AI, Pharmco Products, Inc., Brookfield, CT). A stock solution of 3,000 ppm was prepared from which other desired concentrations (serial dilutions) were prepared. To make 3,000 ppm of deltamethrin, five ampules, of 10 mg each, of 98% deltamethrin were dissolved in 16.336 ml of ethanol. Prior to making the 3,000 ppm solution, 16.336 ml of ethanol was measured out using a 250-ml graduated cylinder (PYREX®, made in Germany). Serial dilutions were used to prepare lower concentrations of deltamethrin that were required for the experiment. For lower concentrations of 1,000, 500, and 100 ppm, a volume of 10 ml deltamethrin of the desired concentrations was prepared; for concentrations of 50 and 25 ppm deltamethrin, the volume of solutions made was 50 ml. For 1 ppm of deltamethrin, a volume of 300 ml was prepared. These final volumes were selected based on the spray volume required for the experiment and also to ensure that the desired volume of 3,000 ppm deltamethrin stock solution could be accurately measured out, especially for the

lower concentrations. Pre-calculated volumes of 3,000 ppm of deltamethrin (stock solution) were measured and mixed with pre-calculated volumes of ethanol. To make 10 ml of 1,000, 500, 250, and 100 ppm solutions, 3.4, 1.7, 0.84, and 0.33 ml of 3,000 ppm deltamethrin, respectively, were measured out using a pipette (Gilson®, Middleton, WI). These volumes were mixed with 6.6, 8.3, 9.16, and 9.67 ml of 100% ethanol, respectively. To make 50 ml solutions of 50 and 25 ppm, 0.83 and 0.42 ml of 3,000 ppm deltamethrin, respectively, were used. These volumes were mixed with 49.17 and 49.58 ml of 100% ethanol, respectively. To make a 300 ml solution of 1 ppm, 0.1 ml of 3,000 ppm deltamethrin stock solution was measured out and mixed with 299.9 ml of 100% ethanol. Volumes of ethanol used were calculated using the formula $C_1V_1 = C_2V_2$.

Concentration-Time-Response Contact Toxicity Bioassay. *Time to Knockdown.* Solutions of technical grade deltamethrin were used to conduct contact toxicity tests. Fluon® (Polytetrafluoroethylene, Sigma Aldrich Saint Louis, MO) was applied to the inner rim of each 9-cm glass Petri dish to prevent insects from escaping. For each species and concentration, there were three sub-replicates. Control dishes were sprayed with ethanol only. For each concentration, 0.3 ml of the deltamethrin solution was sprayed on the inside bottom of a glass Petri dish using an airbrush (Patriot airbrush connected to HD mini regulator and airbrush compressor; TCP Global, SAN DIEGO, CA). This procedure was conducted inside a fume hood. Petri dishes were allowed to dry for 24 h before placing insects on them.

For each species, twenty 1- to 3-wk-old adults were selected from laboratory cultures and placed in each Petri dish. Insects were exposed in the treated dishes for 24 or

48 h. Insects were observed for knockdown every 15 min until all insects were knocked down. The final count was conducted after 24 or 48 h, depending on the exposure period.

Lethal Toxicity. Insects which were exposed in the treated dishes for 24 or 48 h were then transferred to clean (untreated) glass Petri dishes containing 5 g of 95% whole wheat kernels (hard red winter wheat) + 5% (by weight) brewer's yeast (*R. dominica* and *S. oryzae*) or 95% all-purpose wheat flour + 5% (by weight) brewer's yeast (*T. castaneum*). Insects were observed for knockdown and/or mortality 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h after transfer to clean dishes containing their respective diets. Insects were categorized as knocked down when they were lying on their backs but able to move legs and/or antenna or insects were moving too slowly compared to insects in the control dishes (Arthur 1997). Insects were considered dead when they could not move their body parts after being prodded using a camel's hair brush. Each treatment (concentration x exposure time combination) had three temporal replications. The experimental design was a randomized complete block design with sub-replication.

Data Analyses. Knockdown data were subjected to probit analyses to determine the time to knockdown 50%, 95%, and 99% of insects; these times will be referred to as KT_{50}, KT_{95}, and KT_{99}, respectively, hereafter. Mortality was assessed 3 d after transfer to clean Petri dishes containing the respective diet for each species. Mortality data were analysed to determine concentrations that kill 50%, 95%, and 99% of insects; these concentrations will be referred to as LC_{50}, LC_{95}, and LC_{99}, respectively, hereafter. Probit analyses were conducted by using PoloPlus (LeOra Software, 2005). The criterion for precision was the width of 95% confidence limit.

Results

Concentration-Time-Response Contact Toxicity. *Time to Knockdown.* Knockdown (KD) assessments were made at 15 min intervals after the initial exposure to different concentrations, namely, 1, 25, 50, 100, 250, 500, 1,000, and 3,000 ppm of deltamethrin (DM) for *T. castaneum*, *S. oryzae*, and *R. dominica*. The percentage of insects knocked down (KD %) in dishes treated with technical grade absolute ethanol only (control dishes) was ≤ 5% except for *R. dominica* where 14.4 % were knocked down on an average. The KT_{50}, KT_{95}, and KT_{99} times decreased with increasing concentration of deltamethrin in both the 24- and 48-h exposure periods (Tables 1–3). A concentration of 3,000 ppm produced the shortest KT_{50}, KT_{95}, and KT_{99} values. In relation to KT_{99} for 3,000 ppm and the 24-h exposure period, values for *T. castaneum*, *S. oryzae*, and *R. dominica* were 54.8, 59.8, and 75.6 min, respectively. For the 48-h exposure period, these values were 48.8, 101, and 79.7 min, respectively. Based on KT_{99} confidence intervals, there were no significant differences among KT_{99} values for all three species. For concentrations ≥ 25 ppm, 99% of insects of all species were knocked down within 4 h. For concentration < 25 ppm, insects recovered when transferred to their respective diets after the 24- or 48-h exposure periods.

Lethal Toxicity. Concentrations of deltamethrin required to kill 50, 95, and 99% of the individual in samples of *T. castaneum*, *S. oryzae*, and *R. dominica* were compared (Table 4). Probit analysis of mortality data for 3 d after transfer of insects to untreated Petri dishes with diet show that adults of *T. castaneum* were more susceptible to deltamethrin than *S. oryzae* and *R. dominica* (Table 4). The relevance of 3 d is that it was assumed any individual that was unresponsive 3 d after transfer to untreated Petri dishes

with diet was dead. Based on our data, LC_{95} values for all the three species were less than or ≈ 3,000 ppm for the 48-h exposure period. The relevance of this is that the concentration of deltamethrin in new ZeroFly bags is ≈ 3000 ppm. The fact that the LC_{95} for *R. dominica* for the 24-h exposure period was 4,213 ppm and 3,193 ppm for the 48-h exposure period indicates that this species requires longer exposure times for insects to get killed.

Discussion

In insects, neurotoxic activity of deltamethrin is caused by disruption of axonal transmission of nerve impulses as a result of altering ion permeability of nerve membranes (Siegfried 1993). Deltamethrin is known for its rapid knockdown activity on various insects, including coleopterans (Velki et al. 2014), so the effectiveness of higher concentrations of deltamethrin to adults of *T. castaneum*, *S. oryzae, and R. dominica* observed in this study was expected. Based on our data, deltamethrin at 3,000 ppm was found to be highly effective against the adults of *T. castaneum*, *S. oryzae,* and *R. dominica* because 99% of adult insects were knocked down within ≈ 1.5 h. For a concentration of 1,000 ppm, the KT_{99} value showed that 99% of the insects were knocked down within 2 h. A similar study by Anankware et al. (2014) found that 98% of *S. zeamais* adults were knocked down after 8 h of exposure to a deltamethrin concentration of 1,000 ppm whereas mortalities of 76.7, 53.3, 30, 28, 21.7, 23.3, and 0 % were recorded for 500, 250, 100, 50, 25, 12.5, and 0.0 ppm, respectively. They also observed recovery of insects from knockdown. However, after 72 h at 1,000 ppm, Anankware et al. (2014) reported 96.7% mortality of *S. zemais*. In this study, recovery after the exposed adults were transferred to untreated diet was generally greater for concentrations of ≤ 1,000 ppm

compared to 3,000 ppm; meaning mortality was higher for 3,000 ppm. Lethal toxicity assessment 3 d after insects were transferred to diet suggests that at least 95% adult mortality would be obtained by exposing *T. castaneum*, *S. oryzae, and R. dominica* to 3,000 ppm of deltamethrin for 48 h.

The logical explanation for higher knockdown in the case of *R. dominica* in control Petri dishes may be lack of food and desiccation (Toews et al. 2003); insects were monitored for knockdown in a fume-hood with continuous airflow. Knockdown of *R. dominica* in concrete when treated with distilled water was 72% in the study by Toews et al. (2003). Food was not provided to insects during deltamethrin exposure in our experiment. However, after 24 and 48 h, insects were transferred to diet and maintained at RH 65% to monitor recovery of insects in control dishes.

There are reports on the insecticidal efficacy of deltamethrin against stored-product insects based on direct application to grains or surfaces. According to Arthur (1997), deltamethrin (0.05%) had potential to knockdown or kill almost all *T. castaneum* and *R. dominica* after 24 h exposure. Similarly, Evans (1985) concluded deltamethrin has excellent potential for use as a protectant of either stored corn or wheat while testing efficacy of insecticides (malathion, lindane, primiphos-methyl, fenitrothion, etrimfos, permethrin, and deltamethrin) against different species of insects from Kawanda, Uganda. Athanassiou et al. (2004) reported mortality of *S. oryzae* exposed to 0.25 ppm deltamethrin for 7 d in wheat was 89, 92, and 86%, respectively, after 1, 2, and 3.5 mo, respectively, of storage. However, the effectiveness of deltamethrin applied to grain and surfaces like concrete and wood, might not explain the mechanism of the insecticide in deltamethrin-incorporated polypropylene bags. In treated grains and surfaces, the

mortality of insects might not be solely due to the pesticide because abrasion of insect cuticle by rough surfaces or dust present in grain, concrete, or wooden floors may also play a role in insect mortality (Subramanyam and Roesli 2000, Toews et al. 2003).

Therefore, this study on contact toxicity of deltamethrin, conducted in glass Petri dishes, facilitates the interpretation of data from studies involving exposure of stored-product insects to ZeroFly bag fabric. The glass allows us to determine the effect of deltamethrin on insects at different concentrations and exposure periods. For each species tested, these data can be used to plot mortality at different concentrations over the same exposure period or mortality at the same concentration over different exposure periods. The former can in future be used to gauge the effectiveness of ZeroFly bags (due to efficacy of deltamethrin) after any period of usage within the 2-yr life span of the bag. Thus, this experiment could improve our understanding of the way the ZeroFly bag protects grain stored in it from insect infestation.

Our study corroborates the findings of Anankware et al. (2014), who demonstrated the effectiveness of the ZeroFly bag against *S. zeamais*. A study conducted by Costa (2014) also showed that the ZeroFly bag is highly effective in protecting grain stored in it from stored-product insect pest infestation. Data from this study validate the above findings of Anankware et al. (2014) and Costa (2014), and the Vestergaard Frandsen's (SA, Switzerland) assumption that deltamethrin incorporated in ZeroFly bags is being picked up by insects and causing the knockdown and mortalities seen in ZeroFly fabric exposures. Moreover, these data can be used to estimate the initial concentration of deltamethrin in ZeroFly bags based on insect mortality data that can be easily obtained outside the laboratory.

Residue Analysis by Ghana Standards Authority concluded that residue level of deltamethrin in grains stored in ZeroFly bag was 1 mg/kg which is lower than CODEX Alimentarium MRL of 2 mg/kg for cereal grains. This means cereal grain stored in ZeroFly bags are safe for human consumption. In this regard, the deltamethrin-incorporated bags could be considered as a safe storage method which affords grain protection at a relatively lower cost (Anankware et al. 2014, Costa 2014). However, more research is needed to determine the insecticidal activity of ZeroFly bags to other stored-product insects not tested in this study.

Acknowledgements

We thank Kandara Shakya, Friendly Yang, and Brian Barnett for their technical support. This work was funded by the Vestergaard Frandsen SA, Switzerland; partial funding for the work came from the Oklahoma Agricultural Experiment Station (Project No. OKL2949). This paper reports the results of research only. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by Oklahoma State University, Vestergaard Frandsen SA, Switzerland, or the US Department of Agriculture.

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Table. 1. Time to knockdown in minutes (KT_{50}, KT_{95}, and KT_{99}) for *T. castaneum* adults exposed to different concentrations of deltamethrin (DM) at 24- and 48-h exposure periods.

Exposure Period	Concentration (ppm)	KT_{50} (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
24 h	1	159.3	929.5	1,930.5	2.1 ± 0.11	288.9 (37)
		(132.3 – 208.6)	(543.7 – 2,550)	(953.1 – 7373.1)		[7.81]
	25	38.9	83.7	114.9	4.95 ± 0.21	217.1 (28)
		(33.1 – 44.3)	(71.6 – 105.2)	(93.7 – 158.1)		[7.75]
	50	29.4	68.1	96.5	4.49 ± 0.20	94.0 (28)
		(26.1 – 32.4)	(60.0 – 80.0)	(81.9 – 120.5)		[3.35]
	100	22.0	54.0	78.5	4.28 ± 0.22	159.3 (28)
		(18.0 - 25.7)	(45.1 – 70.7)	(61.8 – 114.9)		[5.69]

Exposure Period	Concentration (ppm)	KT_{50} (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
24 h	250	17.6	49.9	76.7	3.65 ± 0.22	163.0 (28)
		(13.1 – 21.5)	(40.6 - 68.5)	(58.1 - 123.5)		[5.82]
	500	12.3	37.7	59.9	3.38 ± 0.28	75.4 (28)
		(8.9 – 15.1)	(31.8 – 47.9)	(47.2 – 88.3)		[2.69]
	1,000	12.7	35.8	55.2	3.64 ± 0.31	85.1 (28)
		(9.2 - 15.4)	(30.1 - 46.6)	(43.2 - 83.9)		[3.04]
	3,000	10.8	34.0	54.8	3.29 ± 0.31	90.7 (28)
		(6.7 - 13.9)	(28.1 - 45.6)	(41.9 - 90.2)		[3.24]
48 h	1	165.8	679.4	1,218.9	2.68 ± 0.14	166.6 (37)
		(146.7 - 194.3)	(486.3 - 1,137.7)	(787.8 - 2,300)		[4.50]

Exposure Period	Concentration (ppm)	KT_{50} (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
48 h	25	36.5	86.5	123.7	4.39 ± 0.18	219.1 (28)
		(30.6 – 42.0)	(73.0 – 110.5)	(99.0 – 174.6)		[7.82]
	50	30.0	63.1	85.9	5.09 ± 0.24	77.8 (28)
		(27.2 – 32.7)	(56.6 – 72.6)	(74.5 – 103.8)		[2.78]
	100	21.7	50.7	71.9	4.48 ± 0.25	177.9 (28)
		(17.6 - 25.5)	(41.9 - 67.6)	(56.3 - 108.3)		[6.36]
	250	18.7	50.9	77.3	3.77 ± 0.22	144.6 (28)
		(14.6 - 22.3)	(42.2 - 67.6)	(59.9 - 117.1)		[5.16]
	500	17.7	44.7	65.6	4.09 ± 0.26	127.5 (28)
		(14.25 - 20.9)	(37.3 - 58.8)	(51.4 - 97.9)		[4.55]

Exposure Period	Concentration (ppm)	KT_{50} (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
48 h	1,000	14.7	35.8	51.9	4.23 ± 0.34	70.7 (28)
		(11.9 - 16.9)	(30.9 - 44.7)	(42.1 - 72.4)		[2.52]
	3,000	13.0	33.2	48.8	4.06 ± 0.36	74.7 (28)
		(10.0 - 15.4)	(28.2 - 42.4)	(38.9 - 71.9)		[2.67]

KT stands for knockdown time; CI for confidence interval; Heterogeneity value (quotient of chi-square and degrees of freedom)

Table. 2. Time to knockdown in minutes (KT_{50}, KT_{95}, and KT_{99}) for *S. oryzae* adults exposed to different concentrations of deltamethrin (DM) at 24- and 48-h exposure periods.

