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Effectiveness of the ZeroFly® storage bag fabric against stored-product insects

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ABSTRACT

The ZeroFly® Storage Bag is a woven polypropylene bag (PP) that has deltamethrin incorporated in its fibers, and represents a novel approach to reducing stored-product insect pest-related postharvest losses. Fabric samples from ZeroFly bags, polypropylene (PP) bags, jute bags, malathion-treated PP bags, malathion-treated jute bags and GrainPro bags were affixed to the bottom of 9-cm Petri dishes and 20 adults of either *Sitophilus oryzae* (L.) or *Tribolium castaneum* (Herbst) were introduced to determine 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 were made from ZeroFly bags, PP bags, laminated PP bags and jute bags, and used to determine ability of adult *S. oryzae*, *T. castaneum* and *Rhyzopertha dominica* (F.) to chew through the bags and efficacy of ZeroFly bags at preventing insect infestations from outside and to contain infestations within bags. Knockdown assessment for ZeroFly bag fabric showed that time required to knockdown 99% of *S. oryzae* and *T. castaneum* was <3 h. For 72-h exposure to ZeroFly bag fabric, mortalities for *S. oryzae* and *T. castaneum* were 76.7 and 62.2%, respectively; mortality was ≤6% in other fabrics. ZeroFly bag fabric also significantly suppressed progeny production by *S. oryzae* and *T. castaneum* for all exposure periods. No insects from the three species tested were able to chew through miniature ZeroFly bags, indicating the bag fabric will prevent entry or exit of insects.

1. 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). Insects are one of the main causes of food losses during storage (Kader, 2005; Parfitt et al., 2010; Affognon et al., 2015). Postharvest losses can be both quantitative and qualitative (Rees, 2004; Kader, 2005; Hagstrum et al., 2012). Besides consuming grain, insects contaminate grain through deposition of their exuviae, webbing and body fragments, and render food unsuitable for human consumption (Bhargava et al., 2007; FAO and World Bank, 2011; Tefera et al., 2011; Nenaah, 2014). Additionally, insects can alter the micro-environment of the grain 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 (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 feed on the endosperm (Bello et al., 2001; Rees, 2004; Hagstrum et al., 2012). 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

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affordable and effective storage technologies for mitigation of *R. dominica*, *S. oryzae* and *T. castaneum* infestations is extremely important.

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; 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, 2010). However, many subsistence farmers in developing countries lack the resources required for effective use of chemical insecticides (Kaminski and Christiaensen, 2014). Additionally, farmers in developing countries have limited access to the 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).

Storage of grains in jute or polypropylene bags is popular in many developing countries (FAO, 1994; Koona et al., 2007; De Groote et al., 2013; Opit et al., 2015). However, serious post-harvest losses do occur in bagged commodities that are not treated with a protectant insecticide; 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; Opit et al., 2015). The broad-spectrum pyrethroid insecticide, deltamethrin, is incorporated into individual polypropylene fibers, and this insecticide provides a powerful knockdown and/or killing action against stored-product insects, thereby preventing their entry into the ZeroFly bag (Paudyal et al., 2016, 2017). Moreover, problems associated with direct application of insecticides to grain, such as high residue levels, are mitigated by use of the ZeroFly bag (Vestergaard, 2015). The concentration of deltamethrin incorporated into fibers of woven poplypropylene ZeroFly bags during the extrusion process is 3 g/kg or 3000 ppm (Vestergaard, 2013). The ZeroFly bag is effective at preventing insect infestations. For example, Anankware et al. (2014) reported 100% mortality of *Sitophilus zeamais* (Mot) (Coleoptera: Curculionidae) after 48 h of exposure to the ZeroFly bag. Kavallieratos et al. (2017) have assessed the effects of ZeroFly bag fabric on *Trogoderma variabile* (Ballion) (Coleoptera: Dermestidae), *Prostephanus trunctatus* (Horn) (Coleoptera: Bostrichidae), *R. dominica*, *T. castaneum*, *S. oryzae*, *S. zeamais*, and *Dermestes maculatus* (De Geer) (Coleoptera: Dermestidae). Their data showed that mortalities after 5 days of exposure to the inside or outside surfaces of the ZeroFly bag ranged from 0 to 100%; *T. castaneum* and *D. maculatus* mortalities were ~5.6% after 5 days of exposure whereas there was no mortality in *S. zeamais* after a similar exposure time. However, there is still lack of data on the sublethal effects of exposure to ZeroFly bag fabric and ability of different stored-product insect species to chew through ZeroFly bags. Therefore, we 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, we evaluated the ability of *R. dominica*, *S. oryzae* and *T. castaneum* to chew through ZeroFly bag fabric and hence assessed effectiveness of the ZeroFly bags.

2. Materials and methods

2.1. 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* was reared on 95% all-purpose wheat flour 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* 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 *R. dominica*, *T. castaneum* and *S. oryzae* that were used in this study were deposited in the K. C. Emerson Entomology Museum at Oklahoma State University under lot numbers 126, 136 and 137, respectively. One-to three-wk old adults of all three species were used in the experiment.

