Infestation of *Rhyzopertha dominica* first instars on different classes of wheat

By

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B.S., De La Salle-Araneta University, 1983
M.S., Kansas State University, 2004

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Abstract

The lesser grain borer, *Rhyzopertha dominica* (F.), females lay eggs loosely outside of wheat kernels. Larvae hatching from eggs enter wheat kernels to complete immature development. Four laboratory experiments were conducted to understand the wheat kernel infestation by first instars of *R. dominica* at 28°C and 65% r.h. The first experiment compared different kernel to first instar ratios on sound hard red winter (HRW) wheat class, probability of successful infestation, and subsequent adult development as affected by site of feeding on the kernels. Infested kernels were dissected 21 d after infestation to determine stage of development and larval weight. Development of larvae to adulthood was monitored for 50 d from time of infestation. Different kernel to first instar ratios did not affect probability of infestation, entry site preferences, larval development and weight, and days to adult emergence. In the second experiment one first instar was placed with a kernel on each of seven different wheat classes. Wheat kernels were artificially-damaged with a microdrill at the germ, endosperm, and brush end, and the sound kernels served as the controls. At 21 d, 82-90% of artificially-damaged HRW wheat kernels were infested by larvae versus 12% for sound kernels. Five times fewer hard white (HW) wheat sound kernels were infested by larvae compared with infestation in soft white (SW) wheat kernels. Sound kernels of durum, soft red winter (SRW), hard red spring (HRS), and hard white spring (HWS) wheat classes were more resistant to larval infestation than artificially-damaged kernels. Majority of first instars preferred germ as the entry site on HRW, HWW, SRW, and HWS wheat classes. Germ entry promoted faster larval development, leading to heavier larvae, and higher kernel weight losses. Adult emergence was earlier by 3-7 d compared with other sites across all 6 wheat classes, except for SWW class, where adult emergence was nil at 50 d. In the third experiment, speed of larval development on artificially-drilled HRW wheat kernels on different kernel sites. Three-hundred kernels each were drilled near the germ, endosperm, and brush end, and one first instar was placed per kernel in a glass vial. Kernels from glass vials were dissected every 3 d for 30 d and larval head capsule was measured. Larval development was fastest on the germ, followed by endosperm, and brush end. In the fourth experiment short-term feeding by *R. dominica* adults on infestation by first instars on sound wheat kernels was determined. Two laboratory tests were conducted with single and grouped (10) kernels using either two adults or two first instars per kernel. Signs of feeding were monitored for 21 d in single kernels and 7 d in grouped kernels. There was a significant association between adult feeding and larval infestation responses in single and group kernel tests. In conclusion, understanding factors that contribute to first instar establishment in wheat kernels will have impacts in breeding varieties that could be resistant and designing grain-handling equipment to minimize grain damage to mitigate *R. dominica* infestation of wheat.

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Dedication

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Chapter 1

Background

1.1. The Pest

Rhyzopertha dominica (F.), also known as lesser grain borer (LGB), is a primary insect pest of stored grain worldwide (Edde, 2012). It belongs to the order Coleoptera in the family Bostrichidae (Potter, 1935; Surtees, 1963), where the members are also known as wood borers (Haines, 1991; Reed, 2006). Both the adults and larvae are serious pests of wheat (USDA, 1986; Haines, 1991; Bashir, 2002), although *R. dominica* has been reported to infest a wide range of 115 commodities (Hagstrum et al., 2013). It is considered a primary insect pest, because its manner of infestation is to bore directly into grains. The eggs are laid by females either singly or in cluster in the grain mass. After hatching, first instars, which are elongate in form (campoediform larvae) actively move about the grains. They are white in color, slightly yellowish towards the head, with very short antennae. The mouth parts are brownish, and the head is surrounded with long hairs. The larval legs are long with claws and the dorsal and ventral abdominal segments are fringed with long hairs (Potter, 1935). The four instars of *R. dominica* have been determined and reported by Stemley (1962) by measuring the head capsule widths. First instars enter kernels and continue immature development (Osuji, 1982; Stemley, 1962). Egg-to-adult development time of *R. dominica* is influenced by temperature and it varies from 58.8 d at 25ºC to 31 d at 35ºC (Hagstrum et at., 1996). The adults cause severe damage to the grain (USDA, 1986; Golebiowska, 1969). A round hole is created by adults emerging from the kernels. Kernels with adult exit holes are called insect-damaged kernel (IDK), and according the United States Food and Drug Administration the defect action level for IDK is 32 kernels per

100 g of wheat (Reed, 2006). Kernels with internally developing life stages of *R. dominica* contribute to fragment counts in wheat flour, and flour exceeding 75 insect fragments per 50 g of flour is unfit for consumotion (Carson and Edwards, 2009).