Exposure Period	Concentration (ppm)	KT_{50} (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
24 h	1	248.9	1095.6	2024.5	2.56 ± 0.17	163.0 (37)
		(206.3 - 338.6)	(669.0 - 2645.2)	(1080 - 6246)		[4.4]
	25	79.2	152.9	200.9	5.75 ± 0.24	206.6 (28)
		(71.7 – 86.4)	(133.8 – 187.0)	(168 – 265.6)		[7.38]
	50	76.9	132.9	166.6	6.94 ± 0.29	139.2 (28)
		(71.7 - 82.1)	(120.8 - 151.3)	(146.9 – 199.2)		[4.97]
	100	56.2	103.4	133.2	6.21 ± 0.27	266.5 (28)
		(49.3 - 62.5)	(89.9 - 128.6)	(110.9 – 180.4)		[9.51]

Exposure Period	Concentration (ppm)	KT_{50} (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
24 h	250	49.3	87.9	111.7	6.53 ± 0.31	140.9 (28)
		(44.8 – 53.5)	(79.1 – 101.8)	(97.2 – 136.8)		[5.03]
	500	42.1	73.7	92.9	6.76 ± 0.34	164.6 (28)
		(37.5 – 46.3)	(65.6 – 87.3)	(79.9 – 117.6)		[5.88]
	1,000	41.6	69.4	85.8	7.39 ± 0.39	83.2 (28)
		(36.6 – 44.3)	(63.6 – 77.9)	(76.6 – 100.5)		[2.97]
	3,000	31.4	49.5	59.8	8.29 ± 0.57	49.7 (28)
		(29.4 - 33.1)	(45.9 – 54.7)	(54.2 - 68.5)		[1.77]
48 h	1	248.4	1146.0	2159.4	2.47 ± 0.17	381.9 (37)
		(190.0 - 451.8)	(570.8 – 6,757.9)	(889.3 – 20,986)		[10.32]

Exposure Period	Concentration (ppm)	KT_{50} (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
48 h	25	103.4	206.4	274.8	5.48 ± 0.26	264.7 (28)
		(93.4 – 115.5)	(170.2 – 290.2)	(213.3 – 434.9)		[9.45]
	50	79.4	132.7	164.1	7.38 ± 0.31	347.8 (28)
		(70.9 – 87.5)	(116.1 – 165.6)	(137.9 – 222.6)		[12.42]
	100	65.2	114.5	144.6	6.73 ± 0.29	122.6 (28)
		(60.6 – 69.6)	(104.4 – 129.5)	(128.0 – 171.1)		[4.37]
	250	48.8	90.3	116.5	6.15 ± 0.28	145.2 (28)
		(43.9 – 53.2)	(80.9 – 105.1)	(100.8 – 143.9)		[5.18]
	500	44.5	79.4	101.0	6.53 ± 0.31	92.8 (28)
		(41.0 – 47.7)	(72.5 – 89.5)	(89.7 – 119.1)		[3.31]

Exposure Period	Concentration (ppm)	KT_{50} (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
48 h	1,000	39.1 (36.5 – 41.6)	65.9 (60.8 – 73.3)	81.8 (73.5 – 94.7)	7.27 ± 0.4	68.7 (28) [2.45]
	3,000	31.6 (29.2 - 33.9)	54.8 (50.2 - 61.5)	68.8 (61.3 - 80.6)	6.89 ± 0.4	65.9 (28) [2.36]

KT stands for knockdown time; CI for confidence interval; Heterogeneity value (quotient of chi-square and degrees of freedom).

Table. 3. Time to knockdown in minutes (KT_{50}, KT_{95}, and KT_{99}) for *R. dominica* adults exposed to different concentrations of deltamethrin (DM) at 24- and 48-h exposure periods.

Exposure Period	Concentration (ppm)	KT_{50} (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
24 h	1	141.4	443.1	711.2	3.31 ± 0.16	321.0 (37)
		(124.6 – 165.2)	(324.3 – 767.6)	(470.7 – 1485.6)		[8.68]
	25	60.6	92.6	110.4	8.93 ± 0.43	36.5 (28)
		(58.6 – 62.6)	(88.4 – 97.9)	(103.8 – 119.1)		[1.30]
	50	56.1	83.0	97.7	9.65 ± 0.51	80.3 (28)
		(53.2 – 58.8)	(77.6 – 98.6)	(89.4 – 110.8)		[2.87]
	100	53.8	84.9	102.5	8.29 ± 0.41	133.7 (28)
		(49.8 – 57.5)	(77.5 – 96.6)	(91.1 – 122.5)		[4.77]

Exposure Period	Concentration (ppm)	KT_{50} (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
24 h	250	50.8 (47.2 – 54.2)	77.0 (70.6 – 87.5)	91.5 (81.7 – 108.9)	9.06 ± 0.52	126.6 (28) [4.52]
	500	45.7 (42.8 – 48.4)	68.4 (63.3 – 76.2)	80.9 (73.2 – 93.8)	9.35 ± 0.52	93.6 (28) [3.33]
	1,000	41.8 (38.8 – 44.6)	68.0 (66.8 – 90.9)	83.9 (80.2 – 121.4)	7.79 ± 0.42	89.3 (28) [3.19]
	3,000	38.9 (35.7 – 41.9)	62.2 (58.7 – 75.9)	75.6 (66.6 – 91.8)	8.05 ± 0.46	111.7 (28) [3.99]
48 h	1	128.9 (109.9- 155.8)	450.3 (310.3 – 931.6)	756.1 (460.2 – 2025.8)	3.03 ± 0.15	446.2 (37) [12.06]

Exposure Period	Concentration (ppm)	KT_{50} (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
48 h	25	82.2	136.5	168.4	7.47 ± 0.29	435.7 (28)
		(74.2 – 89.9)	(120.8 – 135.1)	(143.8 – 218.3)		[11.78]
	50	64.5	118.7	152.8	6.21 ± 0.27	385.5 (28)
		(55.4 – 72.7)	(101.3 – 155.7)	(124.1 – 223.6)		[13.77]
	100	58.0	100.1	125.5	6.95 ± 0.31	341.0 (28)
		(50.4 – 64.9)	(86.8–127.1)	(104.2 – 175.2)		[12.17]
	250	54.7	90.3	111.1	7.55 ± 0.36	342.4 (28)
		(47.4 – 61.1)	(78.6 – 114.7)	(92.9 – 155.3)		[12.23]
	500	48.1	79.4	93.9	8.00 ± 0.40	168.5 (28)
		(41.0 – 47.7)	(43.6 – 52.2)	(82.0 – 116.1)		[6.01]

Exposure Period	Concentration (ppm)	KT_{50} (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
48 h	1000	42.1	63.3	74.9	9.27 ± 0.53	66.3 (28)
		(39.8 – 44.3)	(60.0 – 69.6)	(68.4 – 85.2)		[2.37]
	3000	36.8	63.5	79.7	6.94 ± 0.38	86.5 (28)
		(33.9 – 39.6)	(57.8 – 72.3)	(70.4 – 94.9)		[3.08]

KT stands for knockdown time; CI for confidence interval; *Heterogeneity value (quotient of chi-square and degrees of freedom).

Table. 4. Probit analyses of *T. castaneum*, *S. oryzae*, and *R. dominica* adult mortality data for 24- and 48-h exposure periods 3 days after transfer to clean diet

	LC_{50} (95% CI)	LC_{95} (95% CI)	LC_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
T. castaneum					
24-h exposure	111.4 (95.2 - 129.4)	1,342.9 (1,023.9 - 1,871.0)	3,767.6 (2,611.6 - 5,937.7)	1.52 ± 0.08	27.6 (22) [1.3]
48-h exposure	48.9 (33.6 - 67.3)	1,282.9 (779.0 - 2,555.6)	4,968.4 (2,502.93 - 13,196.6)	1.16 ± 0.06	73.7 (22) [3.3]
S. oryzae					
24-h exposure	231.7 (165 - 324.4)	2,765.7 (1,559.3 - 6,746.5)	7,726.6 (3,638.7 - 25,723.5)	1.52 ± 0.07	134.8 (22) [6.1]

	LC_{50} (95% CI)	LC_{95} (95% CI)	LC_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
S. oryzae	95.0	1,862.7	6,390.7	1.27 ± 0.06	72.3 (22)
48-h exposure	(69.6 - 125.7)	(1,094.6 - 3,352.5)	(3,332.8 - 16,186.6)		[3.2]
R. dominica					
24-h exposure	66.9	4,213.4	23,433.5	0.92 ± 0.07	64.8 (22)
	(31.2 – 108.9)	(2,042.2 – 14,900)	(7,915.8 – 166,669)		[2.95]
48-h exposure	47.8	3,193.4	18,205	0.90 ± 0.07	57.4 (22)
	(23.9 – 79.5)	(1,633.7 – 8,743.4)	(6,978.5 – 82,509)		[2.61]

LC stands for Lethal Concentration; CI for confidence interval; *Heterogeneity value (quotient of chi-square and degrees of freedom).

CHAPTER IV

Laboratory evaluation of effectiveness of the ZeroFly® Storage Bag fabric against stored-product insect pests

ABSTRACT. The ZeroFly® Storage Bag is polypropylene bag (PP) incorporated with deltamethrin, and a novel approach to reduce postharvest losses caused by stored-product insect pests. Fabric samples from ZeroFly bags, polypropylene (PP) bags, jute bags, malathion-treated PP bags, malathion-treated jute bags, and GrainPro bags were fitted into 9-cm Petri dishes and twenty adults of either *Sitophilus oryzae* (L.) or *Tribolium castaneum* (Herbst) were introduced to determine the contact sensitivity of insects exposed to ZeroFly bag fabric. Knockdown, mortality, and number of progeny were recorded for different exposure periods (24, 48, or 72 h) and oviposition periods (7, 14, or 21 d). Additionally, mini bags from ZeroFly bags, PP bags, laminated PP bags, and jute bags were used to determine the ability of adult *S. oryzae*, *T. castaneum*, and *Rhyzopertha dominica* (F.) to bore and/or chew through the bag and efficacy of ZeroFly bag at preventing insect infestations from outside and to contain infestations within the bag. Knockdown assessment showed that time required to knockdown 99% of *S. oryzae* and *T. castaneum* was < 3 h. For the 72-h exposure period ZeroFly bag, mortalities for *S. oryzae* and *T. castaneum* were 76.7 and 62.2%, respectively; mortality was ≤ 6% in other fabrics. The ZeroFly bag fabric also significantly suppressed progeny production by *S. oryzae* and *T. castaneum* for all exposure periods. Results of miniature ZeroFly bag trials showed that none of the insects was able to bore (chew) through the bag. Implications of results for effectiveness of the ZeroFly bag are discussed.

KEY WORDS. ZeroFly® Storage Bag, stored grain, *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.)

Introduction

Postharvest losses of cereal grains, grain legumes, and oilseeds due to insect pests is an important constraint to food security in developing nations (FAO 1996, 2011, 2013, Rembold et al. 2011, Tefera et al. 2011). Food losses due to insect pests and the subsequent economic, health, and environmental problems that result are irreversible; a large amount of food is lost to insect infestations during storage and the cost of managing them is enormous (Kader 2005, Parfitt et al. 2010, Affognon et al. 2015). Insect infestations can reduce nutritional quality of grains and also limit food availability; they also increase financial losses incurred by farmers (FAO and World Bank 2011, Baoua et al. 2015). Postharvest losses can be both quantitative and qualitative (Rees 2004, Kader 2005, Hagstrum et al. 2012). Besides consuming grain, insects through their exuviae, webbing, and body fragments make food unfit for human consumption (Bhargava et al. 2007, FAO and World Bank 2011, Tefera et al. 2011, Nenaah 2014). Additionally, insects can change the environment by making it more favorable for fungal growth thereby leading to discoloration, distortion, unpleasant odors, and loss of seed viability (Dunkel 1988, Rees 2004, Gautam et al. 2013).

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) are serious and cosmopolitan stored-product insect pests (USDA 1996, Arthur 1997, Hagstrum et al. 2012). These insects feed on cereal grains and legumes during storage and cause reduction in their quality and quantity (Munro 1996, Hagstrum et al. 2012). *Sitophilus oryzae* and *R. dominica* are internal feeders which mainly feeds on the endosperm

(USDA 1986, Bello et al. 2001, Rees 2004, Hagstrum et al. 2012). A female of *S. oryzae* usually bores a small hole in the kernel surface with her mandible to lay eggs; it then produces a gelatinous fluid to seal the hole (Munro 1996, Hagstrum et al. 2012). A single female of *S. oryzae* can lay 300 to 400 eggs during its average lifespan of 4 to 5 months (USDA 1986). *Rhyzopertha dominica* is a highly destructive pest and its larval and adult stages feed on intact whole grain kernels. A female of *R. dominica* can lay up to 500 eggs loosely outside the grain kernels and upon hatching the larvae enter the grain kernels and feed (USDA 1986, Hagstrum et al. 2012). *Tribolium castaneum* is an external feeding species that preferentially feeds on farinaceous foods but can also feed on cracked, damaged or broken grain (USDA 1986, Subramanyam and Nelson 1999). A single *T. castaneum* female can lay 300 to 400 eggs during its average lifespan of 5 to 8 months (Campbell and Runnion 2003). Adults and larvae of *R. dominica*, *S. oryzae* and *T. castaneum* reduce grain weight, nutritional value, and the germination ability of stored seeds (USDA 1986, Hagstrum et al. 2012). Therefore, the development of affordable and effective storage technologies is extremely important for mitigation of *R. dominica, S. oryzae* and *T. castaneum* infestations.

Control of stored-product insect pests continues to primarily be based on application of synthetic insecticides such as organophosphates, pyrethroids and fumigants because they are effective for the management of insect infestations (Arthur 1997, Zettler and Arthur 2000, Lee et al. 2004, Upadhyay and Ahmad 2011). Chemical control is a necessary part of stored-product insect pest management and can be very effective if it is judiciously used (Phillips and Throne 2009). However, many subsistence farmers in developing countries lack the resources required for judicious use of chemical

insecticides (Kaminski and Christiaensen 2014). Additionally, farmers in developing countries have limited access to the most modern reduced risk insecticides and are rarely sufficiently trained in proper handling and use of insecticides — thereby posing a threat to human health and the environment (USDA 1986, Kaminski and Christiaensen 2014).

Farmers in developing countries still use traditional storage techniques such as, open platforms, baskets or cribs made of plant material, earthenware pots, underground storage, storehouses or warehouses, and use of bags (FAO 1994, Adejumo and Raji 2007). Storage in bags is popular in many developing countries (FAO 1994, Koona et al. 2007, De Groote et al. 2013). However, serious postharvest losses do occur in bagged commodities that are not treated with any grain protectant; losses of up to 60% have been recorded in maize stored using traditional polypropylene bags (Costa 2014). Therefore, a storage technology such as insecticide incorporation in the fabric of storage bags could be an effective and affordable technique for the smallholder farmers to minimize insect infestation during storage (Anankware et al. 2014, Costa 2014).

The deltamethrin-incorporated bag, marketed as the ZeroFly® Storage Bag (hereafter referred as the ZeroFly bag), is a promising new storage technology for mitigating insect pest infestation of bagged food commodities (Anankware et al. 2014). The broad spectrum pyrethroid insecticide, deltamethrin, is incorporated into individual fibers of the polypropylene bag, and this insecticide provides a powerful knockdown and/or killing action against stored-product insects thereby preventing them from gaining entry into the bag. Moreover, it has the advantage of not having the hazards associated with fumigation or the potential for high pesticide residues levels that are associated with inaccurate insecticide applications (Vestargaard 2015). The ZeroFly bag is effective at

preventing insect infestations. For example, Anankware et al. (2014) recorded 100% mortality of *Sitophilus zeamais* (Mot) (Coleoptera: Curculionidae) after 48 hours of exposure to the ZeroFly bag. However, there is still lack of data on the insecticidal activity of the ZeroFly bag against other stored-product insect pests. Therefore, in this study, I evaluated the contact sensitivity of *S. oryzae* and *T. castaneum* adults to deltamethrin-treated fabric samples and effects of exposure to these samples on oviposition. Additionally, I evaluated the ability of three pest species to bore or chew through fabric and thereby assessed effectiveness of the ZeroFly bags.

Materials and Methods

Insects. Adults of *S. oryzae, T. castaneum,* and *R. dominica* used for the experiment were obtained from laboratory cultures maintained at the Department of Entomology and Plant Pathology, Oklahoma State University. *Tribolium castaneum* is an external infesting species and was reared on 95% all-purpose wheat flour (Great Value $_{TM}$, Walmart Inc.) and 5% Brewer's yeast (wt:wt) at a temperature and relative humidity (RH) of 28 ± 1°C and 65 ± 5%, respectively. *Sitophilus oryzae* and *R. dominica* are internal infesting species and were reared on 95% whole-wheat kernels of hard red winter wheat ('Duster' wheat variety) and 5% Brewer's yeast (wt:wt) at 28 ± 1°C and 65 ± 5%. Voucher specimens of *S. oryzae, T. castaneum*, and *R. dominica* that were used in this study were deposited in the K. C. Emerson Entomology Museum at Oklahoma State University under lot numbers 137, 136, and 126, respectively.

