

Efficacy of Pea Protein and Combinations of Pea Protein and Wasps Against Stored-Grain Insects in Large Scale Tests¹

Xingwei Hou², Paul Fields^{3*}, Paul Flinn⁴, Joel Perez-Mendoza⁴ and James Baker⁴

Abstract

Protein-enriched pea flour is toxic and repellent to three major stored grain pests, the rice weevil, *Sitophilus oryzae* (L.), the red flour beetle, *Tribolium castaneum* (Herbst) and rusty grain beetle, *Cryptolestes ferrugineus* (Stephens). A unique and valuable aspect of the protein-enriched pea flour is that it is not toxic several parasitoids that are associated with two of these insect pests. These parasitoids include *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae), a parasitoid of *S. oryzae*, and *Cephalonomia waterstoni* (Hymenoptera: Bethyilidae), a parasitoid of *C. ferrugineus*. Two large scale tests, including one test in combination with the parasitoids, were conducted to evaluate the effectiveness of the pea flour as a grain protectant.

A two-month, 30-tonne-granary trial with barley was conducted in 6 bins, each filled with 11 tonnes of barley. Bins had full-floor aeration, hence insects could leave or enter the grain mass. Results showed that treating all the barley during loading with 0.1% of pea protein had a similar effect as treating the barley in only the top half of the granary with 0.5% of pea protein. Compared with control bins, the treatments with protein-enriched pea flour reduced the size of *S. oryzae* populations by more than 90%, and those of *Cryptolestes ferrugineus* and *T. castaneum* by more than 70%.

A five-month, 330-kg-barrel test with wheat was set up to test the effectiveness of the combination of protein-enriched pea flour with parasitoids against *S. oryzae* and *C. ferrugineus*. The parasitoid *A. calandrae* and *C. waterstoni* reduced the mean number of *S. oryzae* by about 70% but the reduction was not significant because of high variability in the controls. Combinations of parasitoids and pea flour at 0.1% significantly reduced the *S. oryzae* population by 99.8%.

For unknown reasons, the populations of *C. ferrugineus* did not become established in the barrel tests. As a result, we could make no conclusions concerning the effectiveness of the combination of parasitoids and protein-enriched pea flour against this major pest.

Key words: Pea protein; biological control; parasitoid; *Sitophilus*; *Tribolium*; *Cryptolestes*

¹ Draft version submitted to editors.

² Department of Entomology, University of Manitoba, Winnipeg, MB, Canada, R3T 2N2

³ Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, MB, Canada, R3T 2M9, corresponding author: Tel: (204) 983-1468; fax: (204) 983-4604, E-mail address: pfields@agr.gc.ca

⁴ Grain Marketing and Production Research Center, USDA-ARS, Manhattan, KS, USA, 66502

1. Introduction

Insect pests cause damage to stored grain by reducing dry weight, nutritional value, and seed viability (Semple et al., 1992). Synthetic insecticides, such as deltamethrin, malathion, chlorpyrifos-methyl, and the fumigants, phosphine, methyl bromide, are the primary treatments used for grain protection (Harein and Davis, 1992; Arthur, 1996). However, increased concerns by consumers over insecticide residues, the occurrence of insecticide-resistant strains and the precautions necessary for the application of these traditional chemical insecticides call for new approaches to control stored-product insect pests (Zettler and Cuperus, 1990; Subramanyam and Hagstrum 1995; Cochran, 1995).

Higher plants are a rich source of novel insecticides (Prakash and Rao, 1997). The insecticidal activity of many plant derivatives against several stored-product pests has been demonstrated (Jacobson, 1989; Golob et al., 1999; Weaver and Subramanyam, 2000). Azadirachtin from the Indian neem tree (*Azadirachta indica* A. Juss., Meliaceae) (Saxena et al. 1988; Jilani and Saxena, 1990), and pyrethrum from chrysanthemums (Prakash and Rao, 1997) have received the most attention. However, because of the structural complexity of azadirachtin, the instability of pyrethrum, the availability and the high cost of both, the search for other natural insecticides is continuing.

Legume seeds contain a wide range of allelochemicals with toxic and deterrent effects against insect pests (Harborne et al., 1971; Bell, 1978). Yellow split-peas (*Pisum sativum* L.) mixed with wheat resulted in a marked reduction of survival and reproduction rate of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Coombs et al. 1977; Holloway, 1986). Protein-enriched pea flour causes adult mortality and reduced reproduction for several stored-product insect pests (Bodnaryk et al., 1997), and is repellent to many stored-product insects (Fields et al., 2001). Delobel et al. (1998) have isolated a polypeptide from peas that is toxic to stored-product insects.

