Infestation of *Rhyzopertha dominica* (F.) first instars on sound and artificially-damaged hard red winter wheat kernels

Andrada, M., Subramanyam, Bh.*#
Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506, USA

*Corresponding author, Email: sbhadrir@k-state.edu
#Presenting author, Email: sbhadrir@k-state.edu

DOI: 10.14455/DOA.res.2014.23

Abstract

The lesser grain borer, *Rhyzopertha dominica* (F.), is a devastating pest of stored wheat worldwide. Mated females lay eggs loosely outside of wheat kernels, and larvae hatching from eggs enter wheat kernels to complete immature development. Laboratory experiments were conducted using organic hard red winter wheat to understand wheat kernel infestation by first instars of *R. dominica*. Fifty individual sound kernels and 50 kernels artificially-damaged with a microdrill at the germ, endosperm, and brush end of the kernel were used. They were infested with one first instar per kernel in glass vials and stored in a growth chamber at 28ºC and 65% r.h. Successful kernel infestation was verified by dissecting kernels 21 days after infestation. About 82-90% of artificially damaged kernels were infested by larvae in contrast with 12% for sound kernels. The germ (90%) was the preferred site of entry for first instar, followed by brush end (88%), and the endosperm (82%). The development of first instars entering through artificially-damaged brush end, endosperm, and germ of hard red winter wheat kernels was monitored by measuring head capsule width every 3 days for 30 days. Nonlinear models fit to head capsule widths over time for larvae developing in the germ, endosperm, and brush end were significantly different from one another. Larval development was fastest on the germ, followed by endosperm, and brush end.

Keywords: wheat, lesser grain borer, first instars, infestation rate, development

1. Introduction

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is a devastating pest of wheat stored on farms and at elevators in Kansas (Potter, 1935; Reed et al., 2003; Hagstrum et al., 2013) and the world (Sinha and Watters, 1985). It has been reported to infest 115 different commodities (Hagstrum et al., 2013). The eggs are laid by females either singly, or in groups with a number of eggs adhering together forming a raft (Schwardt, 1933; Potter, 1935; Elek, 1994) in the grain mass. The first instars after hatching from eggs are 0.78 mm long, with a head capsule width of 0.13 mm, and campodeiform in shape (Potter, 1935). The first instars are active and can be identified by a terminal median spine (Howe, 1950) at the dorsal surface of the last abdominal segment (Potter, 1935). First instars enter kernels and continue immature development within kernels (Potter, 1935; Stemley, 1962; Reed, 2006).

Studies on the wheat kernel infestation by *R. dominica* first instars are limited. Some authors have reported that *R. dominica* first instars can successfully infest an undamaged wheat kernel (Crombie, 1945; Stemley, 1962) or sound durum kernels (Limonta et. al., 2011). It can establish and attack sound maize grains according to Potter (1935). Osuji (1982) found that 12% of first instars successfully entered undamaged maize kernels. Thomson (1966) stated that moisture content of 8% or higher is critical for first instars to bore into whole and sound milo kernels. The same author reported that germ is the first point of entry on sound milo kernels. Stemley (1962) reproted that after hatching *R. dominica* first instars preferred boring