Obtaining 1- to 3-wk Old Adults. For all three species, insects were reared using 300-ml glass jars containing the respective diets of each species. *Rhyzopertha dominica* and *S. oryzae* diet in each jar weighed 455 g and comprised of 99% whole-wheat kernels of hard

red winter wheat and 1% Brewer's yeast. *Tribolium castaneum* diet in each jar weighed 335 g and consisted of 98% all-purpose wheat flour and 2% Brewer's yeast. Two hundred and fifty adults of each species were allowed to lay eggs for 10 d in each of the jars containing their respective diets and were removed. *Rhyzopertha dominica*, *S. oryzae*, and *T. castaneum* adults found in jars after 9 wk, 8 wk, and 8wk, respectively, were 1- to 3-week-old.

Contact Sensitivity of Insects to Fabrics and Effects of Exposure on Oviposition. *Storage-Bag Fabric Materials.* The experiment investigated six different types of storage-bag fabric which comprised fabric material taken from the following types of bags, ZeroFly bags (delatmethrin-incorporated bags), polypropylene bags without insecticide, jute bags without insecticide, polypropylene bags treated with Malathion 50EC, jute bags treated with Malathion 50 EC, and GrainPro bags. Three bags of each type were used to obtain fabric for the experiment. Fifty-kilogram ZeroFly bags (S. A. Chemin De Messidor 5-7, CH-1006, Lausanne, Switzerland) were100 cm × 57 cm, polypropylene bags (Uline, Pleasant Prairie, WI) were 25.4 cm × 35.7 cm, jute bags (West Springfield, MA) were 62 cm × 38 cm, and GrainPro bags (GrainPro Inc., Concord, MA) were 130 cm × 74 cm.

Insecticide Application. Malathion 50 EC (referred to as malathion, hereafter) (Southern Agricultural Insecticides, Inc, Boone, NC) is an organophosphate insecticide that was used to treat polypropylene and jute bags. Prior to applying the malathion to fabric materials, 0.1 ml of malathion was measured out using a pipette (Gilson®, Middleton, WI) and mixed in 400 ml of distilled water in a 500-ml graduated cylinder (PYREX®, made in Germany) to obtain the application rate of 5 ml in 20 liters of water.

A volume of 0.12 ml of malathion solution was sprayed on a 62-cm^2 fabric sample (62 cm^2), which was then glued to the bottom of a plastic Petri dish. Malathion was applied on the fabric sample using an airbrush connected to an HD mini regulator and airbrush compressor (Patriot airbrush, TCP Global, San Diego, CA).

Experimental Setup. Fabric samples from ZeroFly bags, polypropylene bags, jute bags, malathion-treated polypropylene bags, malathion-treated jute bags, and GrainPro bags were prepared by cutting out single pieces of 9-cm-diameter fabric from three bags of each type using scissors. A single piece of sample fabric was attached to the bottom of the 9-cm-diameter Petri dish using glue (Kwik seal, DAP Product Inc., Baltimore, MD) to ensure that the fabric covered the whole bottom (floor) of the Petri dish. Fluon® (Polytetrafluoroethylene, Sigma Aldrich Saint Louis, MO) was applied to inner rim of each Petri dish (bottom) to prevent insects from climbing the Petri dish walls and escaping.

This study investigated the effects of exposure period (24, 48, or 72 h) and oviposition period (7, 14, or 21 d) on insects exposed to the fabric materials. During exposure of insects to the different fabric materials, data on knockdown and mortality were also collected. For example, in the case of the Zerofly bag fabric, three different bags were used to obtain the 9-cm pieces of fabric glued to the bottom of Petri dishes. In each ZeroFly bag exposure period, with three oviposition periods, three Petri dishes with 9-cm ZeroFly bag fabric glued to the bottom of each Petri dish were assigned to each oviposition period. Each of the three 9-cm pieces of fabric in the three Petri dishes came from a different bag, i.e. one of the three bags referred to above. Thus there were 9 Petri dishes assigned to the 3 oviposition periods within each exposure period. Therefore, for

the three exposure periods (24, 48, and 72 h) involving the ZeroFly bag, there were a total of 27 Petri dishes. For the six different types of fabric material, altogether there were 162 dishes. For each insect species, 162 dishes were used.

Twenty 1- to 3-wk-old adults of *S. oryzae* or *T. castaneum* were obtained from laboratory cultures and placed in each Petri dish. Knockdown of each species was determined every 15 min for 8 h. Final knockdown or mortality counts were conducted after 24, 48, or 72 h, depending on the targeted exposure period. Insects were categorized as knocked down when they were lying on their backs but able to move legs and/or antenna or insects were moving too slowly compared to insects in the control dishes (Arthur 1997). Insects were considered dead when they could not move their body parts after being prodded using a camel's hair brush.

After 24-, 48-, or 72-h exposure, *R. dominica* and *S. oryzae* were transferred to clean glass Petri dishes containing 5 g of 95% whole hard red winter wheat and 5% (by weight) brewer's yeast; *T. castaneum* were transferred to dishes containing 5 g of 95% all-purpose wheat flour and 5% brewer's yeast (*T. castaneum*). After transfer to clean diet, insects were observed after 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h to assess recovery, knockdown, or mortality. Final assessment of mortality was made after 72 h.

To determine progeny production, all adults in Petri dishes with clean diet were removed after 7, 14, or 21 d from the time they were transferred to clean dishes; these translate to 4, 11, and 18 d after the final mortality assessment that happened 3 d after transfer to clean diet. The diet in Petri dishes, after the removal of adults, was then transferred to 236-ml glass jars (Quilted Crystal® Jelly Jars). Twenty-five grams of the respective diets for the three species were added to each jar to ensure enough food for

progeny. Jars were kept in an incubator maintained at 28 ± 1°C and 65 ± 5% RH for 6 wk after which the number of adults in each jar was counted.

Ability of Insects to Chew or Bore Through Storage Bag Fabrics. *Storage Bags.* Four storage bags, ZeroFly bags, jute bags, polypropylene bags, and laminated polypropylene bags were investigated. Empty 50-kg capacity ZeroFly bags (S. A. Chemin De Messidor 5-7, CH-1006, Lausanne, Switzerland) (100 cm × 57 cm), polypropylene bags (Uline, Pleasant Prairie, WI) (25.4 cm × 35.7 cm), jute bags (West Springfield, MA) (62 cm × 38 cm), and laminated polypropylene bags (Central Bag Company, Leavenworth, KS) (64 cm × 30 cm × 7.5 cm) were used to obtain fabric material. Pieces of predetermined size were cut out of each bag using a pair of scissors and miniature bags with dimensions of 16 cm × 21 cm were stitched with thread and a needle but one end of each bag was left open.

Evaluating Effectiveness of Fabrics to Prevent Infestation from Outside. The effectiveness of the four types of bags to prevent infestation by *S. oryzae*, *T. castaneum*, or *R. dominica* from outside the bag was tested using miniature bags. Each bag was filled with 0.5 kg of diet. Diet for *S. oryzae* and *R. dominica* was whole kernels of hard red winter wheat, whereas that for *T. castaneum* was cracked wheat. The bags were sealed by tying the open end of each bag with a 40.64-cm rubber band. A plastic box (21.7 cm × 20.7 cm × 22 cm) was used to contain each of the bags and insects. The lid of each plastic box had six 1-cm diameter holes to allow air movement and to maintain 65% RH inside the box. The relative humidity of 65% was produced by a saturated solution of $NaNO_2$ kept in different container outside the box. Fluon (Polytetrafluoroethylene, Sigma Aldrich Saint Louis, MO) was applied to the top 2 cm of the inner rim to prevent insects from

escaping. Diet-filled bags were individually placed inside the plastic boxes. One gram of wheat was placed inside the plastic box, but outside the bag, to ensure insects had some food. Twenty 1- to 3- wk-old adults of *S. oryzae*, *T. castaneum*, or *R. dominica* were added to each box. Boxes were placed in an incubator maintained at 28°C and 65% RH throughout the experiment. The number of dead and live insects outside each bag were recorded after 0.5, 1, 5, 10, 14, and 28 d. In addition, the number of holes in each bag were recorded after 28 d.

Evaluating Effectiveness of Fabrics to Contain Infestation. Each of the miniature bags was filled with hard red winter wheat (whole kernels) for *S. oryzae* and *R. dominica* and cracked kernels for *T. castaneum.* Fifty 1- to 3- wk-old adults of *S. oryzae*, *T. castaneum*, or *R. dominica* were added each of the bags filled with 0.5 kg of diet. Bags were sealed by tying the open end of each bag with a 40.64 cm (16") rubber band. Bags were individually kept inside a plastic box which had holes on the lid to enable air movement. One gram of wheat was placed inside the plastic box, but outside the bag, to ensure that insects which exited the bag had some food. Boxes were placed in an incubator maintained at 28°C and 65% RH. The numbers of dead and live insects inside or outside the bag, and number of holes in each bag were recorded after 28 d.

Data Analyses. *Contact Sensitivity of Insects to Fabrics and Effects of Exposure on Oviposition*. Data were analyzed by species. The experimental design was a randomized complete block design (RCBD) with three replications and three sub-replications. Knockdown data were subjected to probit analyses to determine the time to knockdown 50%, 95%, and 99% of insects; these times will be referred to as KT_{50}, KT_{95}, and KT_{99}, respectively, hereafter. Mortality was assessed 3 d after transfer of insects to

clean Petri dishes containing the diet. Statistical analyses were conducted with SAS Version 9.4 (SAS Institute, Cary, NC). In relation to progeny production, analysis of variance (PROC MIXED) methods were used for analysis of adult progeny and percent mortality data. Adult progeny data were analyzed assuming a three factor factorial arrangement in a completely randomized design. An arcsine square root transformation was used to alleviate heterogeneous variance issues associated with the percent mortality response variable. Simple effects of each factor were calculated and significance assessed with a SLICE option in an LSMEANS statement. Protected pairwise comparisons were made on the simple effect means. Raw (untransformed) means and standard errors are reported.

Ability of Insects to Chew or Bore Through Storage Bag Fabrics. Data were analyzed by species. All statistical analyses were conducted with SAS Version 9.4 (SAS Institute, Cary, NC). For the experiment to determine the ability of ZeroFly mini bags to prevent insect infestation from outside, analysis of variance (PROC MIXED) methods were used assuming a two factor factorial arrangement in a RCBD. Mortality data were transformed using the arcsine square root transformation. Simple effects of each factor were calculated and significance assessed with a SLICE option in an LSMEANS statement. Protected pairwise comparisons were made on the simple effect means. Raw (untransformed) means and standard errors are reported.

For the experiment to determine the ability ZeroFly mini bags to contain infestations within the bag, an arcsine square root transformation was used to alleviate heterogeneous variance issues associated with the percent mortality response variable. The design for analysis was a RCBD. PROC GLM for a one-way analysis of variance

(ANOVA) was used to determine the effects of type of mini bag on adult insect mortality. The significance of mean differences was determined by Tukey's HSD (honest significant difference) test at $P < 0.05$.

Results

Contact Sensitivity of Insects to Fabrics and Effects of Exposure on Oviposition. *Sitophilus oryzae.* Knockdown assessments were made at 15 min intervals up to 8 h after the initial exposure to different treatments for *S. oryzae*. The percentage of insects knocked down in the different fabrics after 8 h was ≤ 5%, except for the ZeroFly bag fabric where 99% of insects were knocked down after 143.7 min (≈ 2.4 h) (Table 1). For mortality counts, all the main effects and interactions, namely, type of fabric, exposure period, and fabric × exposure period were significant at $P < 0.05$ (Fig. 1A; Table 2). Percent mortality of *S. oryzae* in the ZeroFly bag fabric was significantly higher than in other fabrics (Fig. 1). In the case of the ZeroFly bag fabric, the percent mortality was significantly higher after 72 h (76.7%) than after 24 h (38.3%) and 48 h (56.7%) of exposure (Fig. 1A). These data indicate that mortality of *S. oryzae* will increase with longer exposure to the ZeroFly bag fabric. In the malathion-treated PP or jute fabrics, the mortalities were not significantly different from mortalities in the PP bag, jute bag, and GrainPro bag fabrics; these mortalities were ≤ 6% (Fig. 1). In relation to progeny production, all main effects and interactions were significant with the exception of fabric × exposure period (Table 3). Progeny production was significantly lower in the ZeroFly bag fabric than in other five fabrics. However, numbers of progeny were similar in the PP bag, jute bag, malathion-treated PP bag, malathion-treated Jute bag, and GrainPro bag fabrics (Fig. 2). The number of progeny in ZeroFly bag fabric ranged from 5 to 14, whereas, in the other treatments, the number ranged from 77 to 217 in the 72-h exposure

period (Fig. 2C). These data show the ZeroFly bag fabric is effective in suppressing progeny production of *S. oryzae*.

Tribolium castaneum. Knockdown assessment data show that 99% of *T. castaneum,* when exposed to ZeroFly bag fabric, were knocked down after 78.9 min (Table 1). However, the percentages of insects knocked down in other fabrics were ≤ 5% after 8 h. For mortality counts, all the main effects and interactions, type of fabric, exposure period, and fabric × exposure period were significant at $P < 0.05$ (Fig. 1B; Table 2). Percentage mortality of *T. castaneum* exposed to ZeroFly bag fabric was significantly higher than in other fabrics. The percent mortality of *T. castaneum* exposed to ZeroFly bag fabric for the 24-, 48-, and 72-h exposure period was 18.33, 21.7, and 62.2%, respectively. These data seem to indicate that *T. castaneum* is less sensitive to ZeroFly bag fabric than *S. oryzae*. In the other fabrics, mortalities were not significantly different from each other and were ≤ 3% in all the exposure periods. In relation to progeny production, all the main effects were, significant, but none of the interactions was significant (Table 3). Progeny production of *T. castaneum* was significantly lower in fabric from ZeroFly bags than in fabric from PP bags, jute bags, malathion-treated PP bags, malathion-treated Jute bags, or GrainPro bags (Fig. 3A). The number of progeny in ZeroFly bag was 33 on an average, however, in other fabrics the numbers ranged from 65 to 82 (Fig. 3A). Exposure period and/or oviposition period had significant effect on the progeny production. The numbers of progeny were significantly lower in 72-h than in 24- and 48-h exposure periods (Fig. 3B) and lower in the 7-d oviposition period than in 14- and 21-d oviposition periods (Fig. 3C).

Ability of Insects to Chew or Bore Through Storage Bag Fabrics. *Evaluating Effectiveness of Fabrics to Prevent Infestation from Outside.* *S. oryzae*, *T. castaneum, or R. dominica* were not able to bore or chew through the ZeroFly bag and both types of PP bags (PP and laminated PP mini bags). However, insects were able to enter the jute bag by chewing through the fibers. All three insect species were able to make holes in the jute bag. For mortality data, all the main effects and the type of mini bag × storage period interaction were significant for all insect species (Table 4). Mortality was higher in the ZeroFly mini bags and significantly different from other treatments in all storage periods, for all insect species (Fig. 4). For the ZeroFly mini bags, 100% mortality of *S. oryzae, T. castaneum*, or *R. dominica* adults placed outside the bag was achieved after 5 d (Fig. 4).

Evaluating Effectiveness of Fabrics to Contain Infestation. None of the *S. oryzae, T. castaneum*, or *R. dominica* adults placed inside the ZeroFly bags, PP, and laminated PP was found outside the bags, however, insects were able to get out of the jute bag. Holes made by insects were found only in jute bags. Additionally, significantly higher mortality of insects, of all three species, occurred inside the ZeroFly mini bags compared to other types of mini bags. Mortality in the ZeroFly mini bags after 28 d was ≥ 94%, for all the three species (Fig. 5). Mortality in the PP, laminated PP, and Jute mini bags did not exceed 24%, for all the three species (Fig. 5).

Discussion

Based on our data, and depending on exposure period, *S. oryzae* and *T. castaneum* exposed to the ZeroFly bag fabric can experience knockdown, mortality, and/or reduced progeny production. The toxic effects of deltamethrin, which is incorporated in the fabric

of ZeroFly bags and causes knockdown and/or mortality of *S. oryzae* and *T. castaneum* has also been shown by Anankware et al. (2014) and Costa (2014). For *S. oryzae* and *T. castaneum* exposed to the ZeroFly bag fabric, the time required to knock down 99% of both species is ≤ 3 h. These data are similar to results of Anankware et al. (2014) who reported >93% knockdown of *Sitophilus zeamais* (Mot) (Coleoptera: Curculionidae) after 6 h of exposure to ZeroFly bag fabric.