2.2. Contact sensitivity of insects to fabrics and effects of exposure on oviposition

2.2.1. 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, polypropylene bags without insecticide (control), jute bags without insecticide (control), 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 (Vestergaard S. A., Lausanne, Switzerland) were 100 cm × 57 cm, polypropylene bags (Uline, Pleasant Prairie, WI, USA) were 35.7 cm × 25.4 cm, jute bags (West Springfield, MA, USA) were 62 cm × 38 cm and GrainPro bags (GrainPro Inc., Concord, MA, USA) were 130 cm × 74 cm.

2.2.2. Insecticide application

Malathion 50 EC (referred to as malathion, hereafter) (Southern Agricultural Insecticides, Inc., Boone, NC, USA) is an organophosphate insecticide, and was used to treat polypropylene and jute bags. Prior to applying malathion to fabric materials, 0.1 ml of malathion was measured out using a pipette (Gilson®, Middleton, WI, USA) and mixed in 400 ml of distilled water in a 500-ml graduated cylinder (PYREX®, New York, USA) to obtain the application rate of 5 ml in 20 L of water. A volume of 0.12 ml of malathion solution was sprayed on a 62-cm^2 fabric sample, which was then affixed (glued) to the bottom of a 9-cm plastic Petri dish with 62-cm^2 area. Malathion was sprayed on the fabric sample using an airbrush connected to an HD mini regulator and airbrush compressor (Patriot airbrush, TCP Global, San Diego, CA, USA).

2.2.3. Experimental setup

Fabric samples from ZeroFly bags, polypropylene bags, 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. Three bags each were used to prepare malathion-treated polypropylene bag fabric arenas and malathion-treated jute bag fabric arenas. A single piece of sample fabric was affixed to the bottom of each 9-cm-diameter Petri dish using glue to ensure that the fabric covered the entire bottom (floor) of the Petri dish arena. For malathion-treated polypropylene and jute fabrics, the treated surface was affixed face up to the bottom of the arena. For ZeroFly fabric, the outside surface was affixed face up. Fluon® (Polytetrafluoroethylene, Sigma Aldrich Saint Louis, MO, USA) was applied to the internal sides of each Petri dish arena to prevent insects from climbing the 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 six different fabric materials. Data on knockdown and mortality were collected. To ensure true replication, three bags of each type of fabric were accordingly used. For example, in the case of the ZeroFly bag fabric, three different bags were used to obtain the 62-cm^2 samples for testing; these samples were affixed 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 affixed 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 nine Petri dishes assigned to the three 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 species, 162 dishes were used.

Contact sensitivity and effects on oviposition of exposure to the six fabrics were investigated for only *S. oryzae* or *T. castaneum*. Twenty 1- to 3-wk-old adults of *S. oryzae* or *T. castaneum* were 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 to Petri dishes with six different types of fabrics tested, *S. oryzae* were transferred from Petri dishes containing fabric to a different set of glass Petri dishes, with no fabric, each containing 5 g of 95% whole hard red winter wheat and 5% (by weight) brewer's yeast. Similarly, *T. castaneum* were also transferred to different set of dishes containing 5 g of 95% all-purpose wheat flour and 5% brewer's yeast. After transfer to a new set of Petri dishes, insects were observed (checked) 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 (3 d).

To determine progeny production, all adults in the second set of Petri dishes — dishes with 5 g of diet and no fabric — were removed after 7, 14 or 21 d from the time of transfer. These periods translate to 4, 11 and 18 d after the final mortality assessment was conducted 3 d after transfer to second set of Petri dishes. After the adults were removed, the diet in Petri dishes was transferred to 236-ml glass jars (Quilted Crystal® Jelly Jars). Twenty-five grams of the respective diets for the two 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.

2.3. Ability of insects to chew through storage bag fabrics

2.3.1. Miniature storage bags

Four storage bags, ZeroFly bags, jute bags, polypropylene bags and laminated polypropylene bags were investigated. Empty 50-kg capacity ZeroFly bags (Vestergaard SA., Lausanne, Switzerland) (100 cm × 57 cm), polypropylene bags (Uline, Pleasant Prairie, WI) (35.7 cm × 25.4 cm), jute bags (West Springfield, MA, USA) (62 cm × 38 cm) and laminated polypropylene bags (Central Bag Company, Leavenworth, KS, USA) (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 (hereafter referred as mini bags) with dimensions of 21 cm × 16 cm were stitched with thread and a needle but one end of each bag was left open (unstiched).

2.3.2. 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 mini 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. A relative humidity of 65% was produced using a saturated solution of $NaNO_2$ kept in a different container outside the 21.7-cm × 20.7-cm × 22-cm box (Winston and Bates, 1960). Fluon (Polytetrafluoroethylene, Sigma Aldrich Saint Louis, MO, USA) was applied to the top 2 cm of the internal sides (inner walls) of the box to prevent insects from climbing the walls and escaping. Diet-filled mini bags were individually placed inside plastic boxes. One gram of wheat was placed inside the plastic box, but outside the bag, to ensure insects had food. Twenty 1- to 3- wk-old adults of *S. oryzae*, *T. castaneum*, or *R. dominica* were added to each box. Four boxes containing insects of each species were placed in the incubator each time — the four boxes had one type of mini bag; altogether, there were a total of 12 boxes for all the three species each time. These 12 boxes represented a single temporal replication. Boxes were placed in an incubator maintained at 28 °C and 65% RH throughout the experiment. The numbers 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 was recorded after 28 d. This experiment was replicated over three time blocks (temporal replications) and had a two factor factorial arrangement in a RCBD with repeated measures.