into the germ of wheat kernels. In contrast, some researchers claimed that first instars are unable to penetrate undamaged wheat kernels (Birch, 1944a,b; Howe, 1950) or had little success feeding on hard portion of the kernel (Stemley, 1962). First instars begin searching for food after hatching and nibble on accumulated flour from adult feeding (Stemley, 1962; Thomson, 1966; Golebiowska, 1968) before chewing into broken pieces of wheat (Pajni and Shobha, 1979) or bore directly into grains that have been slightly damaged (USDA, 1986). Semple (1992) stated that first instars are incapable of penetrating or feeding on sound intact rice paddy kernels. Likewise, Chanbang et al. (2008) reported that when the rough rice hull is removed, the rice is vulnerable to attack by first instars at 32^{o}C and 75% r.h. In corn grains, 92% of *R. dominica* first instars are able to enter into mechanically damaged endosperm (Osuji, 1982). Schwardt (1933) reported that first instars can enter by abrasions made by adult feeding. Osuji (1982) pre-drilled holes on maize germ and crown end or endosperm areas and infested kernels with first instars of *R. dominica*. He observed larvae developing in the germ developed to mature larvae and prepupae within 17-19 days as opposed to 40 days for those developing in the endosperm. He further reported that 100% larval entry is achieved by larvae when it entered a transversely-cut maize (germ portion) versus 48% establishment at the other half (crown end portion) of corn. Similarly, longitudinally-split maize revealed a 94% establishment and fed at the germ portions versus 82% at the endosperm areas. The two objectives of our research were to determine the effect of sound and artificially-damaged hard red winter wheat (HRWW) kernels on probability of infestation by *R. dominica* first instars, and its preferred site of entry and the speed of larval development on different sites of the wheat kernels in artificially-damaged kernels.

2. Materials and Methods

2.1. Probability of infestation by first instars on sound and artificially-damaged HRWW kernels

Organic HRWW samples were sourced from Heartland Mills, Marienthal, KS, USA. The wheat was stored in the laboratory freezer (-13°C) for couple of weeks to kill any live insects present. Approximately 600 g were placed in glass jars and held in the growth chamber (Model I-36VL; Percival Scientific, Inc., Boone, IA, USA) at 28°C and 65% r.h. for a week to equilibrate the moisture content. The moisture content of the grain samples mean ± SE) was 11.3 ± 0.54%, which was determined using the Single Kernel Characterization System (SKCS) unit (SKCS 4100 Model, Perten Instruments, Hagersten, Sweden). A stereoscopic microscope (Nikon SMZ 100 Model, Nikon Instruments, Inc., Melville, NY, USA) was used to thoroughly screen and inspect each sound and naturally-damaged kernels. A sound kernel for this experiment was defined as one without any abrasion or damage on the dorsal, lateral, and ventral surfaces of the endosperm and germ portions. The screened sound kernels were placed in separate small glass jars (75 ml) and set aside for use in tests.

2.2. Micro-drilling of individual sound HRWW kernels

A total of 200 individual HRWW sound kernels were retrieved from the jar. One-hundred and fifty kernels were selected and 50 each were artificially-drilled at the brush end, endosperm, or the germ portions using a 0.24 mm diameter micro-drill (TITEX Micro Drill Cobalt, MSC Industrial Supply Co., Melville, NY, USA). The hole at the brush end was micro-drilled close to the dorsal end opposite to the germ portion. The endosperm hole was micro-drilled in the kernel center on the dorsal side. The center of the germ was micro-drilled. All holes were micro-drilled to a depth of approximately 1 mm. An additional 50 sound kernels were used as the control treatment. Each artificially-drilled kernel or a sound kernel was individually placed inside labelled glass vials (4 ml). Each glass vial was inserted into a 24 cell well plates

(Corning Glass Works, Corning, NY, USA) to prevent them from tipping. These plates were placed on plastic trays and held at 28ºC and 65% r.h. prior to larval infestation.