Our data also show that the ZeroFly bag fabric was significantly more effective than the other five fabric materials tested, both in terms of causing mortality and in suppressing progeny production of *S. oryzae* and *T. castaneum* in all exposure periods. The fact that mortalities in malathion-treated PP or -jute bag fabrics were not different from those in untreated fabrics from PP or jute bags may indicate malathion resistance in the insects used for tests. Malathion resistance has been extensively reported in *S. oryzae* and *T. castaneum* throughout the world, and malathion has been replaced by other pesticides because of this reason (Hortan 1984, Zettler and Cuperus 1990, Arthur 1996, Arthur and Subramanyam 2012).

Production of large numbers of adult insects in fabric from PP, jute, or GrainPro bags indicated that insects used were capable of effective oviposition. This means the suppression of progeny production was most likely exclusively due to the ZeroFly bag or malathion-treated fabrics. The ZeroFly bag fabric significantly reduced the number of live progeny produced by *S. oryzae* and *T. castaneum* — although the effect on progeny production varied between the species. Low progeny production in the ZeroFly bag indicates that deltamethrin present in the bag was toxic to *S. oryzae* and *T. castaneum*, and had sublethal effects. Compared to other fabrics tested, there were significantly fewer

progeny produced after exposure to the ZeroFly bag fabric. However, exposure to the ZeroFly bag fabric, even for 72 h, did not result in total progeny production suppression. The 5 to 14 progeny of *S. oryzae* and ≤ 33 progeny of *T. castaneum* found after the 72-h exposure to the ZeroFly bag fabric is likely due to insects surviving the exposure. The survival of insects after 72-h exposure to ZeroFly bag fabric is consistent with data from the ZeroFly mini bags, which show 5 d are required to kill all adult insects outside the bag, trying to find their way into the bag.

Despite the fact that 72-h exposure to the ZeroFly bag fabric does not kill all adult insects, our results from ZeroFly mini bags showed that no test insects were able to bore or chew through the ZeroFly bag fabric. This supports the findings of Anankware et al. (2014) who showed that *S. zeamais* was not able to bore or chew through the ZeroFly bag fabric. The inability of *S. oryzae*, *T. castaneum*, *R. dominica*, and *S. zemais* to chew or bore through the ZeroFly bag fabric indicates that the ZeroFly bag can be used to effectively protect cereal grains and legumes stored in it. In this study, we have also showed that *S. oryzae, T. castaneum*, and *R. domnica* were not able to bore or chew through PP and laminated PP mini bags. These results are supported by the findings of Allahvaisi et al. (2010) who reported that PP is resistant to *Callosobruchus maculatus* Fabr. (Coleoptera: Bruchidae) penetration. Although, no insects gained entry into PP and laminated PP mini bags mortality of insects in these mini bags was significantly lower than in the ZeroFly mini bags, for all storage periods. Starvation of insects may have contributed to increased mortalities in jute, PP, or laminated PP mini bags because only one gram of diet was provided outside each bag and this may not have been enough for

insects to feed on for 28 d the experiment lasted. No insect holes were found in ZeroFly, PP, and laminated PP mini bags, but holes were found in jute mini bags.

In conclusion, we have showed that the ZeroFly bag fabric can cause insect pest knockdown, mortality, and/or reduced progeny production. Our data indicate that the mode of action of the ZeroFly bag is likely through knockdown, mortality, and/or reduced progeny production of insect pests that come into contact with the bag fabric. The ZeroFly bag fabric not only prevents insect entry into bags from outside, but also contains infestation within the bag hence preventing other surrounding bags from infestation.

Acknowledgements

We thank Sandipa Gautam, Kandara Shakya, Friendly Yang, and Ashana shakya for their technical support. This work was funded by Vestergaard Frandsen SA, Switzerland, and partly by Oklahoma Agricultural Experiment Station (OKL02949)**.** Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by Oklahoma State University or Vestergaard Frandsen.

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Table. 1. Time to knockdown in minutes (KT_{50}, KT_{95}, and KT_{99}) for *S. oryzae* and *T. castaneum* adults exposed to ZeroFly bag fabric.

Species	KT_{50} (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope± SE	χ^2 (df) [H*]
S. oryzae	79.6 (71.9 – 86.9)	120.8 (107.5 – 147.5)	143.7 (123.6 – 188.6)	9.06 ± 0.39	355.08 (28) [12.86]
T. castaneum	54.0 (52.7 – 55.3)	70.6 (68.2 – 73.7)	78.9 (75.4 – 83.5)	14.14 ± 0.91	15.3 (28) [0.548]

KT stands for knockdown time; CI for confidence interval; Heterogeneity value (quotient of chi-square and degrees of freedom).

Table 2. ANOVA results for main effects and interactions for fabric materials (Fabric), exposure period for adult *Sitophilus oryzae* and *Tribolium castaneum* in relation to mortality when exposed to the fabric of ZeroFly® Storage Bag (ZeroFly bag), polypropylene bag (PP), jute bag, Malathion 50EC- treated PP bag, Malathion 50EC- treated jute bag, and GrainPro bag.

Source	*Sitophilus oryzae*			*Tribolium castaneum*		
	Df	*F*	*P*	df	*F*	*P*
Fabric	5, 144	201.99	<0.0001	5, 144	134.25	<0.0001
Exposure Period	2, 144	18.18	<0.0001	2, 144	13.96	<0.0001
Fabric × Exposure Period	10, 144	3.58	0.0003	10, 144	9.96	<0.0001

Table 3. ANOVA results for main effects and interactions for fabric materials (Fabric), exposure period (Exp), and oviposition period (Ovipos) for adult *Sitophilus oryzae* and *Tribolium castaneum* in relation to number of progeny when exposed to fabric of ZeroFly® Storage Bags (ZeroFly bag), polypropylene bag (PP), jute bag, Malathion 50EC- treated PP bag, Malathion 50EC- treated jute bag, and GrainPro bag.

Source	*Sitophilus oryzae*			*Tribolium castaneum*		
	Df	*F*	*P*	df	*F*	*P*
Fabric	5, 108	44.75	<0.0001	5, 108	20.52	<0.0001
Exp	2, 108	4.87	<0.0095	2, 108	3.23	0.0432
Fabric × Exp	10, 108	2.19	0.0238	10, 108	0.54	0.8551
Ovipos	2, 108	26.60	<0.0001	2, 108	6.11	0.0031
Fabric × Ovipos	10, 108	0.81	0.6161	10, 108	0.33	0.9728
Exp × Ovipos	4, 108	0.30	0.8787	4, 108	0.14	0.9666
Fabric × Exp × Ovipos	20, 108	0.28	0.9990	20, 108	0.17	1.0000

Table 4. ANOVA results for main effects and interactions for type of mini bag and storage period for adult *S. oryzae, T. castaneum,* and *R. dominica* in relation to mortality when insects were kept outside mini bags, i.e. ZeroFly mini bags, PP mini bags, laminated PP mini bags, and jute mini bags.

Source		*S. oryzae*		*T. castaneum*		*R. dominica*	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Bag type	3	40.89	< 0.0001	186.88	< 0.0001	323.41	< 0.0001
Storage Period	5	20.86	< 0.0001	29.30	< 0.0001	91.52	< 0.0001
Bag type × Storage Period	15	2.53	0.0118	2.91	0.0040	7.07	< 0.0001

Figure Captions

Figure 1. Mortality (Mean ± SE) of *Sitophilus oryzae* (A) and *Tribolium castaneum* (B) exposed to fabric materials from ZeroFly® Storage Bags (ZeroFly bag) (F1), polypropylene bag (PP) (F2), jute bag (F3), Malathion 50EC- treated PP bags (F4), Malathion 50EC- treated jute bag (F5), and GrainPro bag (F6). Means followed by different lowercase letters are significantly different ($P < 0.05$).

Figure 2. Number of progeny (Mean ± SE) of *Sitophilus oryzae* exposed to fabric materials from ZeroFly® Storage Bags (ZeroFly bag), polypropylene bag (PP), jute bag, Malathion 50EC- treated PP bag, Malathion 50EC- treated jute bag, and GrainPro bag. Means followed by different lowercase letters are significantly different ($P < 0.05$).

Figure 3. Number of progeny (Mean ± SE) of *Tribolium castaneum* exposed to fabric materials (A) for different exposure periods (B) and oviposition periods (C) from ZeroFly® Storage Bags (ZeroFly bag), polypropylene bag (PP), jute bag, Malathion 50EC- treated PP bag, Malathion 50EC- treated jute bags, and GrainPro bag. Means followed by different lowercase letters are significantly different ($P < 0.05$).

Figure 4. Mortality (Mean ± SE) of *Sitophilus oryzae* (A), *Tribolium castaneum* (B), and *Rhyzopertha dominica* (C) when insects were placed outside of ZeroFly bag, polypropylene bag (PP), laminated PP bag, and jute bag. Means followed by different lowercase letters are significantly different ($P < 0.05$).

Figure 5. Mortality (Mean ± SE) of *Sitophilus oryzae* (A), *Tribolium castaneum* (B), and *Rhyzopertha dominica* (C) when insects were placed inside the ZeroFly bag, polypropylene bag (PP), laminated PP bag, and jute bag. Means followed by different lowercase letters are significantly different ($P < 0.05$).

Figure 1. Mortality (%) of *Sitophilus oryzae* and *Tribolium castaneum* exposed to different treatments

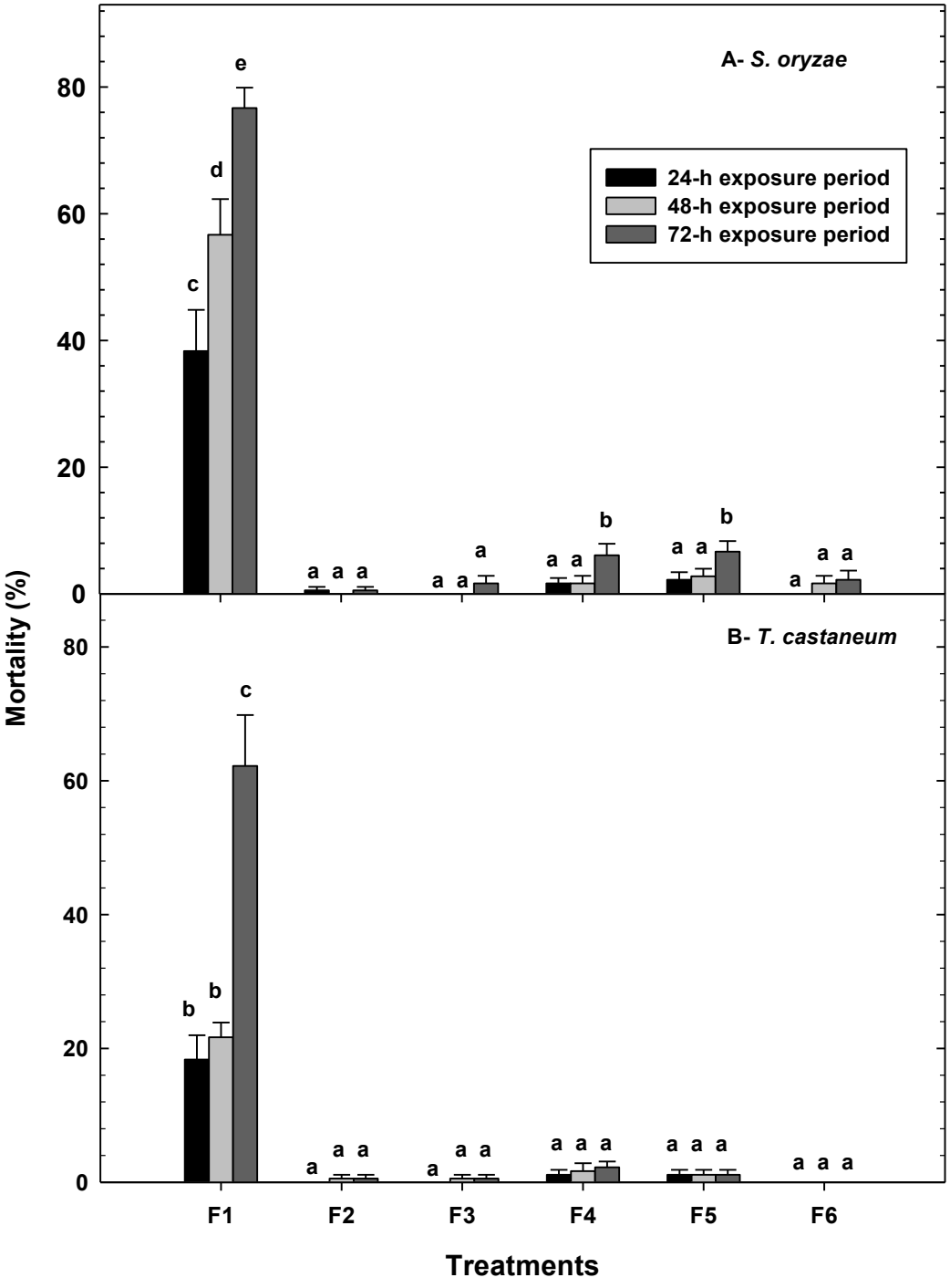


Figure 2. Number of progeny of *Sitophilus oryzae*.

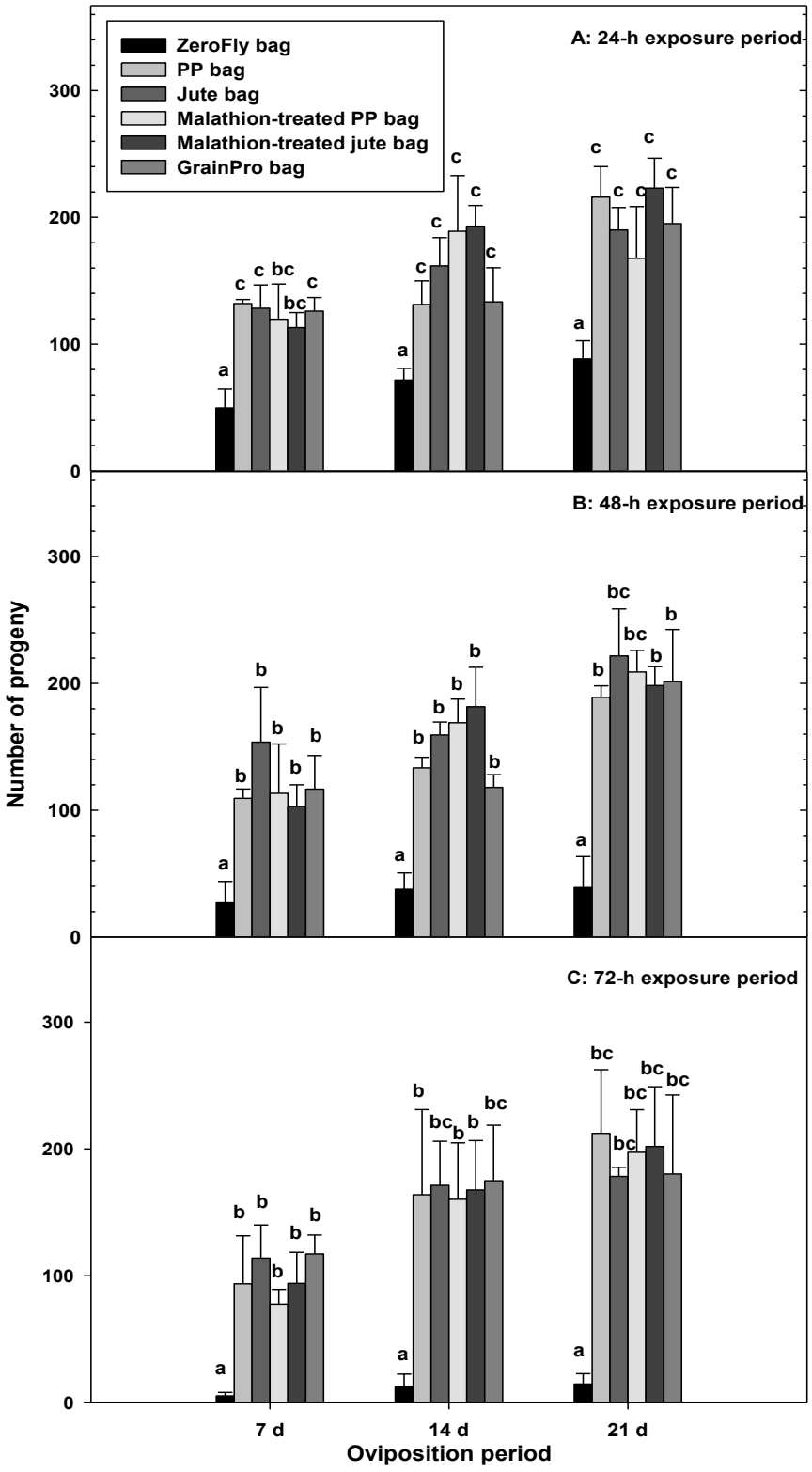


Figure 3. Number of progeny of *Tribolium castaneum*.