Sitophilus oryzae, *Tribolium castaneum* (Herbst) and *Cryptolestes ferrugineus* (Stephens) are cosmopolitan stored-product insects. *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) is an ectoparasitoid of rice weevil. *Cephalonomia waterstoni* (Gahan) (Hymenoptera: Bethyridae) is an ectoparasitoid of the rusty grain beetle. Both parasitoids effectively suppressed the population of *S. oryzae* and *C. ferrugineus* (Press et al., 1983; Cline et al., 1978; Flinn et al., 1996). Our preliminary laboratory tests have shown that neither of these parasitoids were significantly affected by protein-enriched pea flour (unpublished data). The goals of this study were (1) to determine if the protein-enriched pea flour can suppress pest insect populations in barley in large granaries under farm storage conditions, and (2) to determine if the combination of wasps and protein-enriched pea flour can protect wheat from insect attack.

2. Materials and Methods

Protein-enriched pea flour (60% protein, 30% starch, 7% moisture content) was provided by Parrheim Food, Saskatoon, SK.

2.1. Granary trials

The granary trials were conducted at Genlea, Manitoba (48E38'N, 97E09'W) from 17 August to 2 November 1999 with six 30-t capacity farm granaries that had perforated floors. Each granary was filled with approximately 11 t of barley with moisture content of 12.9%. The protein-enriched pea flour was mixed into the barley at the base of an auger running at a rate of 0.5 t/min. There were three treatments with two replications: (1) untreated controls, (2) all barley treated at 0.1% protein-enriched pea flour and (3) only the barley in the top half of the granary treated with 0.5% protein-enriched pea flour.

2.1.1. Insects

Three species, *S. oryzae*, *T. castaneum* and *C. ferrugineus*, were obtained from laboratory cultures reared at 30 °C, 70% relative humidity (RH). *Sitophilus oryzae* was reared on whole kernels of wheat, *T. castaneum* on wheat flour mixed with 5% brewer's yeast, and *C. ferrugineus* on wheat kernels with 5% wheat germ and 5% brewer's yeast.

Sitophilus oryzae and *T. castaneum* were released on August 23 at the rate of approximately 2 insects per species per kg of barley (22,000 insects per granary) and *C. ferrugineus* at approximately 1.4 insects per kg (15,000 insects per granary). During the release, five boxes (30 x 30 x 30 cm) with a screened floor (2 mm² openings) and an open top were buried at the sampling points. One box was located in the centre of the granary and the other four boxes were placed half way between the center and the granary wall at each of the compass points. Three insect species were equally placed in five 4-liter jars containing 2.5 kg of barley taken from the granaries, and poured into the boxes (30 x 30 x 30 cm) which were then filled with untreated barley to the top edge of the boxes. Insects could move freely between the release boxes and the grain mass through the screened floor and the open top. The boxes were removed 10 d after the release date.

2.1.2. Sampling

Ten 1-kg samples were taken from each granary on 21 October 1999 (59 d after release of insects). Five top samples were taken with a 1-liter cup at the surface where the release boxes were buried. Five middle samples were taken at 1 meter below the surface location with a 0.3-liter torpedo probe sampler. Samples were examined in the laboratory, the adult insects were removed from the barley using a sieve with 2 mm² openings, and the species and number of live and dead insects were noted. To estimate internal insects, after adult insects were removed, grain samples were placed in a 4-liter jar and held at 30 °C, 70% RH for 5 wk, and the grain was sieved a second time and the adults were counted.

2.1.3. Temperature

A data logger (CR10, Campbell Scientific Corp., Edmonton, Alberta, Canada) was used to record temperature. In each granary, 12 thermocouples were placed in the grain mass at three depths (5 cm below the surface; 1 m below the surface; and 5 cm above the bottom) and four locations per depth (20 cm from the northern wall; the center; the middle between the middle and the southern and northern wall). Temperatures were checked every 12 min and recorded as the mean for every hour.

2.2. Protein-enriched pea flour combined with parasitoids in barrel tests

Anisopteromalus calandrae was cultured on 3-week-old *S. oryzae* larvae in wheat kernels with 14% moisture content. *Cephalonomia waterstoni* was reared on 4-week old *C. ferrugineus* larvae, which were cultured in the wheat flour mixed with 5% ground wheat germ and 5% ground Brewer's yeast. All cultures were maintained at 30 °C, 70% RH.