2.3. Collection of R. dominica eggs

Bleached flour was initially sifted using a sieve that had 250-µm openings (Seedburo Equipment Co., Chicago, IL, USA) and placed in a clean glass jar (0.95 L). Approximately 20 g of sifted bleached flour was weighed and placed in each of the ten 150-ml round plastic containers with perforated lids covered with a wiremesh screens to prevent insect escape. Unsexed adults of mixed ages *R. dominica* were collected from the laboratory culture jars (insects reared on organic HRRW). Adults were sieved using a 2.12 mm round perforated aluminum sieve with a bottom pan (Seedburo Equipment Co., Chicago, IL, USA) to separate damaged grains, adults, and fine materials, respectively. Sieved adults and fine materials from the bottom pan were further screened over two sets of sieves. The top sieve had 710-µm openings and the bottom sieve had 600-µm openings to separate adults from broken fines and grain dust/debris. Screened *R. dominica* adults were counted and 100 adults were introduced into each plastic container and held in a growth chamber at 28°C and 65% r.h. After 3 days, the bleached flour was sifted using a sieve with 710-µm openings placed on top of a sieve of 250-µm openings with bottom pan. The top sieve retained adults of *R. dominica*. Eggs were retained on the bottom sieve and all of the flour passed through the bottom sieve and collected in the bottom pan. Eggs collcted from each container were placed in 9-cm diameter glass Petri dishes and held at 28°C and 65% r.h. and observed for egg hatchability. Egg hatchability was observed after 5 days and larvae that hatched from eggs were used in tests.

2.4. Larval infestation of HRWW kernels

Each vial with sound or artificially-damaged kernels at the germ, endosperm, or brush end was infested with a first instar of *R. dominica* that hatched within less than 24 hours. Individual larvae were carefully transferred with a camel's hair brush, and placed directly on sound and artificially-damaged kernels. Each vial was sealed with pre-cut 2.5 cm^2 sized parafilm wax (American National Can™, Chicago, IL, USA) and 12 holes with a pin were made for air ventilation. The infested kernels in glass vials were held at 28°C and 65% r.h.

On the 21st day after infestation each individual kernel in vials was inspected under a steromicroscope at the dorsal, lateral, and ventral portions to determine site of entry of larvae into the kernel. After this initial examination, larva from an infested kernel was excised by careful dissection using a scalpel and forceps. Larva was carefully extricated with a slight twisting motion of the cut kernel and it was gently placed using a camel's brush onto clay dough for head capsule width measurement. The head capsule width of each larva was measured using an ocular micrometer that was calibrated with a stage micrometer. Classification of *R. dominica* instar at 21 days was based on the range of head capsule width measurements given in Stemley (1962). Stemley's (1962) individual head capsule data for each of the four instars of *R. dominica* were reanalyzed to calculate mean ± SE width and range. Differences in mean head capsule width among instars (Table 1) was determined by subjected data to one-way analysis of variance (ANOVA) followed by REGWQ multiple comparison test at $\alpha = 0.05$ (SAS Institute, 2008).

The probability of infestation by first instars on sound and artificially-damaged kernels was analyzed by using PROC GLIMMIX (SAS Institute, 2008), and differences among infestation rates were determined by least square means with Bonferroni adjustment at $\alpha = 0.05$ as data were not normally distributed and could not be normalized by transformations.

Table 1 Head capsule width measurements of *R. dominica* instars.

Instar	Number of larvae	Mean ± SE (range) of head capsule width (mm)[1]	Development time in days at 28°C and 70%[2]
1	116	0.139 ± 0.003 (0.108 - 0.18)d	8.8 ± 0.2
2	126	0.202 ± 0.001 (0.192 - 0.217)c	5.7 ± 0.2
3	103	0.316 ± 0.003 (0.254 - 0.375)b	5.4 ± 0.1
4	155	0.465 ± 0.003 (0.400 - 0.525)a	7.8 ± 0.2

[1]Means followed by different letters are significantly different from one another ($P < 0.05$, REGWQ test).
[2]*Source*: Howe (1950).