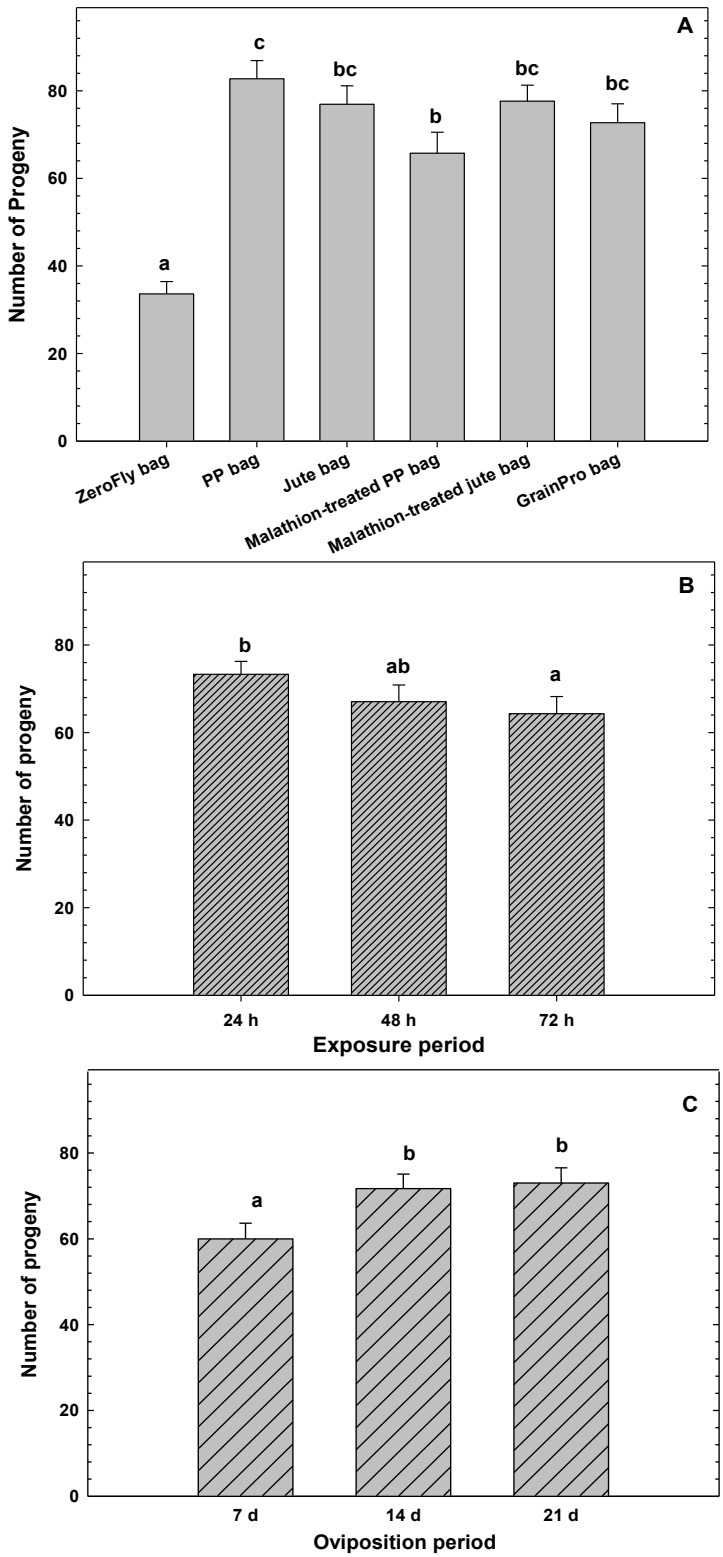


Figure 4. Mortality (Mean ± SE) of *S. oryzae, T. castaneum*, and *R. dominica*

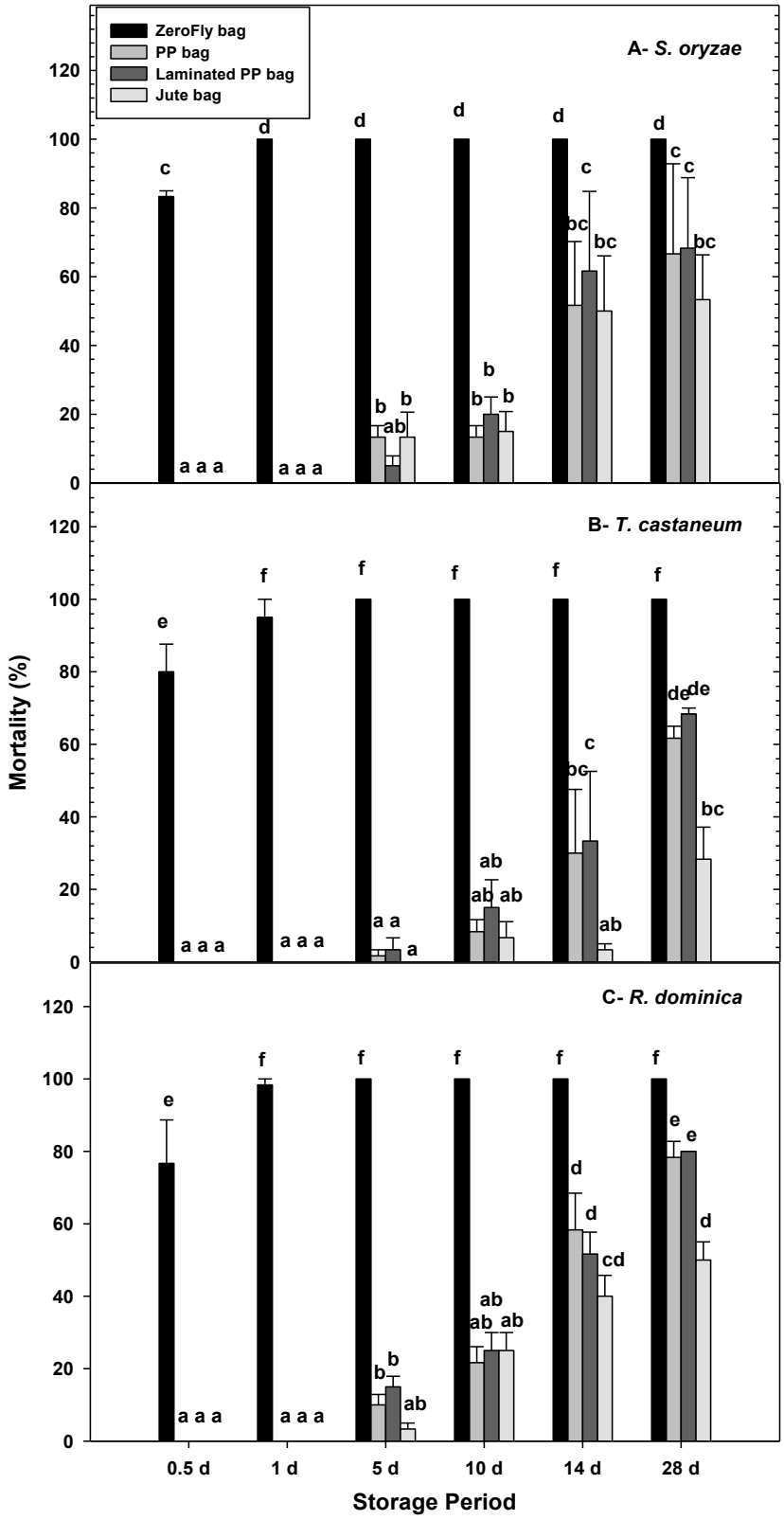
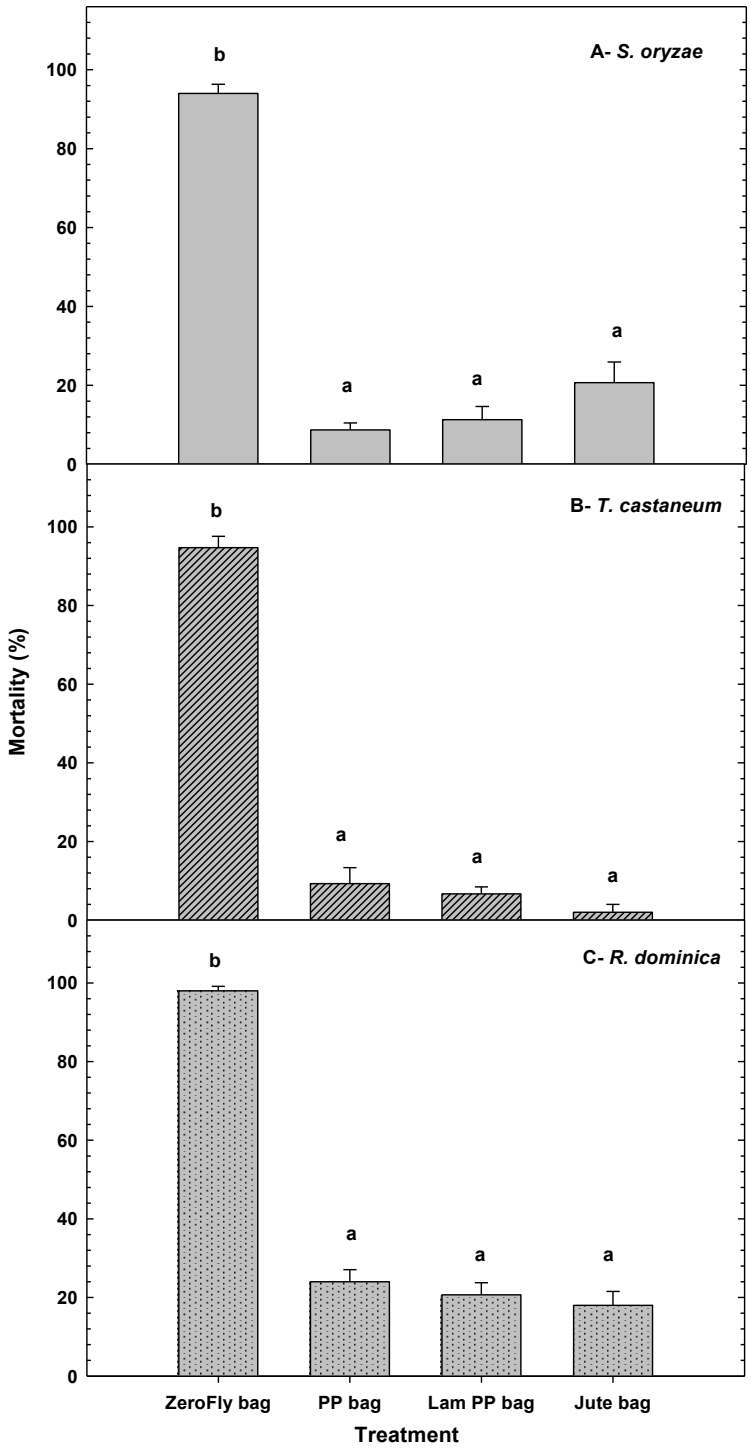


Figure 5. Mortality (Mean ± SE) of *S. oryzae, T. castaneum*, and *R. dominica*



CHAPTER V

Field evaluation of the deltamethrin-incorporated bag (ZeroFly® Storage Bag) as a barrier to insect pest infestation

ABSTRACT The deltamethrin-incorporated polypropylene (PP) bag, ZeroFly® Storage Bag, is a new technology to reduce postharvest losses caused by stored-product insect pests. ZeroFly bags filled with untreated maize were compared with PP bags filled with Betallic Super (80 g pirimiphos-methyl and 15 g permethrin per liter as an emulsifiable concentrate (EC))-treated-maize and PP bags filled with untreated maize (control). The experiment was conducted from February−August 2015, at four sites in different locations of the Middle Belt of Ghana. Moisture content (MC), number of live and dead insects, insect damaged kernels (IDK) and maize weight loss data were collected monthly. ZeroFly bags and Betallic treatment significantly reduced insect damage compared to the control treatment. ZeroFly bags were able to keep IDK level below 5% for 4 mo, but the levels increased to 5.2 and 10.2% after 5 and 6 months, respectively. In the control, IDK increased significantly over time and reached 32% after 6 months. The ZeroFly bag was effective against *Sitophilus*, *Tribolium* and *Cryptolestes* species for 4 months. Mean weight loss of ≤ 3.68% was recorded in ZeroFly bags during 6 months of storage whereas 11.88% weight loss occurred in the PP bags after 6 months. Among the three methods of storage, treating maize with Betallic was more effective in terms of suppressing insect pests' populations, reducing IDK and minimizing weight loss. Based on our results, ZeroFly bags are effective for short-term storage, but could also protect grains for longer if insect-free grains are used to fill the bags.

KEY WORDS ZeroFly bag, stored-product insects, maize, Middle Belt of Ghana

Introduction

Most of the calorie and protein needs of humans are obtained from cereal grains, grain legumes, and oilseeds (Cordain 1999). Maize (*Zea mays* L.) is one of the important cereal grains grown widely throughout the world. It is a major staple food in Africa (FAO 2004, Tefera et al. 2011), and significantly contributes to household food security for smallholder farmers (Baoua et al. 2014). Maize was planted on approximately 31.1 million ha of land in Sub-Saharan Africa during the period 2008-2010, and the average annual production during this period was 56.7 million tons (AGRA 2014). However, due to poor storage techniques, maize producers in developing nations experience considerable losses after harvest (Giga et al. 1991, Boxall 2001, Alonso-Amelot and Avila-Nunez 2011).

Over the past few decades, postharvest loss estimation and measures to effect mitigation of these losses were high on the international agenda (FAO 1999, FAO and World Bank 2011, Abass et al. 2014, Affognon et al. 2015). Despite international focus on postharvest loss issues, farmers in the developing nations still face significant losses. Estimates of postharvest losses vary in literature, and global figures for losses of 9−40% are often quoted (Pimental 2002, FAO and World Bank 2011, Parfitt et al. 2010, Tefera 2012, Hodges et al. 2014). Postharvest losses can arise from inappropriate storage techniques, deterioration by insect pests, and rodents, high ambient temperature, and high relative humidity (FAO and World Bank 2011, Anankware et al. 2013, Abass et al. 2014). However, most of the postharvest losses can be attributed to storage insect pests (Giga et al. 1991, Bett et al. 2007). Infestation of stored maize by insect pests may produce unpleasant odors, make the grain unfit for consumption, reduce nutritional

content, and grain quantity thereby leading to low market price (Hill 1990, Jood and Kapoor 1992, FAO 2004, Mboya 2013).

Stored maize is infested and damaged by a number of insect pests that cause quantitative and qualitative deterioration (FAO 2009). Of the pests that infest maize, beetles and moths are the most important. *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidiae), *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), *Sitotroga cerealella* (Oliver) (Lepidoptera: Gelechiidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) are the most important pests of stored maize (USDA 1986, Rees 2004, Groot et al. 2004, Hagstrum et al. 2012).

Effective grain storage can reduce pest activity and can help secure food quality and quantity until the next harvest season. For storage of cereal grains and grain legumes, farmers in sub-Saharan Africa predominantly use traditional storage techniques such as open platforms, woven baskets, pots, mud rhombus, maize cribs, bamboo storage structures, straw roofed storage structures, underground storage, bag storage, and warehouses (FAO 1994a, Adejumo and Raji 2007). Because most of the aforementioned structures are made of locally available materials, small scale farmers find them economically feasible to construct. Storing cereals in sacks such as jute or polypropylene bags is currently the most common storage technique (FAO 1994a, Koona et al. 2007, De Groote et al. 2013). However, postharvest losses do occur in bagged commodities that are not treated with any grain protectant; losses of up to 60% have been recorded in maize stored using traditional polypropylene bags (Costa 2014).

Grain protectants and fumigants are used by farmers in developed countries to control insect pests of stored maize (White 1995, Arthur 1996, Zettler and Arthur 2000). Some challenges to pesticide use are that most resource-poor smallholder farmers in developing countries find the cost of pesticides prohibitive thereby making them unaffordable and these chemicals have been abused and misused by farmers in ways that pose risks to human health and the environment (Kamanula et al. 2011, Hodges et al. 2014). Therefore, a need exists for the development of reduced risk approaches to managing insect infestations in bagged grain. Additionally, ways of scaling up such reduced risk technologies need to be sought because farmers in developing countries have been too slow to adopt such technologies (USDA 1986, Kaminski and Christiaensen 2014).