Adults are 2-3 mm long (Haines, 1991), and slender in form (USDA, 1986). Its color is polished dark brown or black, and surface of the elytra has roughened ridges and regular rows of punctures (USDA, 1986; Haines, 1991). The head of adults is obscured under the thorax, and each antenna has 10 segments with a loose 3-segmented club (Haines, 1991) at the anterior end. The adults are slow-moving insects in the grain mass compared with other grain infesting insects (Reed, 2006). The mandibles of the adults are very powerful allowing them to bore into wood (USDA, 1986).

1.2. Wheat Classes

In the United States, wheat (*Triticum aestivum* L.) is divided into eight classes based on texture, color, and season grown (http://www.gipsa.usda.gov). These classes are hard white (HW) wheat, soft white (SW) wheat, soft red winter (SRW) wheat, hard red winter (HRW) wheat, hard red spring (HRS) wheat, hard white spring (HWS) wheat, durum wheat, mixed wheat, and unclassed wheat. The wheat class nomenclature involves the following: the first character refers to endosperm texture (hard or soft), the second character relates to color pigmet of the seed coat (red or white), and the last character pertains to what season it is grown (winter or spring).

The three main anatomical structure of a mature seed of wheat consists of the germ or embryo, the bran, and the endosperm (Posner and Hibbs, 2005). Distal to the germ, there is a cluster of hairs called the “brush end”. This kernel hairs are extensions of the pericarp, and differ in size based on wheat varieties. These hairs serve as traps for some unwanted materials and during the milling process may end up in the finished flour (Posner and Hibbs, 2005). A major portion of a mature kernel of wheat has 81-84% starchy endosperm while 1.6% (durum) to 2.7% (bread wheat) for germ portion (Hoseney and Faubion, 1992). The starchy endosperm serves as the main source of finished flour in the wheat milling process. As a result of this valued flour end-product, wheat is among the oldest and most extensively grown of all grain crops worldwide (Wrigley, 2009). On average, covering the period between 2011 and 2015, wheat in the United States is harvested from 46.7 million acres with an estimated value of $ 13.7 billion (http://www.nass.usda.gov).

In the research reported here, there were three sources of wheat classes tested. The HRW wheat were organically-grown and obtained from Heartland Mills, Marienthal, Kansas, the HW wheat class came from Oklahoma with varietal code of OK08707W, and SRW wheat class was from Labette, Kansas. Durum, SW, HRS, and HWS wheats were obtained from the Department of Grain Science and Industry's Milling Laboratory, Manhattan, KS, USA. The same wheat classes were submitted for proximate analysis determination at the Experiemnt Station Chemical Laboratories of the University of Missouri in Columbia. In summary, the proximate analysis profile showed the following results: crude protein ranged from 10.69 to 16.69%; crude fat from 0.71 to 2.24%; crude fiber from 1.40 to 2.21%; and ash content from 1.18 to 1.67%. All of these values were based on w/w percentage basis. Likewise, the quality parameters of these wheat samples were determined through the Single Kernel Characterization System (SKCS) apparatus in the Milling Laboratory of the Department of Grain Science and Industry. Results showed that the moisture contents ranged from 11.28 to 14%; kernel hardness from 18.39 to 88.92; kernel weight from 26.77 to 36.30 mg; and kernel diameter from 2.58 to 2.92 mg.

Overall, this research involved two distinct types of wheat kernels based on their physical condition: a sound and artificially-damaged kernel. A sound or undamaged kernel is a kernel devoid of any abrasions or scratches in its dorsal, ventral, and lateral portions. On the other hand, an artificially-damaged kernel is a kernel micro-drilled at the brush end, endosperm, and germ anatomical portion of the wheat.