2.5. Speed of larval development by site of entry of first instars in artificially-damaged kernels

The procedures explained above were followed for selecting sound organic HRWW kernels, micro-drilling of kernels at the three kernel sites (germ, endosperm, and bush end), and collecting eggs and first instars of *R. dominica*. One artificially-damaged kernel was placed per glass vial as explained above and infested with a first instar of *R. dominica*. There were several vials with infested kernels that were damaged at the germ, endosperm, and brush end. Every 3 days for 30 days (9 sampling occasions), 10 vials of each treatment category were inspected and kernels dissected to extricate larvae and their head capsule widths were measured. No sampling was done past the 30 days because larvae were turning into pupae. Data on the head capsule widths of larvae developing on germ, endosperm, and brush end as a function of time were fit to the same non-linear regression, $y = a + bx^2$. Differences in the speed of development of larvae by site of kernel entry were determined by pair-wise comparions of regression models using the model comparison procedure ($\alpha = 0.05$) of Draper and Smith (1981).

3. Results and Discussion

3.1. Probability of infestation by first instars on sound and artificially-damaged HRWW kernels

Only 12% of sound kernels were infested as opposed to 82-90% for artificially-damaged kernels (Table 2). There were significant differences among the four treatments in infestation rates ($F = 20.27$; df = 3, 196; $P < .0001$) (Table 3). However, infestation rates in artificially-damaged kernels at the germ, endosperm, and brush end were not significantly different from one another ($P > 0.05$), but infestation rates of each of the three treatments were significantly different ($P < 0.05$) from infestation rate observed in sound kernels (Table 3).

Table 2 Probability of infestation on sound and artificially-damaged (AD) kernels by *R.dominica* first instars at 21 days.

Treatment	Infested kernels	Uninfested kernels	% Infestation*
Sound kernels	6	44	12
AD-brush end	44	6	88
AD-endosperm	41	9	82
AD-germ	45	5	90

$n = 50$ kernels for each treatment.
*Significant ($P < 0.0001$; PROC GLIMMIX, fixed effects; Type III SS).

Table 3 Results of least squares means test with Bonferroni adjustment comparing pair-wise infestation rates shown in Table 2.

Treatments compared	*t*-value (df = 196)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	0.84	1.0000
AD-brush end vs. AD-germ	-0.32	1.0000
AD-endosperm vs. AD-germ	-1.14	1.0000
AD-endosperm vs. Sound	6.16	< 0.0001*
AD-brush end vs. Sound	6.47	< 0.0001*
AD-germ vs. Sound	6.53	< 0.0001*

AD = Artificially Damaged.
*Significant ($P < 0.05$).

The lack of defects on the surface of sound kernels may have deterred larvae from successfully entering the kernels. Only 6 kernels out of 50 were infested. Of these, 3 kernels had larvae entering through the germ and 3 through the endosperm (Table 4). In artificially-damaged kernels, larvae entered and established at the damaged site. In the germ damaged kernels, all 45 larvae entered only through the germ. In endosperm damaged kernels, except for one larva that entered through the germ, all larvae (40) entered through the damaged germ. Similarly, in brush end damaged kernels 43 larvae entered and established at this site compared to one larva that entered through the endosperm. These results suggested that kernel damage enhances successful larval establishment. Several authors have reported damage to kernel surface to facilitate successful larval entry and establishment of *R. dominica* (Schwardt, 1933; Birch, 1944a,b; Pedersen, 1992; Semple, 1992). The low infestation found in sound kernels could be due to first instar mortality, because these tiny larvae require food to survive for shorter time periods before entering kernels to complete development (Golebiowska, 1968). Starving larvae may be apparently weakened (Breese, 1960) to gainfully enter a sound kernel.

3.2. Site of entry by first instars in sound and artificially-damaged HRWW kernels

A greater percentage of kernels damaged at the germ were infested (90%) followed by brush end damaged kernels (86%), and endosperm damaged kernels (80%), although differences among these infestation rates were not significant. However, when the head capsule width data were examined, generally larvae at 21 days developing in the germ were 4th instars as opposed to 3rd instars or occasionally 4th instars developing in brush end or endosperm (Table 4).