Twelve steel barrels (168 cm high 58 cm diameter), each containing 330 kg wheat with 13.5% moisture content, were set up in a room maintained at 25 °C, 40-70% RH. Four treatments with 3 replicates per treatment were prepared: (1) untreated controls, (2) parasitoids alone, (3) 0.1% pea flour + parasitoids, and (4) 0.04 % pea flour + parasitoids. On 18 June 2001, *S. oryzae*, *T. castaneum* and *C. ferrugineus* were released on the surface of the wheat at a density of 2 insects per kg wheat. After 29 days, a single inoculative release of *C. waterstoni* and *A. calandrae* at the age of 1-7 days was conducted. The release density was 2 parasitoids per species per kg of wheat, or a 1:1 parasitoid: host ratio based on the initial release rate of the pest insects. The barrels was sealed with a fine cloth screen. On 29 October 2001, or about 5 months after the wheat was infested, three grain samples (about 1 kg each) were gently vacuumed from each barrel. Sampling locations were the top layer (20 cm from the surface), the middle layer (68 cm from the top), and the bottom layer (24 cm from the bottom). The number of insects in the samples was determined.

2.3. Data Analysis

Data from the granary and the barrel tests were analyzed by using SAS PROC GLM (SAS Institute Inc., 2000). Number of insects in grain samples were transformed with logarithm of $X + 0.0001$. Treatment means were tested for significance with SAS CONTRAST.

3. Results and Discussion

3.1. Granary trials

The grain temperature decreased constantly at a rate of 1.8 °C per week. The grain temperature was 23.2 °C at the start of this study, and ranged from 6 to 9 °C when samples were taken. Protein-enriched pea flour reduced the population of parent *S. oryzae* and *C. ferrugineus*, and the offspring of *S. oryzae* in the top layer (Table 1). No live parent *S. oryzae* were found in the top layer in either the pea-protein treated granaries or in the middle layer in 0.1% entire treated granaries. Protein-enriched pea flour reduced the population of *T. castaneum* in the middle layer. Overall in the granaries, compared with populations in the untreated controls, the population of *S. oryzae* was reduced by 100% for parents ($F = 28.8$, $df = 1$, $P = 0.0007$) and 81% for offspring ($F = 6.3$, $df = 1$, $P = 0.0367$) in 0.1% protein-enriched pea flour treatments, and 94% for parents ($F = 17.6$, $df = 1$, $P = 0.0030$) and 94% for offspring ($F = 8.1$, $df = 1$, $P = 0.0216$) in 0.5% half pea flour treatments. Parent population of *T. castaneum* was reduced by 74% in 0.1% treatment ($F = 5.0$, $df = 1$, $P = 0.0558$) and 81% in 0.5% treatment ($F = 9.78$, $df = 1$, $P = 0.0141$). Parent population of *C. ferrugineus* was reduced by 89% ($F = 0.63$, $df = 1$, $P = 0.4488$) in 0.1% treatment and 94 in 0.5% treatment ($F = 4.7$, $df = 1$, $P = 0.0623$). Neither pea flour treatment significantly reduced the offspring of *T. castaneum* or *C. ferrugineus* ($P > 0.05$).

Protein-enriched pea flour apparently altered the distribution of insects between layers. The top layer had significantly more offspring of *S. oryzae* ($F = 31.1$, $df = 1$, $P = 0.0307$) and *T. castaneum* ($F = 78$, $df = 1$, $P = 0.0126$), and less offspring of *C. ferrugineus* ($F = 72346$, $df = 1$, $P = 0.0004$) than middle layer in the untreated granaries (Table 1). However, no significant difference in the number of offspring between two layers was found for all three species in both pea flour treated granaries ($P > 0.05$). Protein-enriched pea flour also changed the distribution of parent *C. ferrugineus* between layers.

3.2. Protein-enriched pea flour combined with parasitoids in barrel tests

With *S. oryzae*, only the combination of 0.1% protein-enriched pea flour with parasitoids significantly reduced the population size by 99.8% ($P < 0.05$) (Fig. 1). The high variability of population density in the control barrels made it difficult to detect significant differences between the treatment and control. For example, the 92% reduction in the population size in treatment of 0.04% pea protein flour combined with parasitoids *A. calandra* and *C. waterstoni* was not significantly different from the controls. Similarly, the parasitoids *A. calandra* and *C. waterstoni* reduced the population size by 72% but was not significantly different from the controls. Although, a single inoculative release of *A. calandrae* at a 1: 1 parasitoid: host ratio reduced the *S. oryzae* population by about 70%, a higher ratio of parasitoids to hosts would probably result in a more significant reduction, particularly if only a single release is used.