2.3.3. Evaluating effectiveness of fabrics to contain infestation

Mini bags were made as previously described. Each of the mini bags was filled with 0.5 kg of 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 to each of the bags containing 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 plastic boxes which had holes on the lid to enable air movement. One gram of wheat was placed inside each plastic box, but outside the bag, to ensure that insects which exited the bag had food. Four boxes containing insects of each species were placed in the incubator each time — the four boxes had one type of mini bag; altogether, there were a total of 12 boxes for all the three species each time. These 12 boxes represented a single temporal replication. 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. This experiment was replicated over three time blocks (temporal replications) and had a one factor factorial arrangement in a RCBD.

2.4. Data analyses

2.4.1. Contact sensitivity of insects to fabrics and effects of exposure on oviposition

Data were analyzed by species. The experimental design was a completely randomized design (CRD) with three replications.

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

Species	KT50 (95% CI)	KT_{95} (95% CI)	KT_{99} (95% CI)	Slope ± SE	χ^2 (df) [H]
Sitophilus 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]
Tribolium 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).

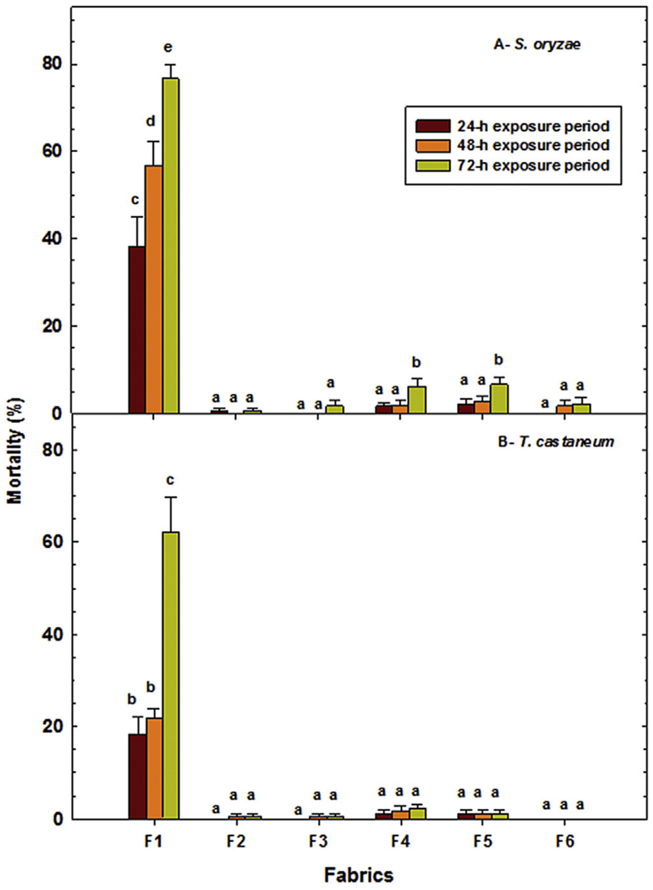

Fig. 1. Mortality (Mean ± SE) of *Sitophilus oryzae* (A) and *Tribolium castaneum* (B) exposed to fabric materials from ZeroFly® Storage Bags (ZeroFly bag) (F1), polypropylene bag (PP) (F2), jute bag (F3), Malathion 50EC- treated PP bags (F4), Malathion 50EC- treated jute bag (F5) and GrainPro bag (F6).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

Knockdown data were subjected to probit analyses to determine the time to knockdown 50%, 95% and 99% of insects; these times will hereafter be referred to as KT_{50}, KT_{95} and KT_{99}, respectively. Probit analyses were conducted using PoloPlus (LeOra Software, 2005). Mortality was assessed 3 d after transfer of insects to clean Petri dishes containing diet. Statistical analyses were conducted with SAS Version 9.4 (SAS Institute, Cary, NC, USA). Analysis of variance (PROC MIXED) methods were used for analysis of percent mortality and adult progeny (resulting from oviposition) 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.

2.4.2. Ability of insects to chew through storage bag fabrics (mini bags)

Data were analyzed by species. All statistical analyses were conducted with SAS Version 9.4. 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 with repeated measures. Mortality data were accordingly transformed. 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, mortality data were accordingly transformed. 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 means differences were determined by Tukey's HSD (honest significant difference) test at $P < 0.05$.

Table 2
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*	df	*F*
Fabric	5, 144	202.0	5, 144	134.3
Exposure Period	2, 144	18.2	2, 144	14.0
Fabric × Exposure Period	10, 144	3.6	10, 144	10.0

In all cases $P < 0.01$. Table 2 relates to Fig. 1.

Table 3
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 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, 108	44.75	<0.01	5, 108	20.52	<0.01
Exp	2, 108	4.87	<0.01	2, 108	3.23	0.04
Fabric × Exp	10, 108	2.19	0.02	10, 108	0.54	0.86
Ovipos	2, 108	26.60	<0.01	2, 108	6.11	<0.01
Fabric × Ovipos	10, 108	0.81	0.62	10, 108	0.33	0.97
Exp × Ovipos	4, 108	0.30	0.88	4, 108	0.14	0.97
Fabric × Exp × Ovipos	20, 108	0.28	0.99	20, 108	0.17	1.00

Table 3 relates to Figs. 2 and 3.