There are several reasons that attract first instar to enter through the germ portion of the grain. The aleurone cells at the germ area are thinner than those covering the endosperm (Hoseney and Faubion, 1992; Hoseney, 1998). The germ is softer portion of the wheat kernel, and therefore the easiest point of entry for the first instars (Potter, 1935). Preference of *R. dominica* first instars to bore through the germ portion of sorghum (milo) kernels was reported by Thomson (1966). Birch (1944a) also observed 80% of the 400 first instars entered at the germ end of wheat of 10% moisture (wet basis) in tests at 30 and 34°C. The fact that larvae established in damaged brush end and endosperm suggests that first instars may enter kernels through any suitable opening.

Table 4 Influence of site of entry on head capsule widths of *R. dominica* first instars at 21 days postinfestation.

Treatment	Site of larval entry	Mean ± SE head capsule width (mm)	Number of kernels infested	Instar classification
Sound kernels	Brush end	No infestation	0	---
	Endosperm	0.380 ± 0.010	3	3rd
	Germ	0.420 ± 0.003	3	4th
AD-brush end	Brush end	0.390 ± 0.006	43	3rd
	Endosperm	0.45	1	4th
	Germ	No infestation	0	---
AD-endosperm	Brush end	No infestation	0	---
	Endosperm	0.390 ± 0.009	40	3rd
	Germ	0.44	1	4th
AD-germ	Brush end	No infestation	0	---
	Endosperm	No infestation	0	---
	Germ	0.430 ± 0.004	45	4th

AD = Artificially Damaged.

3.3 Speed of development of larvae at different artificially-damaged kernel sites

Larvae developing in the germ area had larger head capsule widths, followed by those developing in the endosperm. The smaller head capsule widths were associated with larvae developing in the brush end (Fig. 1). Pair-wise model comparison procedures indicated that the model describing head capsule widths for larvae developing in the three kernel sites were significantly different from one another: brush end vs endosperm (F = 6.83; df = 2, 17; P = 0.006), brush end vs germ (F = 15.68; df = 2, 15; P = 0.0002), and endosperm vs germ (F = 12.43; df = 2, 16; P = 0.0005).

Our results show that first instars of *R. dominica* that established in artificially-damaged germ of wheat kernels developed the fastest when compared to those developing in the endosperm and the brush end. It is well know from previous studies that first instars generally enter and establish in the germ of wheat kernels (Potter, 1935; Birch, 1944a, 1944b; Stemley, 1962). The reasons for faster development of larvae developing in the germ may be related to availability of nutrients (Hoseney and Faubion, 1992; Serna-Saldivar, 2010). Further research that we plan to conduct will elucidate reasons for observations reported in this paper.