As was the case with *S. oryzae*, compared to the controls, the *T. castaneum* population reduced 28, 44, and 82% in the treatments of parasitoids only, parasitoids with 0.04% pea flour and parasitoids with 0.1% pea flour respectively (Fig. 1). However, none of these differences was statistically different due to the high variability among barrels. Since neither parasitoid we released attacks *T. castaneum*, the reduction of the population might result from the interaction among species or the effect of protein-enriched pea flour.

In this test, the population of *C. ferrugineus* was too low (Fig. 1) to assess the biological significance of any treatment effects. Even though *C. ferrugineus* will feed and develop well in whole wheat with dockages, there was essentially no population increase in either the control or treatment barrels on 29 October. It is not known whether biotic or abiotic factors or some combination of such factors resulted in the lack of population growth.

Protein-enriched pea flour significantly reduced populations of *T. castaneum* and *C. ferrugineus* in the granary test but not in the barrel test. The reasons could be that in the granary test, insects could leave the grain mass easily through perforated floors or from the top of the grain mass. Protein-enriched pea flour is both toxic and repellent to stored-product insects (Bodnaryk et al., 1997; Fields et al. 2001). However, in the barrel test, insects could not leave the barrels.

One limitation of using pea flour to control stored-product insects in commercial granaries is that *Rhyzopertha dominica* (F.), the lesser grain borer is not controlled at 0.1% pea flour (Bodnaryk et al., 1997). *Anisopteromalus calandrae* parasitizes a wide range of stored-product insects including *R. dominica* (Chatterji, 1955; Ahmed, 1996). Unlike diatomaceous earth which is toxic to *A. calandrae* (Perez-Mendoza et al., 1999), protein-enriched pea flour has no or little effect on *A. calandrae*. A proper combination of parasitoids and protein-enriched pea flour

may be complementary, and increase the efficacy of protein-enriched pea flour to insects other than *Sitophilus spp.*

Acknowledgements

We thank Tannis Mayert, Colin Demianyk, and Andrea Heida for technical assistance, Agri-Food Research and Development Initiative and the University of Manitoba for financial support and Parrheim Foods for providing the protein-enriched pea flour.

References

- Ahmed, K.S., 1996. Studies on the ectoparasitoid, *Anisopteromalus calandrae* How. (Hymenoptera: Pteromalidae) as a biocontrol agent against the lesser grain borer, *Rhyzopertha dominica* (Fab.) in Saudi Arabia. *Journal of Stored Products Research* 32, 137-140.
- Arthur, F.H., 1996. Grain protectants: current status and prospects for the future. *Journal of Stored Products Research* 32, 293-302.
- Bell E.A., 1978. Toxins in seeds. In: Harborne, J.B. (Ed.), *Biochemical Aspects of Plant and Animal Coevolution*. New York, Academic Press Inc., pp. 143-161.
- Bodnaryk, R.P., Fields, P.G., Xie, Y.S., Fulcher, K.A., 1997. Insecticidal factor from peas. USA Patent, 5,955,082.
- Cline L.D., Press, J.W., Flaherty, B.R., 1978. Suppression of rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae), inside and outside of burlap, woven polypropylene, and cotton bags by the parasitic wasp, *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). *Journal of Economic Entomology* 78, 835-838.
- Chatterji S., 1955. Studies on the biology of *Aplastomorpha calandrae* Howard (Insecta: Hymenoptera: Chalcidae) parasitic on some storage pests. *Proceedings of zoological Society of London* 8, 11-23.
- Cochran, D.G., 1995. Insect resistance to pyrethrins and pyrethroids. In: Casida, J.E., Quistad, G.B. (Eds.), *Pyrethrum flowers: production, chemistry, toxicology and uses*. Oxford University Press. New York, pp. 234-248.
- Coombs, C.W., Billings, C.J., Porter, J.E., 1977. The effect of yellow split-peas (*Pisum sativum* L.) and other pulses on the productivity of certain strains of *Sitophilus oryzae* (L.) (Col. Curculionidae) and the ability of other strains to breed thereon. *Journal of Stored Products Research* 13, 53-58.
- Delobel, B., Grenier, A., Gueguen, J., Ferrasson, E., Mbailao, M., 1998. Use of a polypeptide derived from a PA1b legume albumen as insecticide. Patent No. WO 99/58695.
- Fields, P.G., Xie, Y.S. Hou, X., 2001. Repellent effect of pea (*Pisum sativum*) fractions against stored-product insects. *Journal of Stored Products Research* 37, 359-370.
- Golob, P., Moss, C., Dales, M., Fidgen, M. Evans, J., Gudrups, I., 1999. The use of spices and medicinals as bioactive protectants for grains. Food and Agricultural Organization of the United Nations, Rome.
- Flinn, P.W., Hagstrum, D.W., Mcgaughey, W.H., 1996. Suppression of beetles in stored wheat by augmentative releases of parasitic wasps. *Environmental Entomology*. 25, 505-511.