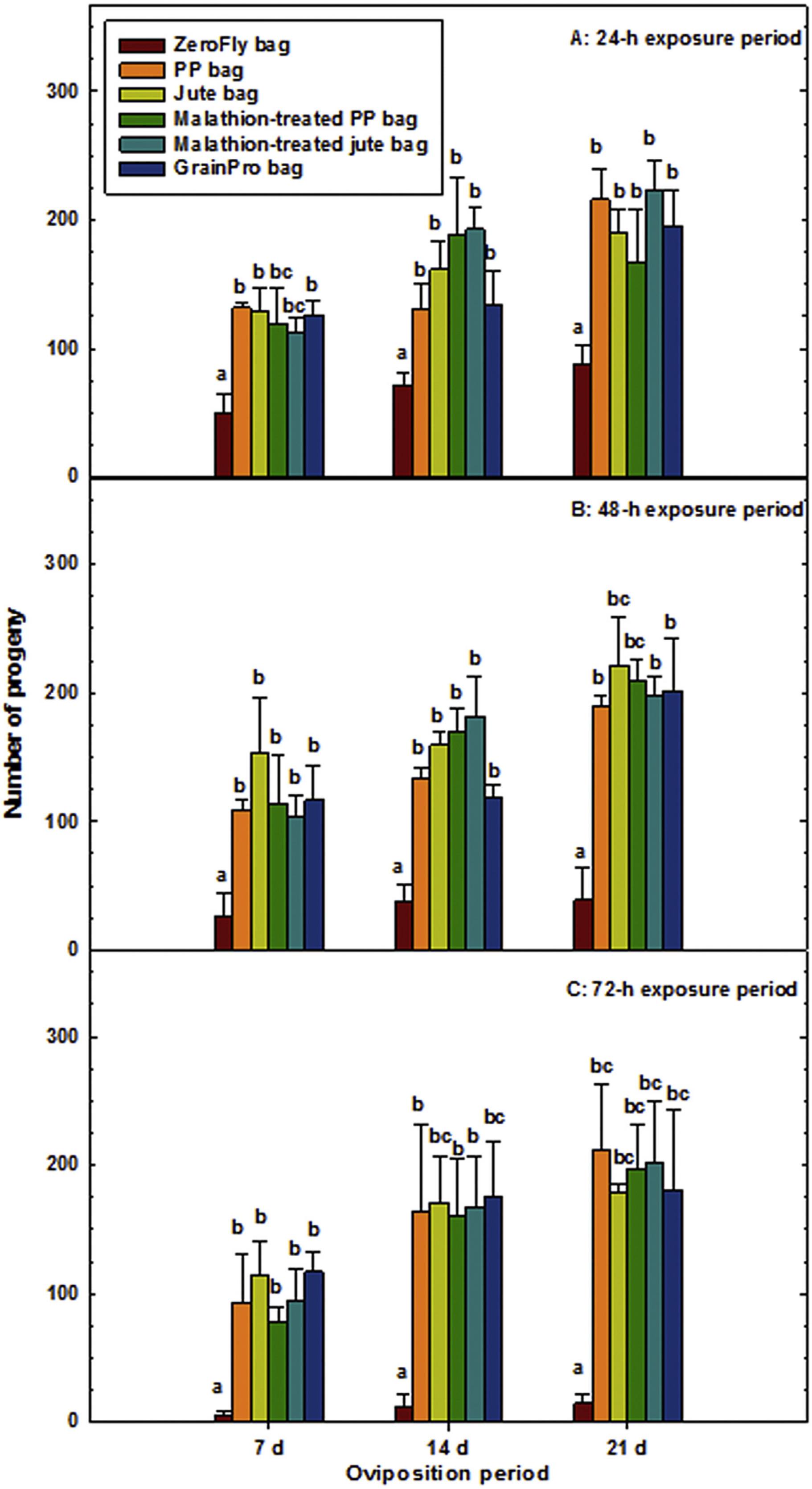

Fig. 2. Number of progeny (Mean ± SE) of *Sitophilus oryzae* exposed to fabric materials from ZeroFly® Storage Bags (ZeroFly bag), polypropylene bag (PP), jute bag, Malathion 50EC-treated PP bag, Malathion 50EC- treated jute bag and GrainPro bag. Means with different lowercase letters are significantly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