In rural parts of Ghana, most maize produced by smallholder farmers is traditionally stored in jute or polypropylene bags (FAO 1994a, Akramov and Malek 2012, Anankware and Bornu-Ire 2013). These bags often do not protect the grains against insect pests, leading to heavy losses. Reducing postharvest losses in bagged grain could increase smallholder farmers' income and also improve food insecurity. Vestergaard Frandsen SA, Switzerland has developed a deltamethrin incorporated polypropylene bag (ZeroFly® Storage Bag), which has great potential to reduce postharvest losses of cereal grains and grain legumes stored in it for use in developing countries (Anankware et al. 2014, Costa 2014). The ZeroFly bag is designed to give protection to commodities by preventing the entry of insect pests thereby facilitating preservation of cereal grains and grain legumes. However, there are little published data on field trials with the ZeroFly Storage Bag. Therefore, this study was conducted in the Middle Belt of Ghana to evaluate

the effectiveness of the ZeroFly bag to protect maize from infestation by stored-product insect pests.

Materials and Methods

Study Sites. The experiment was conducted in three major maize growing areas located in the transitional ecological zone of Ghana: Ejura (located at a longitude $1^{0}5^{'}$ W and $1^{0}39^{'}$ W and latitudes $7^{o}9^{'}$ N and $7^{o}36^{'}$ N), Techiman (located at a longitude $1^{o}42^{'}$ 54 W and latitudes $6^{o}37^{'}25$ N), and Wenchi (longitude: 2° 6' 0" W and latitude: 7° 45' 0" N). The experiment lasted from February to August, 2015, and was set up at four warehouses (hereafter referred to as sites), two at different locations in Ejura, one in Techiman, and another in Wenchi. Sites selected for the experiment were either near maize storage areas or market areas where insect pressure was high.

Treatments. There were three treatments assigned to each site. The first treatment was Betallic Super EC (Batch number- Eas-13 g-13193, Hockley International Ltd, Manchester M22 5lb, UK)-treated maize in 50-kg untreated polypropylene (PP) bags. The maize treated with Betallic Super EC which contains two active ingredients, namely, 80 g pirimiphos-methyl and 15 g permethrin per liter as an emulsifiable concentrate (EC). According to the Betallic label, an application rate of 300 ml in 15 liters of water for application on twenty 100-kg bags (maxi bags) of grain is recommended. In this study, 7.5 ml of Betallic was mixed with 0.38 liter of water and used to treat each batch of 50 kg of clean, dry, insect pest-free maize; a hand sprayer (Bentronic pressure sprayer) of 2-liter capacity was used to apply the insecticide. Therefore, total volume of 135 ml and 6.84 liters of Betallic and clean water, respectively, were applied on nine 100-kg bags (0.9 tonnes) of maize used in the Betallic

treatment, in the four sites. Treated maize was thoroughly mixed using a wooden spade to ensure uniformity, and the treated maize was air-dried before being used to fill 50-kg untreated PP bags; filled bags were sealed by sewing using thread and a needle. The bags were then stacked on pallets at the four sites.

The second treatment was untreated maize in untreated 50-kg PP bags (control). Clean, dry, insecticide- and insect pest-free maize with a MC of 11−13% was used to fill untreated PP bags. No insect pest-control measures were conducted during the 6 months maize storage period; which was also the duration of the experiment.

The third treatment consisted of untreated maize in 50-kg deltamethrin (DM) incorporated PP bags [ZeroFly® Storage Bags (hereafter referred as ZeroFly bags) made by Vestergaard Frandsen (VF)] — here each of the three storage methods is referred to as "treatment", although each of them is not a treatment as defined in statistics. ZeroFly bags were obtained from Vestergaard Frandsen's local distributor in Nigeria (Turner Wright Nigeria Limited 15, Adenekan Salako Close, Ogba, Lagos, Nigeria), whereas, untreated polypropylene bags and Betallic Super EC (distributed by Bentronic productions, P. O. Box Ks 14318, Kumasi, Ghana) were obtained from a local market in Ejura, in the Ashanti region of Ghana. According to the producer's label, the ZeroFly bag contains 3 mg/kg deltamethrin and unique technology is used to incorporate insecticide into the PP fabric in such a way as to allow slow and controlled release of the active ingredient for at least two years.

Maize and its Pre-Experimental Disifestation. Ghana is a major maize producing African country and a majority of producers grow white maize varieties (Ragasa et al. 2013). Therefore, a white variety of maize called "Obatanpa" was used for

the experiment. Maize was bought from a single local farm in Ejura to ensure uniformity of maize used in the experiment. The maize was cleaned, dried to a moisture content of 11.5% (John Deere moisture meter measurement) and then fumigated using Phostoxin® tablets before the experiment was set up to ensure that the maize used was free from all life stages of insect pests. Twenty six Phostoxin tablets were placed in a stack of seventy two 50-kg bags (mini bags). Thirty tablets are recommended for 28.32 m^3; one tablet produces 25 ppm of phosphine (hydrogen phosphide or PH_3) in 28.32 m^3 (http://www.researchfumigation.com/msds/weevil-cid-applicator-manual.pdf). The stack of seventy two 50-kg bags had a volume of 24 m^3 (6 m x 4 m x 1 m). Therefore, the number of tablets required for a 24 m^3 stack was calculated as 26. One tablet per m^2 (of the 6 m x 4 m area) and two tablets were placed in the center to give a total of 26 tablets for the 24 m^2 base area. The stack was covered with a gas-tight tarpaulin to ensure gas leakage was as minimal as possible. The estimated PH_3 concentration inside the stack after complete decomposition of the tablets was 764.71 ppm. Fumigation lasted 5 d after which the tarpaulin was removed. Fourteen days after the removal of the tarpaulin, the fumigated maize was used to set up the treatments referred to above.

Methodology. Maize was transported to each site in bags provided by the maize supplier. Filling of 50-kg bags for the three different treatments was done at each site, starting with maize and bags which were untreated (no pesticide in either the maize or bag). This was done to prevent insecticide cross- contamination among treatments through maize and/or bags. In each of the four sites, there were six sub-replicates for each treatment, i.e. six maize-filled bags were assigned to each treatment; each stack of six bags was on a separate pallet to prevent bags from absorbing moisture from the floor. The

pallets were placed 4 m apart from each other. There were eighteen 50-kg bags per site. Additionally, 5 mouse and rat traps were placed in each site to minimize rodent damage to bags of stored maize — evidence of the presence of mice and rats had been seen during preparation of the sites for the experiment. The experimental design used was randomized complete block design (RCBD) with four replications and six sub-replicates for each treatment replicate. Each site represented a replication.

Sampling and Data Collection. Bags were sampled at the start of the experiment, just after the experiment had been set up, and this was in February 2015. Monthly sampling was then conducted during the period March−August. Bags in each stack of six were randomly numbered 1–6 to facilitate sampling. The assignment of numbers to bags was by randomly picking numbered and folded pieces of paper, each with one of the six numbers written on, which had been shuffled in a small plastic container and accordingly assigning numbers to bags. Three bags from each treatment were randomly selected for sampling during each sampling event. The order of sampling for the three treatments, from first to last, was the control, ZeroFly bags and Betallic treatment. The rationale for the order of sampling was to avoid insecticide cross contamination among treatments.

Moisture Content (MC). Moisture content in each bag sampled was assessed using two moisture meters. The bag selected for sampling in each treatment was opened by undoing the thread seal. A moisture meter developed by the USDA-ARS Center for Grain and Animal Health Research, Manhattan KS, referred to in this study as the PHL meter, was inserted in a bag of maize, left to stabilize over a 3-min period, and then the temperature (°C) and MC (%) readings were taken. Three different readings were taken

from different positions for each bag and the average values were calculated. A John Deere moisture meter (Manufactured by agraTronix™; Moisture Check Plus™, USA for Deere & Company; Batch SW08120) was also used to measure the MC of maize in each of the bags data were collected using the PHL meter. Three measurements were taken and the average MC was calculated for each bag.

Grain Sampling. A 1.2-m open-ended trier (grain probe) (Seedburo Equipment, Chicago, IL) was used to sample maize from bags. Three triers, one for each treatment, were used for sampling. Samples were taken from the center and two opposite sides near the inner surface of each bag. Samples from each bag were mixed thoroughly in a basin to ensure homogeneity. A sample of 250 g was then weighed out using a dial spring scale (CAMRY, Yongkang, China). The 250-g maize sample was placed in a labeled plastic bag and taken to the laboratory for data collection. The maize from each bag that remained in the basin, after the 250-g sample was weighed out, was put back into the bag from where the samples were taken; this was done immediately after all required data had been taken. Sampled bags were sealed by sewing using thread and a needle immediately after unused maize from the samples taken had been put back. To avoid insecticide cross contamination, hand gloves were changed after handling samples for each treatment. Floors in the different sites were always kept clean and mice traps were replaced regularly.

For each of the 250-g samples collected, data on number of insects of each species, number of insect damaged kernels (IDK), percentage of IDK, and weight of damaged and undamaged kernels. All samples were processed at Kwame Nkrumah University of Science and Technology Insectary, located in the Department of Crop and

Soil Sciences. Percent weight loss due to insect damage was determined using the count and weight method (FAO 1992).

$$\% \text{ Weight loss} = \frac{(Wu \times Nd) - (Wd \times Nu)}{Wu(Nd + Nu)} \times 100$$

Where W_u is the weight of undamaged grain, N_u is the number of undamaged grain, W_d is the weight of damaged grain, and N_d is the number of damaged grain.

Statistical Analyses. Statistical analyses were performed with SAS Version 9.4 (SAS Institute, Cary, NC). Treatment effects were assessed using analysis of variance methods (PROC MIXED). A repeated measures model in a randomized complete block design was utilized, with site as the blocking factor and month as the repeated factor. An autoregressive covariance structure was used to model the correlations within treatment and across months. Analyses of the numbers of IDK and numbers of live and dead insects were conducted with the use of a square root transformation. A square root transformation was used to correct for heterogeneous variances and the lack of normality of the count response variable. The simple effects of treatment given month were assessed with protected planned contrasts (SLICE option in an LSMEANS statement). In case of percent MC, IDK, and weight loss, data analyses were conducted with the use of an arcsine transformation to stabilize variances but untransformed percentages are reported.

Results

Moisture Content. For moisture content measured by the PHL or JD meters, there was no significant interaction between storage period and treatment (Table 1). For moisture content measurements taken on the same bags of maize at approximately the

same time, there was a ≤ 2% difference in the moisture content measured by the PHL and JD meters (Fig. 1). Moisture content measurements for the PHL moisture meter ranged from 11 to 13.9% (Fig. 1A), whereas those for the JD meter ranged from 13 to 14.8% (Fig. 1B).

Insect Damaged Kernels (IDK). The interaction between treatments and storage period was significant for the number of IDK and percentage of IDK (Table 1). Our results show that the ZeroFly bag and Betallic treatments significantly reduced insect damage compared to untreated PP bags with untreated maize treatment (control). Mean numbers of IDK were not significantly different during the entire storage period in the Betallic treatment (Fig. 2). In the control treatment, IDK increased significantly from 11% per 250-g maize sample in June to 32% in August. However, in the case of ZeroFly bag treatment, numbers of IDK were not significantly different during the period from May to August; the number of IDK increased from 3% in May to 10% in August (Fig. 2).

Insect Infestation Level. *Sitophilus* spp. There was significant interaction between storage period and treatment in relation to the number of live *Sitophilus* spp. (Table 2). There were no live adult *Sitophilus* in samples from the Betallic and ZeroFly bag treatments during the first 2 mo of storage. In the Betallic treatment, the numbers of *Sitophilus* were not significantly different during entire storage period (Fig. 3). In the case of the ZeroFly bag treatment, numbers of *Sitophilus* in bags were not significantly different during the period February to July, but the number increased numerically to 15 at the end of the study in August. The number of live *Sitophilus* was significantly higher in bags in the control treatment where 32 insects were found in June, but the number decreased to 20 in August (Fig. 3A). Although, there was no significant interaction

between storage period and treatment for dead *Sitophilus*, depending on the month, the numbers of dead insects were numerically or significantly higher in the ZeroFly bags than in the other two treatments. On average, 31 dead *Sitophilus* per sample were found in ZeroFly bags at the end of storage in August. These data indicate that ZeroFly bags are likely contributing to increased mortality of *Sitophilus* spp. but are not doing so effectively enough to result in the same kind of population suppression observed in the Betallic treatment.

Tribolium spp. There was significant interaction between storage period and treatment for both live and dead *Tribolium* spp. (Table 2). The numbers of live *Tribolium* were not significantly different for samples from the ZeroFly bag and Betallic treatments in all storage periods; in both treatments, the mean number of insects per sample was below 1 during the 6 mo of storage. In the control treatment, mean number of live insects increased significantly to 6.5 in June, but decreased to 1.7 and 2.1 in July and August, respectively; the July and August number of live *Tribolium* spp were statistically similar to the number of insects in March (Fig. 4A). In all the treatments, dead *Tribolium* spp. numbers were below 2 (Fig. 4B).

Cryptolestes spp. There was significant interaction between storage period and treatment for both live and dead *Cryptolestes* spp. (Table 1). Despite the lack of significant differences in the number of live insects in the Betallic and ZeroFly bag treatments, there were numerically more insects in the latter treatment (11.5 ± 6.48) than in the former treatment (0.8 ± 0.42) at the end of study (August) (Fig. 5A). In the control treatment, the numbers of live insects were significantly higher than in the other two

treatments, and numbers increased significantly with storage time from 45 in July to 92 in August (Fig. 5A).

Grain Weight Loss. In relation to maize weight loss, the interaction between storage period and treatment was significant (Table 1). There were no significant differences among the three treatments during the first 3 mo of storage. In the control treatment, percentage weight loss increased significantly from June to August when 11.9% weight loss occurred. No significant weight loss occurred in the Betallic treatment where weight loss did not exceed 1% during entire storage period of 6 mo. In the ZeroFly bag treatment, there was no significant weight loss until August when 3.7% weight loss occurred (Fig 6).

Discussion

Based on our data, maize moisture content at the start of the storage period (February) was 11−13%, a MC level considered safe for storage of maize (FAO, 1994b, 2011). However, moisture content increased over the storage period, in all treatments, and was significantly higher after 6 months of storage in August. The MC levels in August were 13–14.8%. It is possible that insects infesting maize contributed to increase in MC but other factors may have played a bigger role in the increase given that the Betallic treatment had low insect infestation. Increase in MC levels may have been due to the influence of ambient relative humidity — relative humidity in the Middle Belt of Ghana increases during the period February−August (Jarvis et al., 2014). Moisture content is an important physiological variable that always needs to be considered for successful grain storage and should be monitored regularly; storage at MC levels of 12-

13% helps to reduce deterioration and microbial growth (FAO, 2011; Costa, 2014). The JD moisture meter costs $250 and is too exorbitant for smallholder farmers in developing countries. Conversely, the PHL meter is a relatively low cost ($50 – $70) moisture meter produced by the USDA-ARS, Center for Grain and Animal Health Research, Manhattan, KS. The PHL meter is designed to give MC measurements using both temperature and relative humidity of air surrounding the grain (Opit et al., 2014). Based on our data, there was approximately a 2% difference in the measurements of the PHL and JD meters.

One of the variables the Ghana Standards Authority uses to grade maize is the percentage of maize kernels that are damaged, i.e. percentage IDK (%) (GSA, 2013). The percentage IDK threshold accepted by the Ghana storage industry, wholesalers, retailers and consumers is 5%. Although other factors such as diseased, discolored, broken, stained, germinated and shriveled kernels are used to determine grades and acceptability of maize, a level > 5% IDK results in maize getting rejected (GSA, 2013). In the current study, percentage IDK increased significantly with storage time in the control treatment and levels of 11, 16 and 32% per 250-g maize samples were found in June, July and August, respectively; these value are far higher than the 5% threshold referred to above. In contrast, the Betallic treatment was able to keep percentage IDK levels well below 2% during entire storage period. In the ZeroFly bag treatment, IDK levels were below 5% until June (3.1%), but levels increased to 5.2 and 10.2% in July and August, respectively.

Based on our data, the ZeroFly bag and Betallic treatments were highly effective in suppressing insect infestation and damage levels compared to the control treatment (untreated PP bag). The common way of storing maize in Ghana is using untreated PP bags that contain untreated maize (the negative control treatment in the present study). In

the control treatment, the number of *Sitophilus* spp., *Tribolium* spp. and *Cryptolestes* spp. were generally significantly higher than in other two treatments.

The Betallic treatment was most effective at suppressing stored-product insect pest levels. The ZeroFly bag was quite effective only up to 4 months of storage. After 4 months of storage, the insect levels (*Sitophilus*, *Tribolium* or *Cryptolestes*) in the ZeroFly bag increased significantly and resulted in percentage IDK levels > 5%. The increased infestation levels in the ZeroFly bags after 4 months of storage seem to indicate that insects (eggs and/or larvae) that may have survived the phosphine fumigation before maize was bagged do not contact the surface of the ZeroFly bag enough for their levels to be significantly reduced. For stored-product insects to be killed by the ZeroFly bag fabric, they need to contact the fabric for long periods of time (≥ 24 h) (Paudyal et al., unpublished data). It is also likely that the repeated sealing and unsealing of the ZeroFly bags during grain sampling may have created breaches in the deltamethrin barrier that allowed easy access of insects into the bags. Anankware et al. (2014) showed that the ZeroFly bag causes 100% mortality of *S. zeamais* after 48 h of exposure to ZeroFly bag fabric. This may indicate that ZeroFly bags are likely contributing to increased mortality of insects but are not doing so effectively enough to result in the same kind of control observed in the Betallic treatment.