- Harborne J.B., Boulter D., Turner B.L. 1971. Chemotaxonomy of the Leguminosae. Academic Press Inc., London.
- Harein, P.K., Davis, R., 1992. Control of stored-grain insects. In: Sauer, D.B. (Ed.), Storage of Cereal Grains and Their Products, 4th edition. AACCC Inc. USA.
- Holloway, G.J., 1986. The potency and effect of phytotoxins within yellow split-pea (*Pisum sativum*) and adzuki bean (*Vigna angularis*) on survival and reproductive potential of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Bulletin of Entomological Research 76, 287-295.
- Jacobson, M., 1989. Botanical pesticides: past, present, future. In: Arnason, J.T., Philogene, B.J. R., Morand, P. (Eds.), Insecticides of Plant Origin. American Chemical Society, Washington, DC, pp. 1-10.
- Jilani, G., Saxena, R.C., 1990. Repellent and feeding deterrent effects of turmeric oil, sweetflag oil, neem oil, and a neem-based insecticide against lesser grain borer (Coleoptera: Bostrychidae). Journal of Economic Entomology 83, 629-634.
- Perez-Mendoza, J., Baker, J.E., Arthur, F.H., Flinn, P.W., 1999. Effects of Protect-It on efficacy of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) parasitizing rice weevils (Coleoptera: Curculionidae) in wheat. Environmental Entomology 28, 529-534.
- Prakash. A., Rao, J., 1997. Botanical Pesticides in Agriculture. CRC Press Inc., Baton Rouge, Florida.
- Press, J.W., Cline, L.D., Flaherty, B.R., 1983. Suppression of residual populations of the rice weevil, *Sitophilus oryzae*, by the parasitic wasp, *Anisopteromalus calandrae*. Journal of Georgia Entomological Society 19, 102-110.
- Saxena, R.C., Jilani, G., Abdul-Kareem, A., 1988. Effects of neem on stored grain insects. In: Jacobson, M. (Ed.), Focus on Phytochemical Pesticides, Vol. 1, The Neem Tree. Boca Raton, Florida: CRC Press Inc., pp. 97-111.
- SAS Institute Inc., 2000. SAS On-line Doc[®], Version 8. SAS Institute Inc., Carey, NC.
- Semple, R.L., Hicks, P.A., Lozare, J.V., Castermans, A., 1992. Towards integrated commodity and pest management in grain storage: A Training Manual for application in humid tropical storage systems. Food and Agricultural Organization of the United Nations, Regent.
- Subramanyam, Bh., Hagstrum, D. W., 1995. Resistance measurement and management. In: Subramanyam, Bh., Hagstrum, D. W. (Eds.), Integrated Management of Insects in Stored Products. Marcel Decker, Now York, pp. 331-397.
- Zettler, J.L., Cuperus, G.W., 1990. Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrychidae) in wheat. Journal of Economic Entomology 83, 1677-1681.
- Weaver, D., Subramanyam, Bh., 2000. Botanicals. In: Subramanyam, Bh, Hagstrum, D. (Eds.), Alternatives to Pesticides in Stored-Product IPM. Dordrecht, Kluwer Academics Publishers, pp. 303-320.