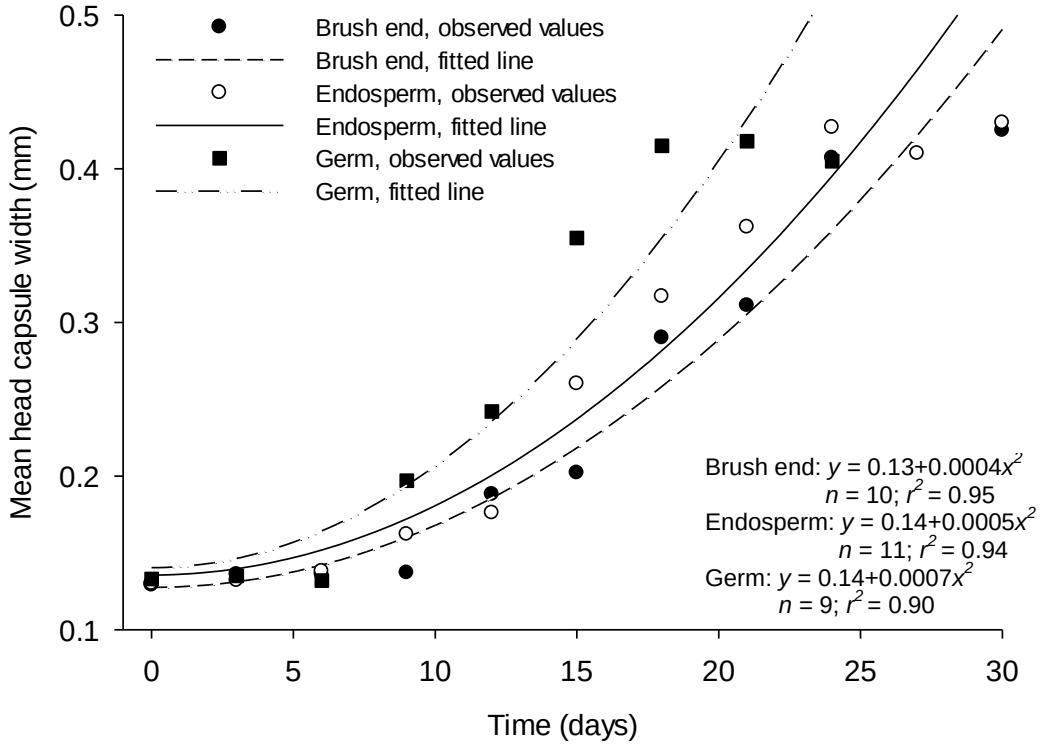

Figure 1 Observed and fitted lines showing relationship between head capsule widths of *R. dominica* larvae by site of entry as first instars.

4. Conclusions

Our results show that germ is the preferred site of entry for first instars of *R. dominica,* and reasons for such behavior still need to be determined. It is clear from the data reported here that larvae established in the germ tend to develop faster compared to larvae developing in non-germ portions of kernels. In the future it is important to relate development of larvae within wheat kernels to nutrients available in different anatomical portions of the kernels. One outcome of this study shows that preventing any damage to kernels during harvesting, handling, and storage will reduce *R. dominica* first instar infestation. Understanding factors that contribute to first instar establishment in wheat kernels will have impacts in breeding varieties that could resistant *R. dominica* infestation.

Acknowledgement

The senior author of this paper acknowledges the Philippine National Food Authority officials for granting study leave to pursue a Ph.D. degree at Kansas State University. Research reported here was funded by a partial grant from the Kansas State University Agricultural Experiment Station.

References

Birch, L.C., 1944a. The mortality of the immature stages of *Calandra oryzae* (L.) (small strain) and *Rhizopertha dominica* (Fab.) in wheat of different moisture contents. Australian Journal of Experimental Biology and Medical Science 23, 141-145.

Birch, L.C., 1944b. The influence of temperature on the development of the different stages of *Calandra oryza*e (L.) and *Rhizopertha dominica* (Fab.) (Coleoptera). Australian Journal of Experimental Biology and Medical Science 23, 29-35.

Breese, M.H., 1960. The infestibility of stored paddy by *Sitophilus sasakii* (Tak.) and *Rhyzopertha dominica* (F.). Bulletin of Entomological Research 51, 599-630.

Chanbang, Y., Arthur, F.H., Wilde, G.E., Throne, J.E., Subramanyam, Bh., 2008. Methodology for assessing rice varieties for resistance to the lesser grain borer, *Rhyzopertha dominica* (F.). Journal of Insect Science 8, 1-5.

Crombie, A.C., 1945. On competition between different species of graminivorous insects. In*:* Proceedings of the Royal Society Biological Sciences 132, 362-395.

Draper, N.R., Smith, H., 1981. Applied Regression Analysis. Second edition. Wiley and Sons, New York, NY, USA.

Elek, J.A., 1994. Methods for collecting eggs and monitoring egg-hatch and immature development of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Journal of Stored Products Research 30, 261-265.

Golebiowska, Z., 1969. The feeding and fecundity of *Sitophilus granarius* (L.), *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.) in wheat grain. Journal of Stored Products Research 5, 143-155.

Hagstrum, D.W., Klejdysz, T., Subramanyam, Bh., Nawrot, J., 2013. Atlas of Stored-Product Insects and Mites. American Association of Cereal Chemists, International, Inc., St. Paul, Minnesota.