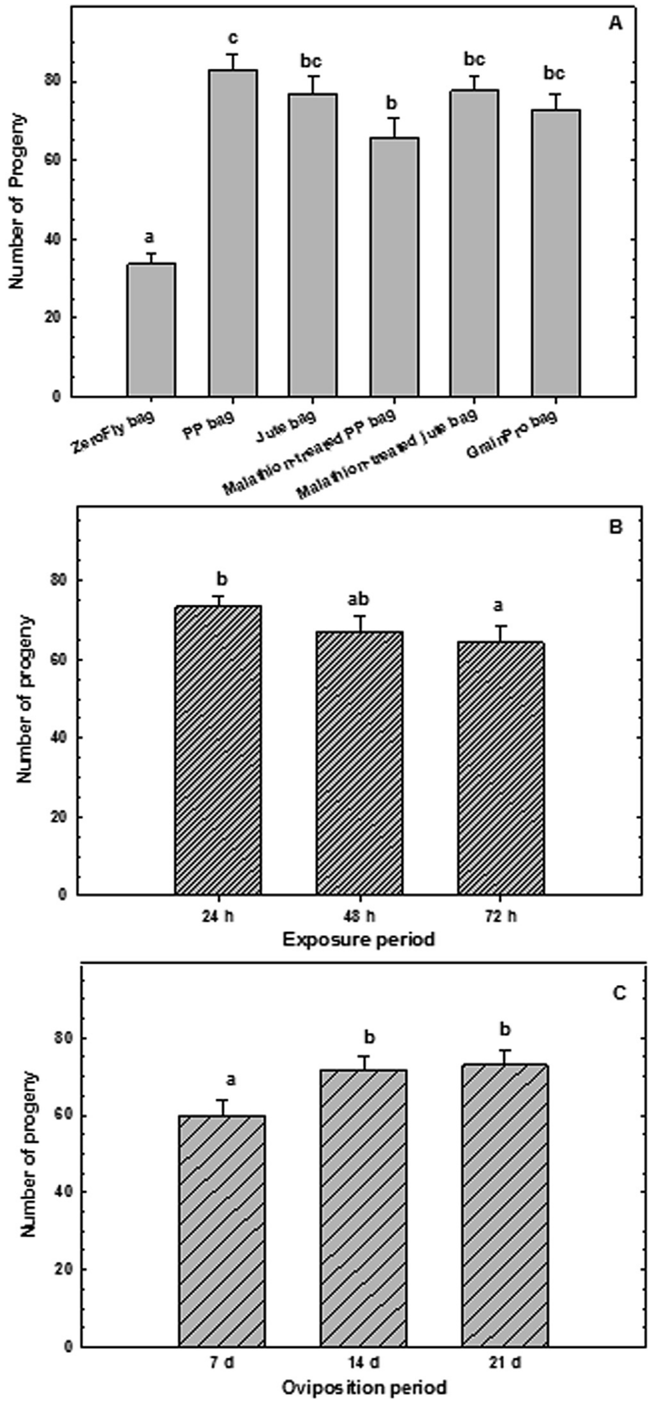

Fig. 3. Number of progeny (Mean ± SE) of *Tribolium castaneum* exposed to different fabric materials (A) for different exposure periods (B) and oviposition periods (C) from ZeroFly® Storage Bags (ZeroFly bag), polypropylene bag (PP), jute bag, Malathion 50EC-treated PP bag, Malathion 50EC- treated jute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

3. Results

3.1. Contact sensitivity of insects to fabrics and effects of exposure on oviposition

3.1.1. *Sitophilus 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 interaction — 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). In malathion-treated PP or jute fabrics, PP bag, jute bag and GrainPro bag fabrics, mortalities were ≤6% (Fig. 1). In both malathion-treated fabrics, *S. oryzae* mortality was higher after 72 h. In relation to progeny production, all main effects were significant, whereas all the interactions were not significant with the exception of fabric × exposure period (Table 3). Progeny production was significantly lower in the ZeroFly bag fabric than in the other five fabrics during all exposure and oviposition periods. However, numbers of progeny were similar in the PP bag, jute bag, malathion-treated PP bag, malathion-treated jute bag and GrainPro bag fabrics. In relation to the longest exposure period (72 h), 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 (Fig. 2C). These data show the ZeroFly bag fabric is effective in suppressing progeny production of *S. oryzae*.

3.1.2. *Tribolium castaneum*

At least 99% of *T. castaneum* were knocked down after 78.9 min of exposure to the ZeroFly fabric (Table 1). However, the percentages of insects knocked down in other fabrics were ≤5% after 8 h. For percentage mortality, all the main effects and interaction — 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 indicate that *T. castaneum* is less sensitive to ZeroFly bag fabric than *S. oryzae* (Fig. 1). 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 from five other fabrics (Fig. 3A). The mean number of progeny in ZeroFly bags was 33, 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 the 72-h exposure period than in the 24-h exposure period (Fig. 3B) and lower in the 7-d oviposition period than in 14- and 21-d oviposition periods (Fig. 3C).

3.2. Ability of insects to chew through storage bag fabrics

3.2.1. Evaluating effectiveness of fabrics to prevent infestation from outside

Based on observation of insect activity, *S. oryzae*, *T. castaneum*, *or R. dominica* were not able to chew through the ZeroFly bag and both types of PP bags (PP and laminated PP mini bags). However, insects were able to create holes and enter the jute bag. For mortality data, all the main effects (mini bags and storage period) and the type of mini bag × storage period interaction were significant for all insect species (Table 4). Mortality was significantly higher in the ZeroFly mini bags 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). Although the mortalities in jute, PP, or laminated PP mini

Table 4

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*	*df*	*F*	*df*	*F*
Bag type	3, 7.25	40.89	3, 15.8	186.88	3, 11.5	323.41
Storage Period	5, 36.2	20.86	5, 38.7	29.30	5, 33.9	91.52
Bag type × Storage Period	15, 34.8	2.53	15, 37.7	2.91	15, 33.4	7.07

In all cases $P < 0.01$. Table 4 relates to Fig. 4.

bags were significantly lower than ZeroFly bags, mortalities increased over time (Fig. 4).