In the present study, mean weight losses of ≤ 0.44% and ≤ 3.68% were recorded in Betallic and ZeroFly bag treatments, respectively, during the 6 months storage period. However, losses of 2.23, 3.88 and 11.88% were recorded in June, July and August, respectively, in the control PP bag treatment. This study corroborates with the study by Costa (2014) who found that postharvest losses (including weight losses) of 59 and 54%

were recorded in maize stored for 90 d in traditional PP bags in field experiments conducted in Uganda and Burkina Faso, respectively. However, losses in ZeroFly bags were 2.7% and 2.4% after 90 d of storage in Uganda and Burkina Faso, respectively. This implies that, in sub-Saharan Africa, severe and significant postharvest losses due to insects occur in maize stored in PP bags without any control measures.

From observation during our study, the ZeroFly bag appeared to act as a barrier to insects from outside (Fig. 7). Observations during visits to field sites showed that insects were not present on the outside surface of ZeroFly bags (Fig. 7B). However, large numbers of insects were found crawling on the surfaces of PP bags in the control treatment (Fig. 7A). Our data indicate that the ZeroFly bag is effective in suppressing insect population levels, but it is not as effective as admixing maize with Betallic. The fact that the effectiveness of the ZeroFly bag results mostly from preventing insect entry into bags means maize (grains) put into in bags at the start of storage needs to be insect-free to avoid damaging insect levels developing inside bags during storage. Additionally, the bags must retain their physical integrity during the storage period (no holes, well-sealed immediately after each opening) to effectively protect the grain in bags from insect infestation. Based on the need for insect-free grain during filling of ZeroFly bags, it is our recommendation that these bags be used by commercial aggregators and large scale farmers who are better equipped to properly disinfest grain before bagging. Commercial aggregators and large scale farmers in the developing countries can afford the cost of fumigation or any other effective insecticide in order to keep the commodities clean and insect-free before storage in ZeroFly bags. Smallholder farmers in developing countries usually have neither the knowledge nor the resources required for good disinfestation of

grain for bagging. These smallholder farmers typically store their untreated grains in PP bags, and are probably not the best target group for ZeroFly bag storage technology.

Acknowledgements

This work was funded by the Vestergaard Frandsen SA, Switzerland; partial funding for the work came from the Oklahoma Agricultural Experiment Station (Project No. OKL2949). This paper reports the results of research only. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by Oklahoma State University, the USDA-ARS, or Vestergaard Frandsen. Oklahoma State University and the US Department of Agriculture are equal opportunity providers and employers.

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Table 1. ANOVA results for storage period and treatment for moisture content measured by both JD and PHL meters, number of insect damaged kernels, percentage of insect damaged kernels (%), and weight loss per 250-g samples from the Betallic, Control, and ZeroFly bag treatments.

Response Variable	Source	df	F	P
Moisture Content (PHL meter)	Treatment	2, 14.4	3.42	0.0609
	Storage period	6, 47	46.49	<0.0001
	Treatment × Storage period	12, 47.8	0.89	0.5618
Moisture Content (JD meter)	Treatment	2, 8.47	1.96	0.1992
	Storage period	5, 35.4	12.57	<0.0001
	Treatment × Storage period	10, 36.9	1.03	0.4412
Number of Insect Damaged Kernels	Treatment	2, 7.08	15.93	0.0024
	Storage period	6, 48.2	19.89	<0.0001
	Treatment × Storage period	12, 48	5.00	<0.0001
Insect Damaged Kernels (%)	Treatment	2, 5.5	14.68	0.0062
	Storage period	6, 44.4	18.19	<0.0001
	Treatment × Storage period	12, 44.5	4.78	<0.0001
Weight loss (%)	Treatment	2, 6.73	9.02	0.0125
	Storage period	6, 46.4	19.25	<0.0001
	Treatment × Storage period	12, 46.3	5.22	<0.0001

Table 2. ANOVA results for storage period and treatment for number of live and dead *Sitophilus* spp., *Tribolium* spp., and *Cryptolestes* spp. per 250-g samples from Betallic, Control, and ZeroFly bag treatments.

Insects	Source	Live insects			Dead insects		
		df	F	P	df	F	P
Sitophilus spp.	Treatment	2, 7.8	9.14	0.0090	2, 5.67	2.23	0.1933
	Storage period	6, 48.4	6.46	<0.0001	6, 40.5	6.06	0.0001
	Treatment × Storage period	12, 48.2	3.66	0.0006	12, 41.1	1.62	0.1230
Tribolium spp.	Treatment	2, 30.5	53.03	<0.0001	2, 5.76	6.57	0.0327
	Storage period	6, 50.1	9.26	<0.0001	6, 41	5.05	0.0006
	Treatment × Storage period	12, 50.7	9.25	<0.0001	12, 41.7	2.58	0.0116
Cryptolestes spp.	Treatment	2, 6.82	1.47	<0.2938	2, 4.58	3.81	0.1060
	Storage period	6, 48.9	9.09	<0.0001	6, 26.9	6.92	0.0002
	Treatment × Storage period	12, 48.4	6.16	<0.0001	12, 28.9	2.32	0.0318

Figure Captions

Figure 1. Moisture content (%) (Mean ± SE) measured by PHL (A) and JD (B) meter per 250-g of maize kernels in the Betallic Super-treated maize-filled PP bags (Betallic Super), untreated maize-filled PP bags (control), and ZeroFly® Storage Bags (ZeroFly bags). Maize was sampled at the start of storage (February) and at monthly intervals thereafter for six months. Means followed by different lowercase letters are significantly different ($P < 0.05$).

Figure 2. Number (A) and percentage (B) of insect damaged kernels (IDK) (Mean ± SE) per 250 g of maize kernels in the Betallic Super-treated maize-filled PP bags (Betallic Super), untreated maize-filled PP bags (control), and ZeroFly® Storage Bags (ZeroFly bags). Maize was sampled at the start of storage (February) and at monthly intervals thereafter for six months. Means followed by different lowercase letters are significantly different ($P < 0.05$).

Figure 3. Numbers of live (A) and dead (B) *Sitophilus* spp. (Mean ± SE) per 250 g in Betallic Super-treated maize-filled PP bags, untreated maize-filled PP bags (control), and ZeroFly® Storage Bags (ZeroFly bags). Maize was sampled at the start of storage (February) and at monthly intervals thereafter for six months. Means followed by different lowercase letters are significantly different ($P < 0.05$).

Figure 4. Numbers of live (A) and dead (B) *Tribolium* spp. (Mean ± SE) per 250 g in Betallic Super-treated maize-filled PP bag, untreated maize-filled PP bag (control), and ZeroFly® Storage Bag (ZeroFly bag). Maize was sampled after 0, 1, 2, 3, 4, 5, 6 months of storage. Means followed by different lowercase letters are significantly different ($P < 0.05$).

Figure 5. Numbers of live (A) and dead (B) *Cryptolestes* spp. (Mean ± SE) per 250 g in Betallic Super-treated maize-filled PP bags, untreated maize-filled PP bags (control), and ZeroFly® Storage Bags (ZeroFly bags). Maize was sampled at the start of storage (February) and at monthly intervals thereafter for six months. Means followed by different lowercase letters are significantly different ($P < 0.05$).

Figure 6. Percentage weight loss (Mean ± SE) per 250-g sample obtained from Betallic Super-treated maize-filled PP bags, untreated maize-filled PP bags (control), and ZeroFly® Storage Bags (ZeroFly bag). Maize was sampled at the start of storage (February) and at monthly intervals thereafter for six months. Means followed by different lowercase letters are significantly different ($P < 0.05$).

Figure 7. A Polypropylene bag with live insects on the surface (A) and the outside view of ZeroFly bag (B)

Figure 1. Moisture content (MC) data for the PHL meter (A) and JD meter (B).

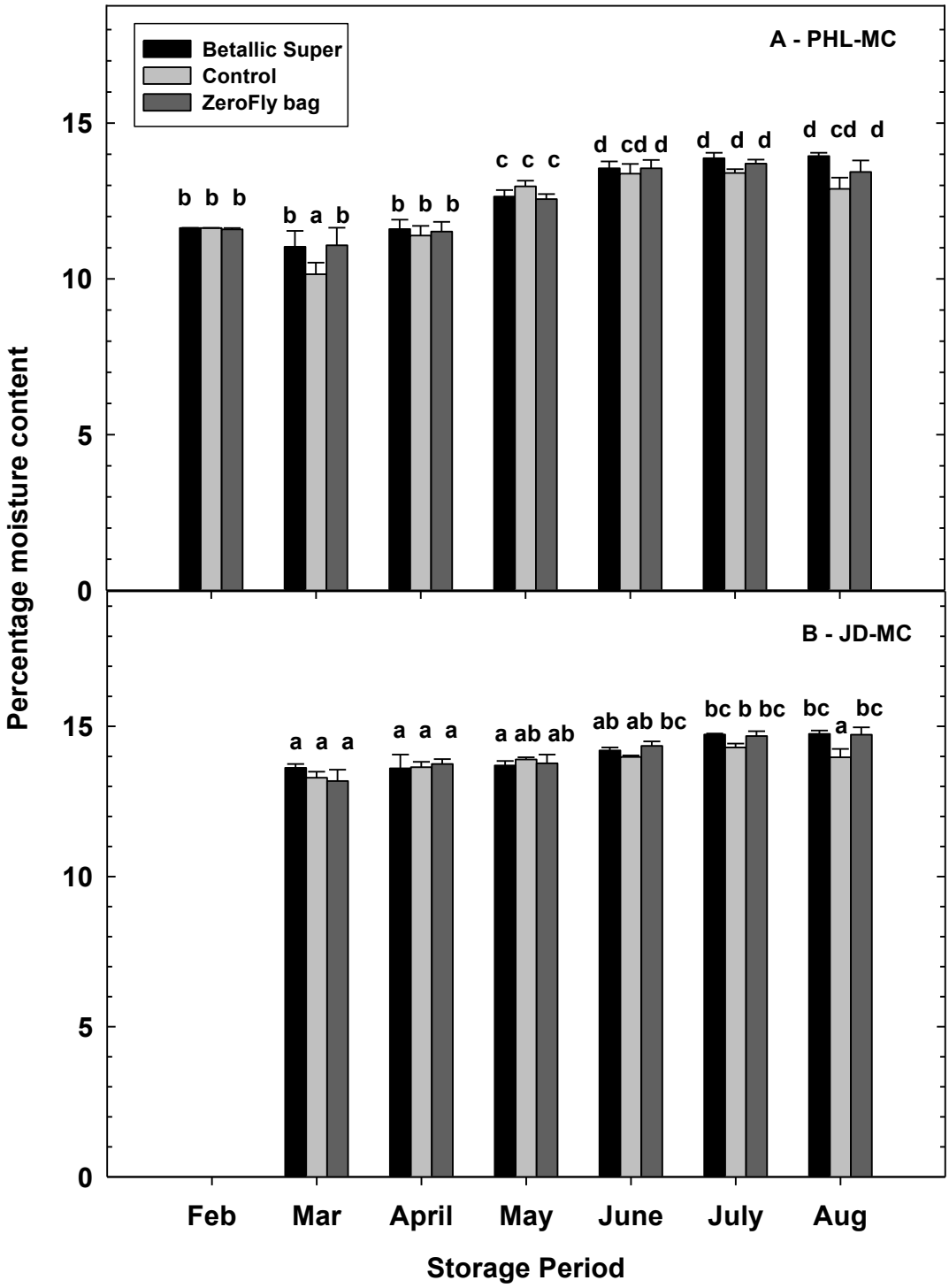

Figure 2. Number of insect damaged kernels (IDK) (A) and percentage IDK (%) (B).

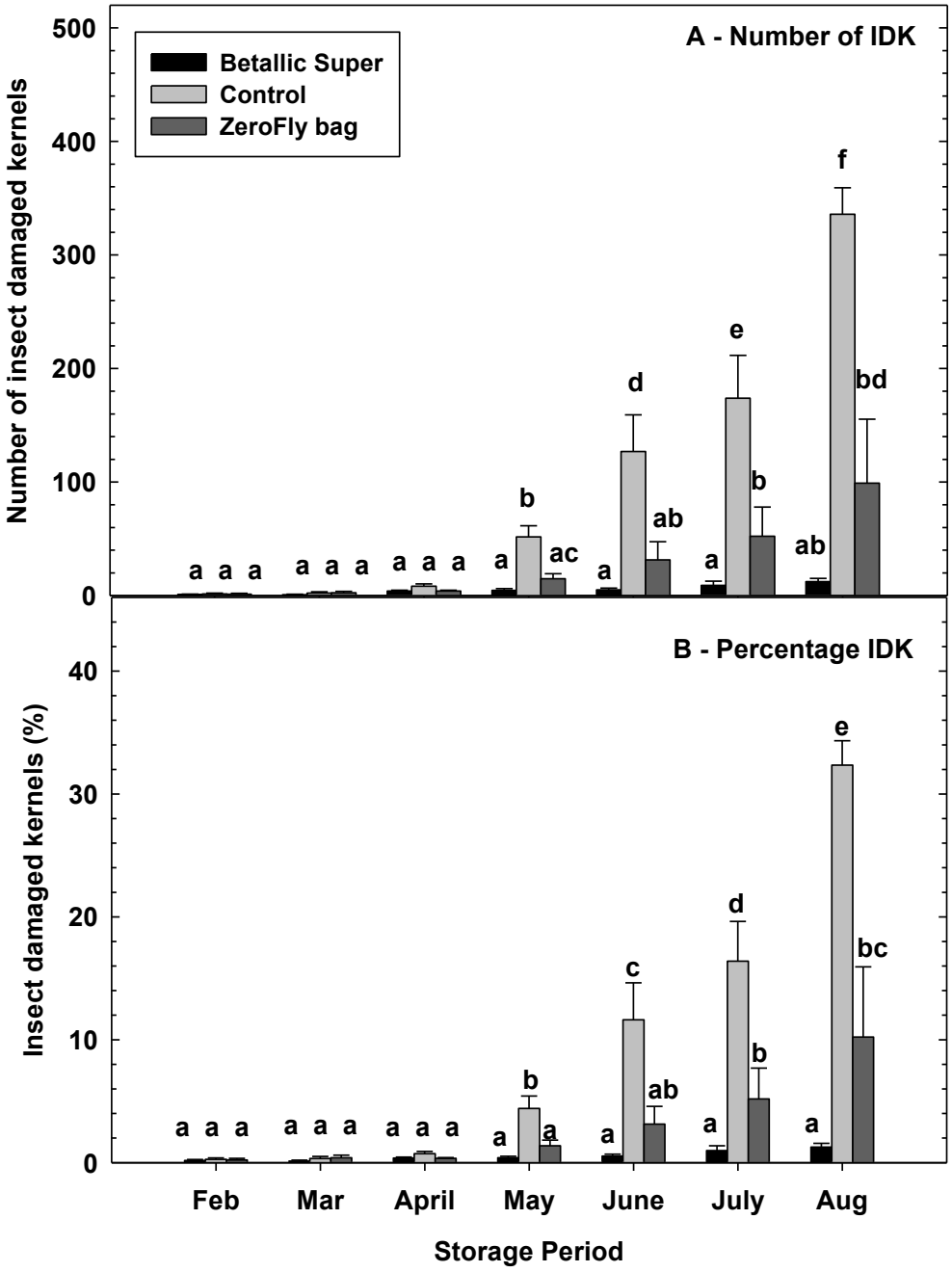

Figure 3. Number of live (A) and dead (B) *Sitophilus* spp. for all storage periods