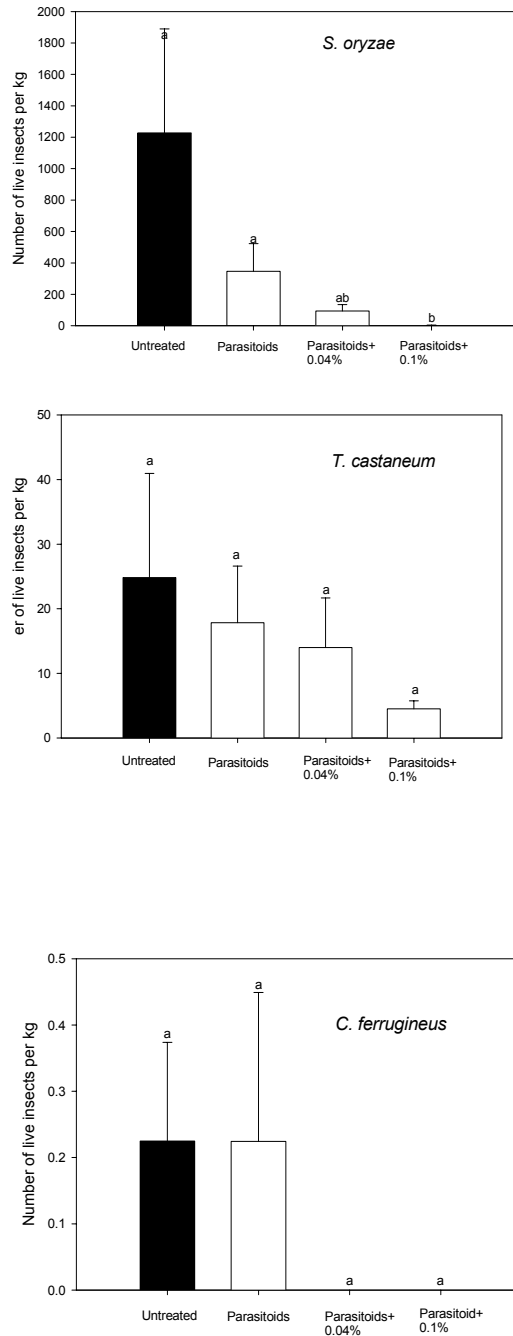


Figure 1. Number of live adults in wheat taken on 29 October 2001 from barrels each containing 330 kg wheat with following treatments: (1) untreated, (2) parasitoids (*A. calandreae* and *C. waterstoni* at 2 insects per species per kg), (3) 0.04% protein-enriched pea flour and parasitoids and (4) 0.1% protein-enriched pea flour and parasitoids (n = 3). A: *S. oryzae*; B: *T. castaneum*; C: *C. ferrugineus*.

Table 1. The number (mean \pm SE) of live *Sitophilus oryzae*, *Tribolium castaneum* and *Cryptolestes ferrugineus* found in the barley samples taken from different layers of granaries treated with pea-protein flour at 0.1% all grain treated, 0.5% top half treated, or all untreated as control at different times. The number of offspring was recorded when barley samples were cultured five weeks at 30 °C, 70% RH, after parent adults were removed (n=2).

Insect	Generation	Layer	Number of Live Insects per 1 kg sample (\pm SEM) ¹			ANOVA	
			Control	0.1% All Treated	0.5% Top Half Treated	F	P
<i>S. oryzae</i>	Parent	Top	0.7 \pm 0.1 a	0 \pm 0 b	0 \pm 0 b	6732	<0.0001
		Middle	0.9 \pm 0.8 a	0 \pm 0 a	0.1 \pm 0.1 a	3.49	0.1646
	Offspring	Top	72 \pm 10 a *	15 \pm 15 b	2 \pm 2 b	7.25	0.007
		Middle	8 \pm 5 a	0.1 \pm 0.1 b	3 \pm 2 c	22.15	0.0263
<i>T. castaneum</i>	Parent	Top	0.6 \pm 0.3 a	0.3 \pm 0.1 a *	0.2 \pm 0.2 a	0.9	0.4946
		Middle	0.5 \pm 0.2 a	0 \pm 0 b	0 \pm 0 b	366.57	0
	Offspring	Top	0.5 \pm 0.4 a *	0.2 \pm 0.1 a	0.2 \pm 0.2 a	0.58	0.6108
		Middle	0 \pm 0	0 \pm 0	0 \pm 0	NA	NA
<i>C. ferrugineus</i>	Parent	Top	17 \pm 3 a *	2 \pm 1 b	0.7 \pm 0.3 b	14.65	0.0283
		Middle	0.2 \pm 0.02 a	0.2 \pm 0.01 a	0.2 \pm 0.2 a	0.72	0.5565
	Offspring	Top	0 \pm 0 a *	0.1 \pm 0.1 a	0.1 \pm 0.1 a	0.5	0.6495
		Middle	1.8 \pm 0.3 a	0.3 \pm 0.3 a	0.4 \pm 0.4 a	0.74	0.5472

¹ Different letters in the same row indicates significant difference between treatments (P<0.05, SAS Contrast).

* There were differences between top and middle layer within the treatment (P<0.05, SAS Contrast).