3.2.2. Evaluating effectiveness of fabrics to contain infestation

None of the *S. oryzae*, *T. castaneum* or *R. dominica* adults placed inside the mini ZeroFly bags, PP and laminated PP were able to chew their way out of the bag. However, insects were able chew their way out of the jute bag. Holes created by insects were found only in jute bags. Additionally, significantly higher mortality of all three species occurred inside the ZeroFly mini bags compared to other types of mini bags (Fig. 5). Mortalities in the ZeroFly mini bags after 28 d was ≥94%, for all species (Fig. 5). Mortalities in the PP, laminated PP and jute mini bags did not exceed 24% after 28 days, for all three species (Fig. 5; Table 5).

4. Discussion

Sitophilus 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 stored-product insect pests have been shown by Anankware et al. (2014) and more recently by Kavallieratos et al. (2017). In the present study, we showed that 99% of *S. oryzae* and *T. castaneum* exposed to the ZeroFly bag fabric were knocked down in ≤3 h. Anankware et al. (2014) reported > 93% knockdown of *S. zeamais* after 6 h of exposure to ZeroFly bag fabric. Kavallieratos et al. (2017) reported 100% knockdown of all *T. variable*, *P. truncatus*, *R. dominica* and *T. castaneum* after only 1 h exposure to the outside and inside surfaces of the ZeroFly bag. Of the seven stored-product insect species tested by Kavallieratos et al. (2017), *T. castaneum*, *D. maculatus*, and *S. zeamais* were the most tolerant species when exposed to ZeroFly bag fabric — mortality for *T. castaneum* and *D. maculatus* after 5 d of exposure was only ~5.6%; after 5 days of exposure there was no mortality in *S. zeamais*.

For both *S. oryzae* and *T. castaneum*, there was significantly higher mortality after 72 h than 24 h of exposure. Additionally, data from the present study showed that *T. castaneum* is less sensitive to ZeroFly bag fabric than *S. oryzae*. These findings are supported by data from the Kavallieratos et al. (2017) study where mortality of different stored-product insect pests exposed to ZeroFly bag fabric was influenced by insect species, exposure interval, and whether exposure was to the outside or inside surfaces of the bag. Expectedly, in this and the Kavallieratos et al. (2017) study, mortality increased with the time that insects were exposed to ZeroFly bag fabric. In some aspects, the response of *T. castaneum* in these two studies was rather different. Mortalities of *T. castaneum* exposed for 24-, 48- and 72-h in this study were 18.3, 21.7 and 62.2%, respectively. However, in Kavallieratos et al. (2017), mortality of *T. castaneum* after 5 d of exposure to the inside or outside surface of the ZF bag was only 5.6%. Differences in the strains of insects used, number of insects placed on each arena (10 versus 20), and/or criteria used for categorizing an insect as knocked down or dead may account for this discrepancy.

In the present study, exposure of *S. oryzae* to ZeroFly bag fabric for 24, 48 and 72 h (3 d) resulted in 38.3, 56.7 and 76.7% mortality, respectively. Additionally, 100% mortality of *S. oryzae*, *T. castaneum* and *R. dominica* adults occurred after 5 d of being closely confined outside ZeroFly mini bags. These data are consistent with the fact that mortality increases with time insects are exposed to ZeroFly bag fabric. In the case of *S. oryzae*, data in this study are supported by those from Kavallieratos et al. (2017) where 100% mortality occurred after 5 d of exposure to the outside surface of the ZeroFly bag fabric in Petri dish arenas.

The large numbers of adult insects (progeny) produced after *S. oryzae* and *T. castaneum* exposure to fabric from PP, jute or GrainPro bags shows that insects used in this experiment were fecund. Therefore, the marked suppression of progeny production in the ZeroFly bag fabric was likely due to exposure of parental adults to deltamethrin incorporated in the fibers. Low progeny production in insects exposed to the ZeroFly bag fabric 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 5–14 progeny of *S. oryzae* and ≤33 progeny of *T. castaneum* associated with 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 and prevent entry into the bag. The fact that 72 h exposure to ZeroFly bag fabric did not result in total progeny production suppression shows why it is important to store insect free grain in the ZeroFly bags. Occasional exposure of insects in the bag to fabric does not result in certain mortality nor substantial progeny suppression.

Although the 72-h exposure to the ZeroFly bag fabric did not kill

Table 5

Effects of type of mini bag on adult *S. oryzae, T. castaneum* and *R. dominica* mortality when insects were kept inside 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*	*F*	*F*
Bag type	3, 6	151.84	47.13	126.72

In all cases $P < 0.01$. Table 5 relates to Fig. 5.