Hoseney, R.C., 1998. Principles of Cereal Science and Technology. Second edition. American Association of Cereal Chemists, Inc., St. Paul, MN, USA.

Hoseney, R.C., Faubion, J.M., 1992. Physical properties of cereal grains. In Sauer, D.B. (Ed.), Storage of Cereal Grains and their Products. Fourth edition. American Association of Cereal Chemists, Inc., St. Paul, Minnesota, pp.1-38.

Howe, R., 1950. The development of *Rhizopertha dominica* (F.) (Col., Bostrichidae) under constant conditions. The Entomologist's Monthly Magazine 11, 1-5.

Limonta, L., Morosini, M.C., Locatelli, D.P., 2011. Development of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) on durum wheat kernels and semolina. Journal of Entomological and Acarological Research 43, 33-38.

Osuji, F.N.C., 1982. Development of the lesser grain borer, *Rhyzopertha dominica*, in maize kernels as affected by site of larval entry. Entomologia Experimentalis et Applicata 31, 391-394.

Pajni, H.R., Shobha, A., 1979. Some observations on the biology of *Rhyzopertha dom*inica Fabr. (Coleoptera: Bostrichidae). Bulletin of Grain Technology 17, 209-212.

Pedersen, J.R., 1992. Insects: Identification, damage, and detection. In Sauer, D.B. (Ed.), Storage of Cereal Grains and their Products. Fourth edition. American Association of Cereal Chemists, Inc., St. Paul, Minnesota, pp. 435-489.

Potter, C., 1935. The biology and distribution of *Rhizopertha dominica* (Fab.). The Transactions of the Royal Entomological Society of London 83, 449-482.

Reed, C.R., 2006. Managing Stored Grain to Preserve Quality and Value. AACC International, St. Paul, MN, USA.

Reed, C.R., Hagstrum, D.W., Flinn, P.W., Allen, R.F., 2003. Wheat in bins and discharge spouts, and grain residues on floors of empty bins in concrete grain elevators as habitats for stored-grain beetles and their natural enemies. Journal of Economic Entomology 96, 996-1004.

SAS Institute, 2008. SAS/STAT User's Guide, version 9.2. SAS Institute, Cary, NC, USA.

Schwardt, H.H., 1933. Life history of the lesser grain borer. Journal of the Kansas Entomological Society 6, 61-66.

Semple, R.L., 1992. Host plant and varietal resistance to post harvest insect attack. In Towards integrated commodity and pest management in grain storage. A training manual for application in humid tropical storage systems. Section 11–Biological control methods. Proceedings and Selected Papers from the Regional Training Course on Integrated Pest Management Strategies in Grain Storage Systems. The National Post Harvest Institute for Research and Extension (NAPHIRE), Department of Agriculture, June 6-18, 1988, Philippines, pp. 1-21.

Serna-Saldivar, S.O., 2010. Cereal Grains: Properties, Processing, and Nutritional Attributes. CRC Press Inc., Boca Raton, FL, USA.

Sinha, R.N. and Watters, F.L., 1985. Insect Pests of Flour mills, Grain Elevators, and Feed mills and their Control. Agriculture Canada Publication 1776E. Canadian Government Publishing Centre, Ottawa, Canada.

Stemley, P.G., 1962. The life history and behavior of an internal feeding stored grain insect, *Rhizopertha dominica* (Fab.), by use of X-ray. Ph.D. dissertation. Kansas State University, Manhattan, KS. pp. 1-88.

Thomson, V., 1966. The biology of the lesser grain borer *Rhizopertha dominica* (Fab.). Bulletin of Grain Technology 4, 163-168.

USDA, 1986. Stored-grain Insects. USDA-ARS Agriculture Handbook No. 500, U.S. Government Printing Office, Washington, D. C., USA.