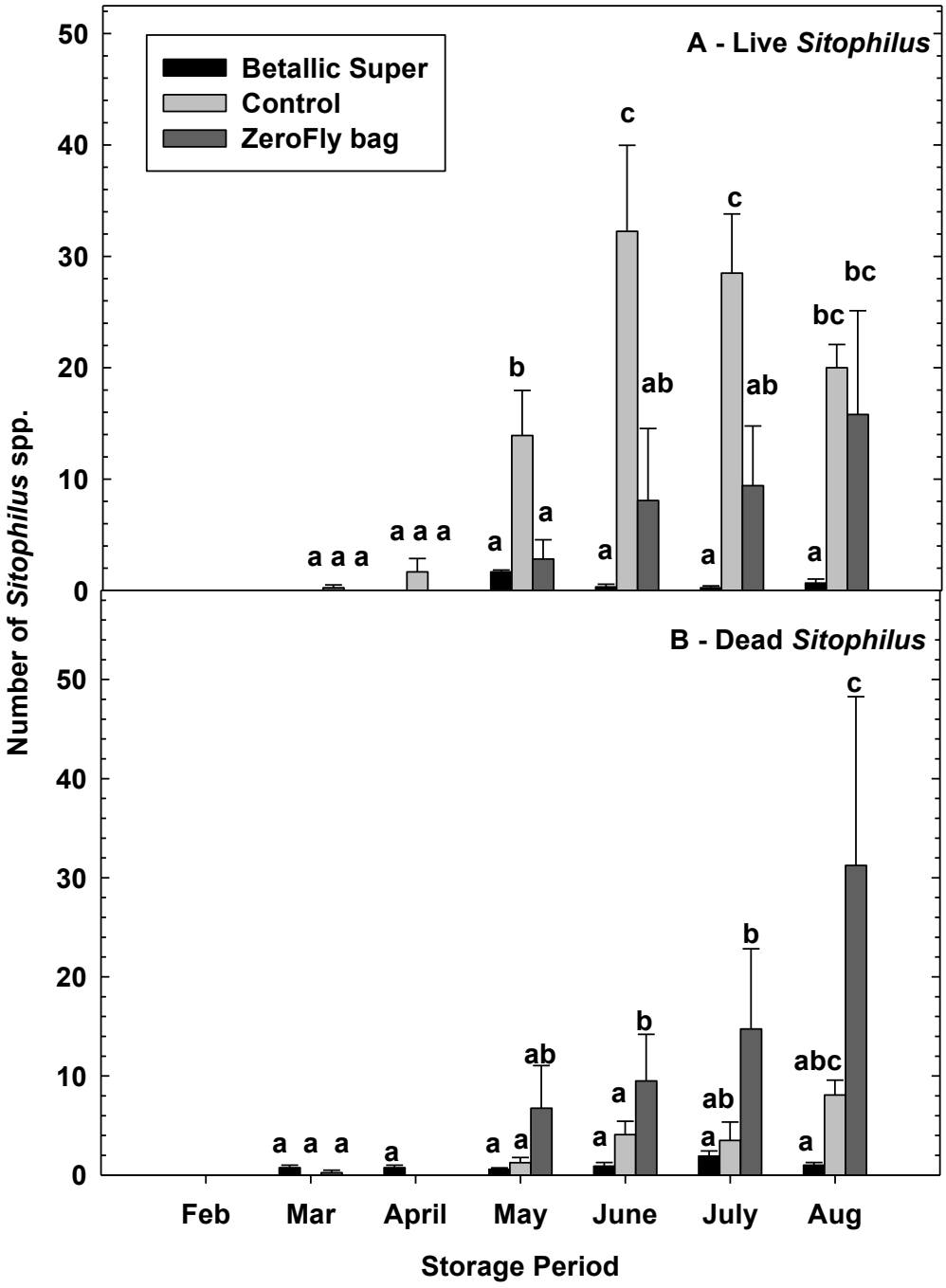

Figure 4. Number of live (A) and dead (B) *Tribolium* spp. in all storage periods

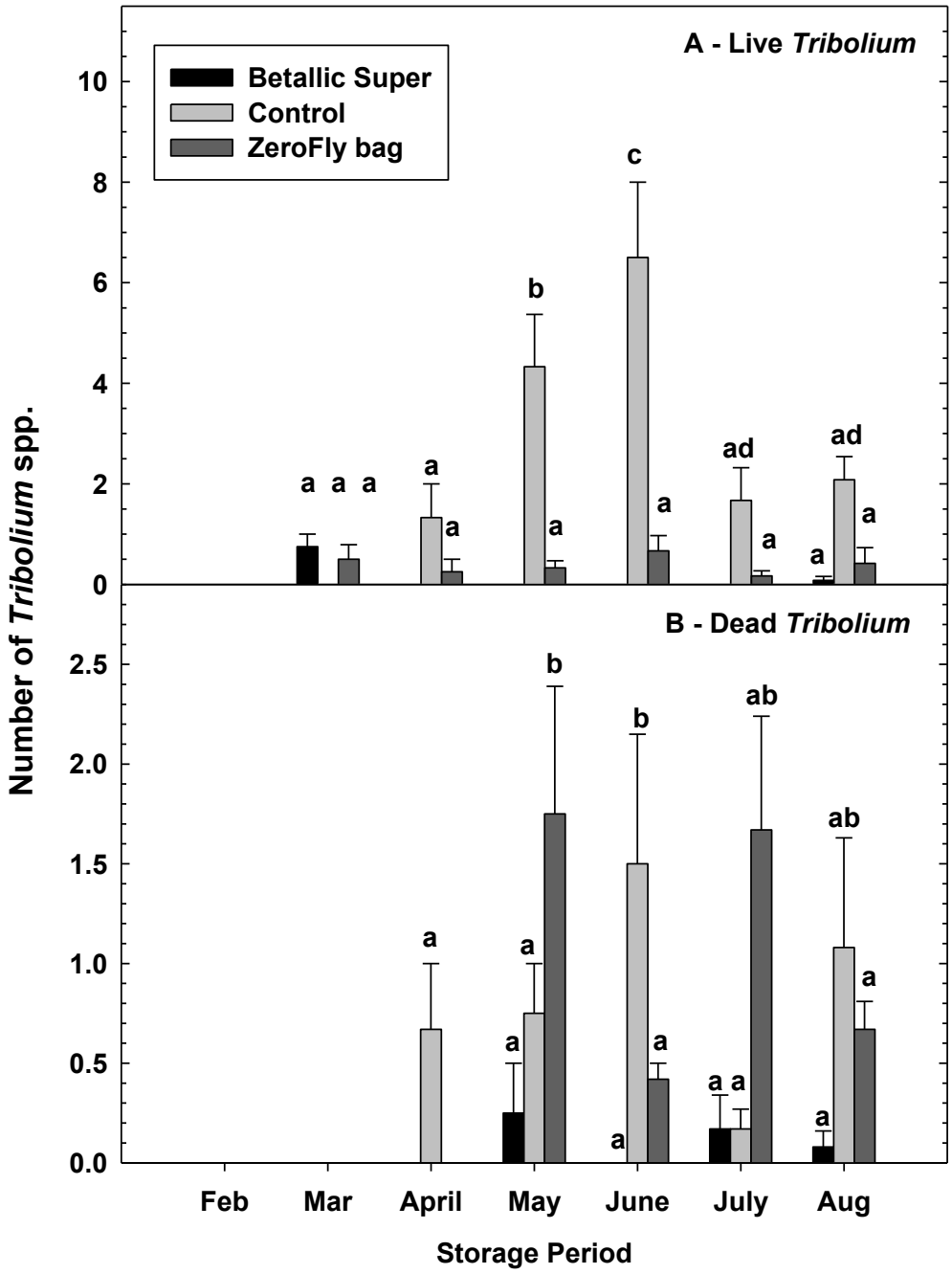

Figure 5. Number of live (A) and dead (B) *Cryptolestes* spp. in all storage periods

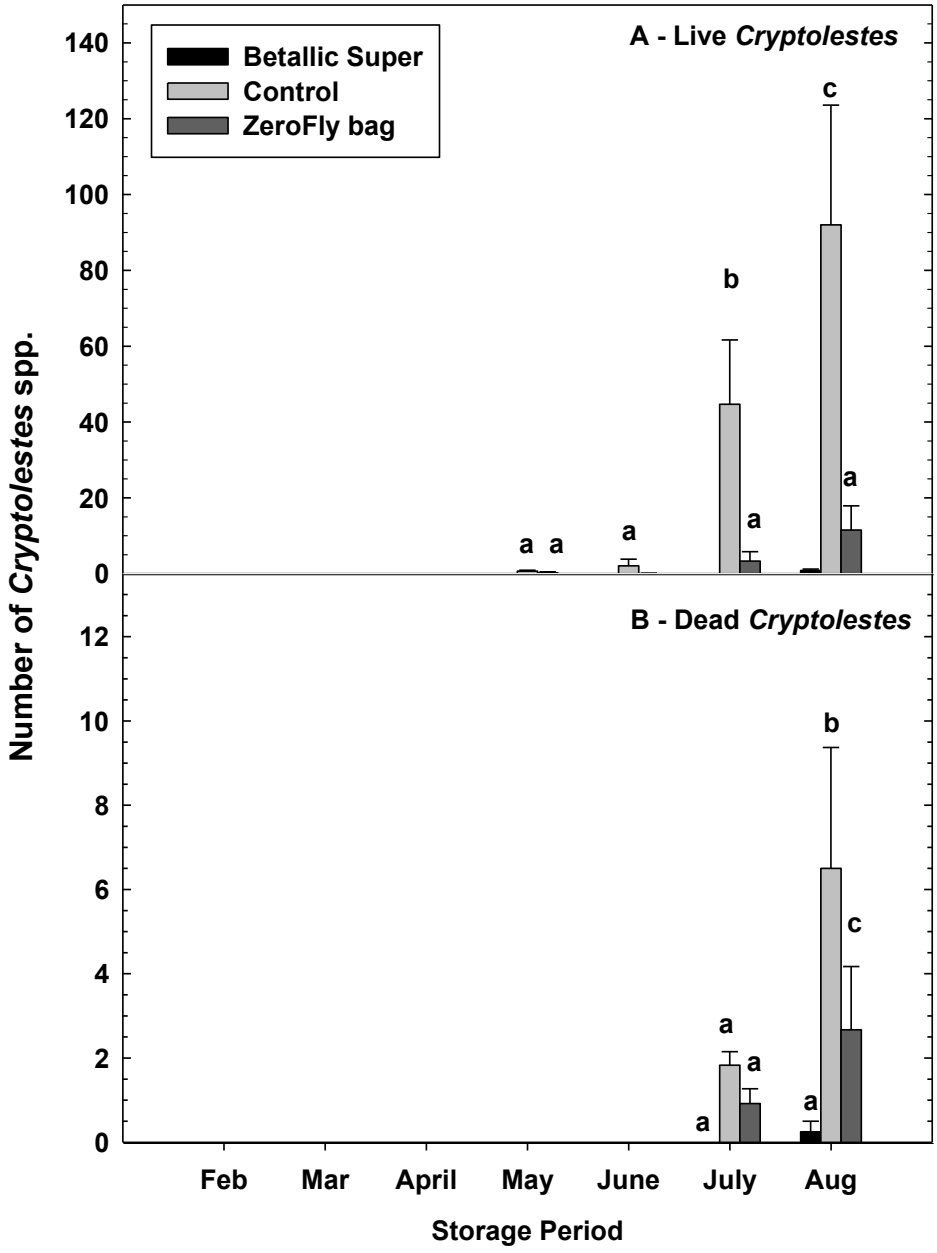

Figure 6. Weight loss (%)

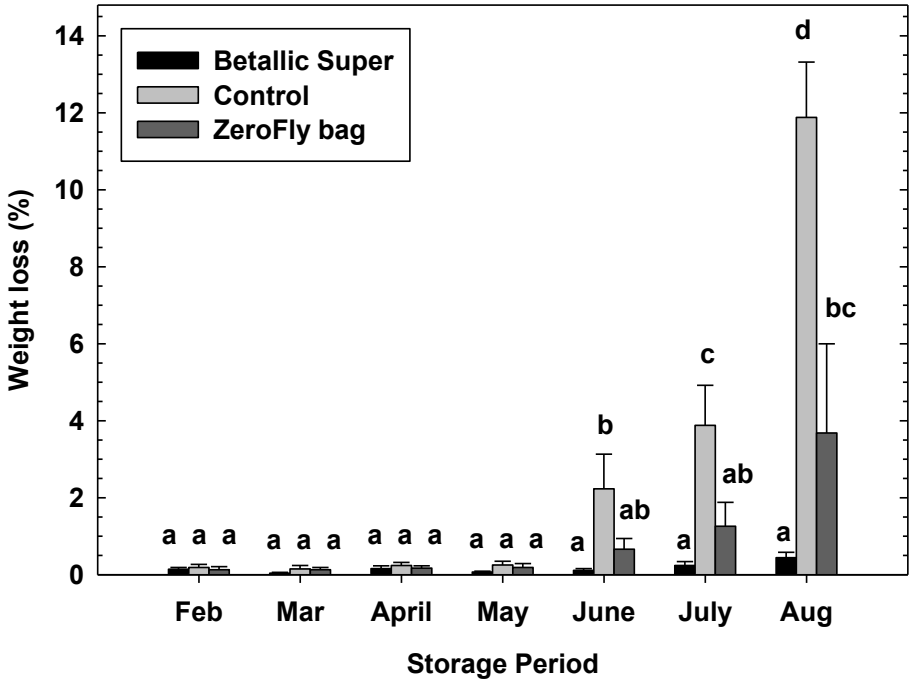

Figure 7A. Outside view of ZeroFly bag

Figure 7B. Live insect outside the PP bag (Control)

CHAPTER VI

CONCLUSION

Postharvest losses due to insect infestation and lack of effective control strategies for use by farmers and/or aggregators in developing countries have resulted in the development of a new innovative tool, the ZeroFly Storage Bag, for the control of key pests during storage. Successful scale up of ZeroFly bags requires good data on the effectiveness of these bags against stored-product insect pests. The ZeroFly bag is made of woven polypropylene (PP), with deltamethrin incorporated in the individual fibers. Given the lack of published baseline data on the contact toxicity of deltamethrin and its efficacy when incorporated in PP bags, studies were conducted to address these issues. The first objective was to investigate the response by adults of *Tribolium castaneum*, *Sitophilus oryzae*, and *Rhyzopertha dominica* to different concentrations of deltamethrin. Eight different concentrations, namely, 1, 25, 50, 100, 250, 500, 1000, and 3000 ppm of deltamethrin were tested over two exposure periods, 24 or 48 h. The second objective was to measure the sensitivity of stored-product insect pests to fabric of ZeroFly bags, PP bags, jute bags, Malathion-treated PP bags, Malathion-treated jute bags, and GrainPro bags — data on knockdown, mortality, and number of progeny for *S. oryzae* and *T. castaneum* were collected. Additionally, the ability of *T. castaneum*, *S. oryzae*, and *R. dominica* to bore or chew through fabric was evaluated using miniature bags. The experiment for the third objective, a field evaluation of the effectiveness of the ZeroFly

bag to protect maize from infestation by stored-grain insect pests, was conducted in Middle Belt of Ghana.

Data showed that at concentrations ≥ 25 ppm on glass, 99% knockdown of *T. castaneum*, *S. oryzae*, and *R. dominica* was achieved within 4 h after exposure. LC_{95} values for all species, for the 48-h exposure period, were ≈ 3,000 ppm, the concentration of deltamethrin in new ZeroFly bags. The study on contact toxicity of deltamethrin, conducted in glass Petri dishes, facilitates the interpretation of data from studies involving exposure of stored-product insects to ZeroFly bag fabric. Data on contact toxicity of deltamethrin improve our understanding of the mode of action of ZeroFly bags against insect pests which results in protection of grain stored in them from infestation.

Based on sensitivity data of insects in contact with the ZeroFly bag, the mode of action of the ZeroFly bag is through knockdown, mortality, and/or reduced progeny production. Relative to five other fabrics — fabric from PP bags, jute bags, malathion-treated PP bags, malathion-treated jute bags, and GrainPro bags — ZeroFly bag fabric was significantly more effective, both in terms of causing mortality and in suppressing progeny production of *S. oryzae* and *T. castaneum* in all exposure periods. When *S. oryzae* and *T. castaneum* were exposed to the ZeroFly bag fabric, mortality was significantly higher in the 72-h exposure period than in 24- and 48-h exposure periods. Data from the miniature ZeroFly bags experiments showed that no live insects were found inside and outside the bags, and none of the insects from the three species tested

was able to bore (chew) through the bag after 28 d of storage. This indicates that the ZeroFly bag can effectively protect cereal grains and grain legumes stored in them by preventing insect entry after bagging.

A field experiment to evaluate the effectiveness of ZeroFly bags to protect maize from infestation by stored-grain insect pests was conducted in the Middle Belt of Ghana (in collaboration with students from Kwame Nkrumah University of Science and Technology (KNUST)). A lower number of insect damaged kernels and smaller populations of insects were found in ZeroFly bags than in PP bags. Based on data from the field experiment, ZeroFly bags were found to have potential for use in the reduction of postharvest grain losses in bagged cereal grains and legumes. However, greater benefits of using ZeroFly bags are realized if insect-free grains or legumes are stored in bags.

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