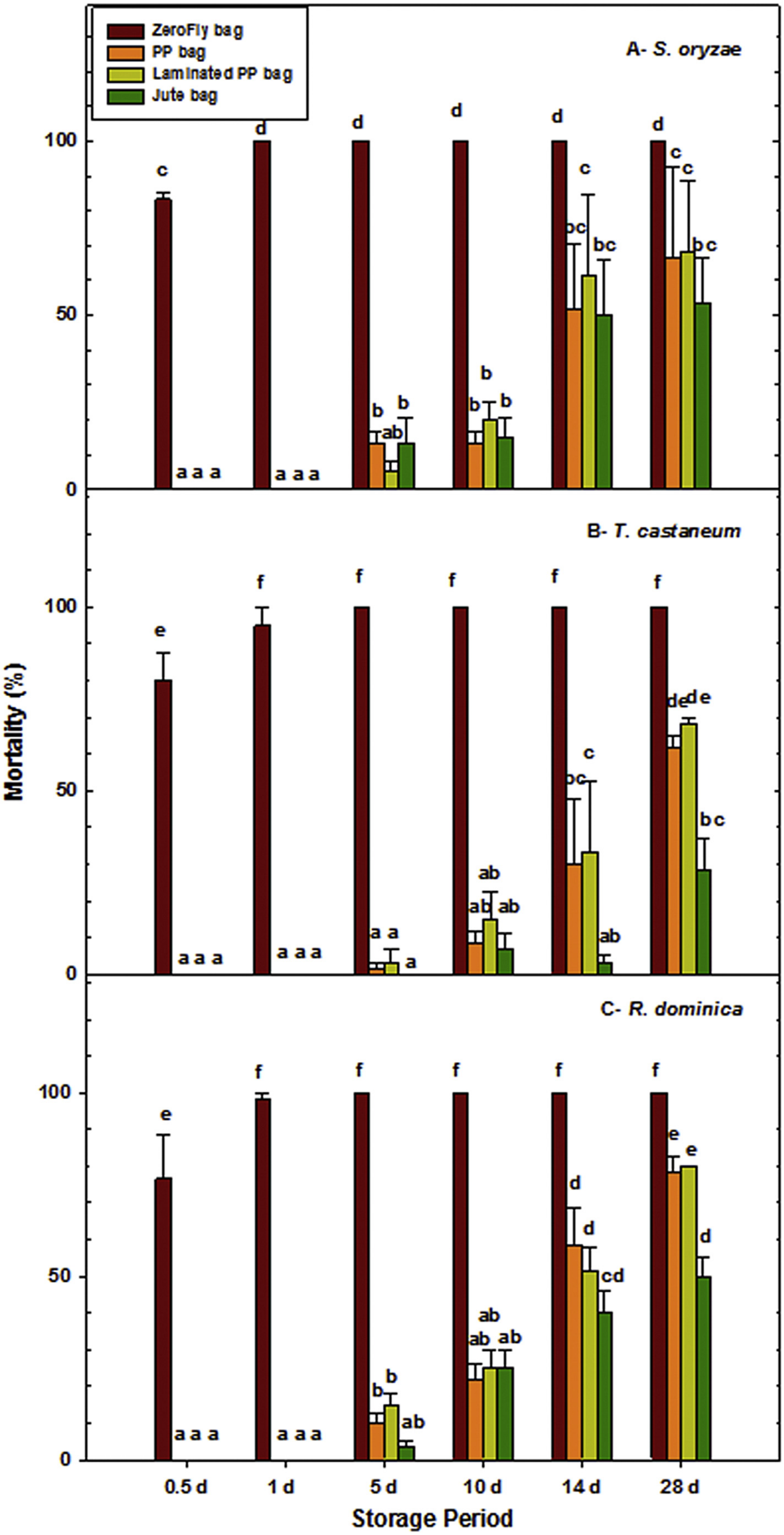

Fig. 4. Mortality (Mean ± SE) of *Sitophilus oryzae* (A), *Tribolium castaneum* (B) and *Rhyzopertha dominica* (C) when insects were placed outside of ZeroFly bag, polypropylene bag (PP), laminated PP bag and jute bag. Means with different lowercase letters are significantly different ($P < 0.05$).

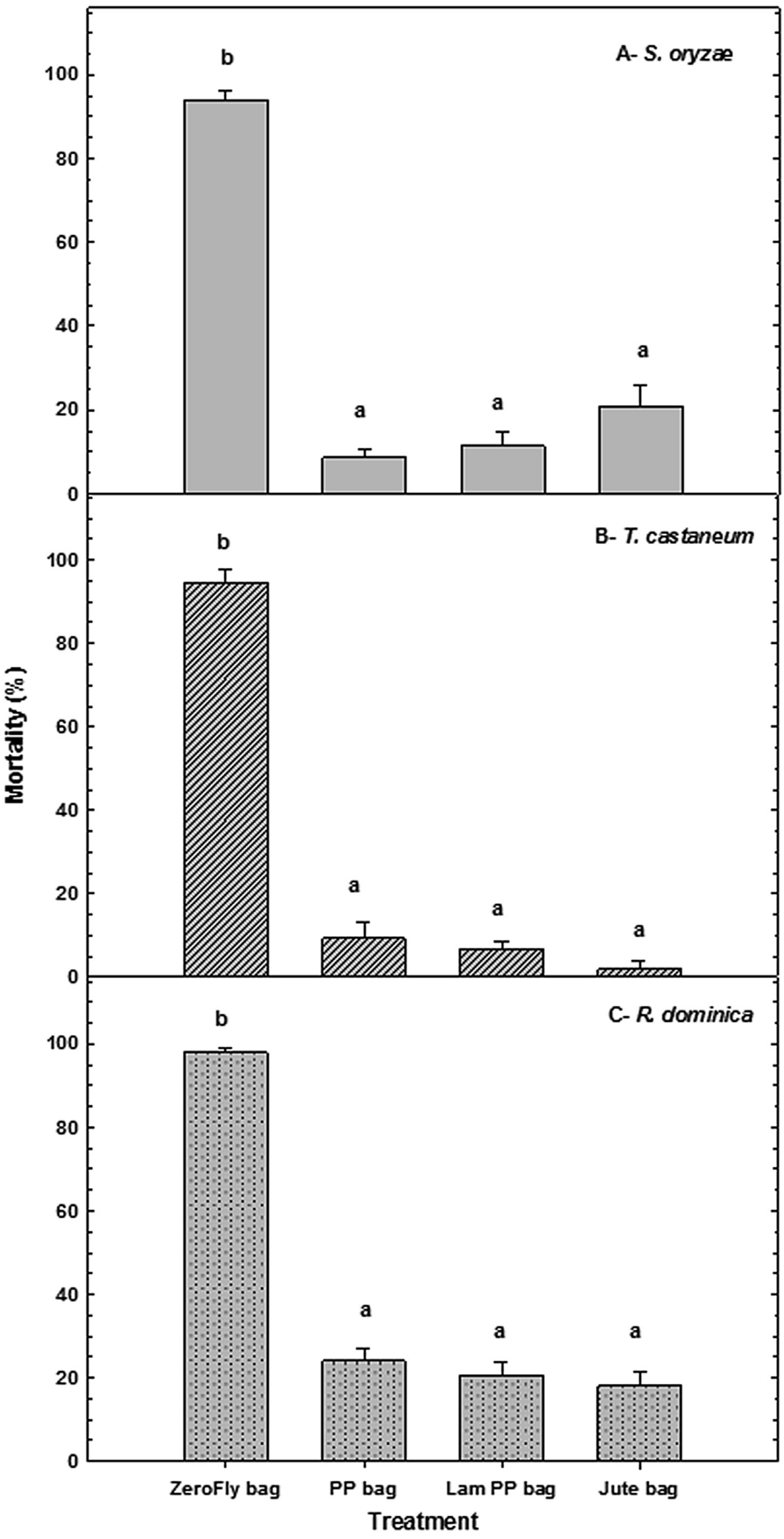

Fig. 5. Mortality (Mean ± SE) of *Sitophilus oryzae* (A), *Tribolium castaneum* (B) and *Rhyzopertha dominica* (C) when insects were placed inside the ZeroFly bag, polypropylene bag (PP), laminated PP bag and jute bag for 28 d. Means with different lowercase letters are significantly different ($P < 0.05$).

all adult insects, data from ZeroFly mini bags showed that no test insects chewed through the ZeroFly bag fabric. This supports the findings of Anankware et al. (2014) who showed that *S. zeamais* was not able to chew through the ZeroFly bag fabric. The inability of *S. oryzae*, *T. castaneum*, *R. dominica* and *S. zeamais* to chew 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. dominica* were not able to chew through PP and laminated PP mini bags. These results are similar to those reported by Allahvaisi et al. (2010) indicating PP prevented penetration by *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Although no insects entered the PP and laminated PP mini bags in the current test, 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 the 28-d duration of the experiment. No insect holes were found in ZeroFly, PP and laminated PP mini bags, but holes were found in jute mini bags. For clarification, stored-product insects could easily gain access to the some of the 100-kg non-laminated coarse PP woven maize bags that are commonly used in sub-Saharan Africa because of the openings that result in them after bags are filled. These bags are different from the PP and laminated PP mini bags used in this study that insects failed to breach.

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). It is possible that with long intensive use of ZF bags, stored-product insects could develop resistance to this deltamethrin-incorporated bag. Additionally, stored-product insects that are already resistant to deltamethrin may be able to chew though the bag fabric. That being said, it is important to note that there is a marked difference between exposure of insects by walking on the deltamethrin-incorporated ZeroFly bag fabric surface and exposure due to chewing on fabric containing a deltamethrin concentration of 3 g/kg — the latter would be expected to have more dire effects on an insect than the former. Additionally, existence in the field of many other conspecifics that are susceptible to deltamethrin due to lack of exposure may significantly slow the development of resistance and, implicitly, any threat to reduced efficacy of ZeroFly storage bags.

Based on this study, ZeroFly bag fabric can cause direct effects of knockdown and mortality, and sublethal effects such as reduced progeny production. The ZeroFly bag fabric not only prevents insect entry into bags, but also prevent insects inside the bag from escaping, thereby preventing the spread of the infestation. The ZeroFly bag is currently commercially available for mitigating stored-product insect problems in stored grain (Vestergaard, 2015). Our data show that the ZeroFly bag fabric is effective at causing knockdown, mortality, and in suppressing progeny production of *S. oryzae* and *T. castaneum*. Based on the present study, the ZeroFly bag is most likely going to be effective against stored product pests in the field if insect-free grain is stored in these bags.

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