1.3. Justification for research

There have been different studies on how first instars of *R. dominica* infest kernels fo wheat, corn, and paddy rice. It has been mentioned that first instars are unable to feed on hard portion of the kernel or penetrate undamaged kernels (Birch, 1945a; Golebiowska, 1969; Edde, 2012). First instars begin searching for food after hatching and nibble on flour from accumulated adult feeding before chewing into broken pieces of wheat grain (Pajni and Shobha, 1979) or kernel that has been damaged by the adults. Semple (1992) reported that *R. dominica* first instars are incapable of penetrating or feeding on sound intact rice paddy kernels, but are able to exploit kernels with physical defects on husks. In contrast, other researches reported that first instars can establish and attack sound corn kernels with signs of hard brown testa around the bottom container several days from egg hatching (Potter, 1935). Three varieties of unhusked or brown rice are found to be vulnerable to attack by first instars ar 32ºC and 75% r.h. (Chanbang et al. 2008). Based on postharvest losses studies in wheat caused in particular by *R. dominica*, Rao and Wilbur (1972) reported wheat weight loss of about 9.5% when larvae were fed for 20 d and weight loss per kernel was within the range of 6.5 to 19.4% for a period of four weeks after adult emergence, primarily due to adult feeding. In one study, first instars placed near near artificially-damaged germ of corn kernels developed 60 times faster (35-38 d) than those placed near artificially damaged endosperm (58-65 d) at 27ºC and 67% r.h. (Osuji, 1982). Aside from physical characteristics such as kernel hardness and hull integrity in the case of rice, oat, and barley, the chemical composition of wheat kernel such as moisture content and enzymatic activity can also affect larval establishment in wheat kernels. Gutierrez et al. (1990) stated that inhibitors of α-amylases are implicated in plant defense mechanisms. They further stated that cereal storage pests generally have a markedly higher α-amylase activity compared to other insects investigated.

The work reported herein is based on the preceding research outputs by including and focusing on *R. dominica* first instar infestation on different wheat classes, as this aspect has not been fully explored. It also includes a study on preferential feeding by first instars on germ, endosperm, and brush end and speed of development of first instars when placed on these kernel anatomical portions.

The objectives of this study were to conduct a series of experiments to compare successful *R. dominica* first instar infestation at various kernel to larval ratios and track larvcal development until adult emergence on sound and artificially-damaged kernels on different classes of wheat. Additional investigations were made on the influence of site of entry either at the germ, endosperm, or brush end on the speed of larval development. Lastly, we sought to determine the role of short-term feeding by *R. dominica* adults on the successful infestation of first instars on wheat sound kernels.

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Chapter 2

Probability of infestation by *Rhyzopertha dominica* first instars on sound and artificially-damaged kernels of hard red winter wheat

2.1. Abstract

Two laboratory experiments were conducted using organic hard red winter wheat kernels to understand probability of infestation by the lesser grain borer, *Rhyzopertha dominica*, first instars. In the first experiment 50 individual sound kernels in glass vials were infested with 1, 2, 3, 4, or 5 first instars per kernel, and held in growth chamber at 28ºC and 65% r.h. Kernels were examined individually under a steromicroscope 21 d after infestation, and kernels with flour residue, indicative of successful entry by first instars, were dissected. Out of the 250 kernels across all kernel to larval ratios, 12-34% of kernels were infested. These infestation rates were not significantly different from one another. Infestation data were pooled across kernel to larval ratios. Out of 65 infested kernels, first instars entered through germ, brush end, endosperm, and germ and brush end 41.3, 28.4, 27.0, and 3.2% of the time, respectively. In the second experiment, 50 individual sound kernels and 50 kernels artificially-damaged with a micro-drill at the germ, endosperm, or brush end were infested at a kernel to larval ratio of 1:1. After 21 d infested kernels were dissected to measure head capsule width, larval weight, and kernel weight loss. About 82-90% of artificially-damaged kernels were infested by larvae in contrast with 12% for sound kernels. Similarly, germ was the preferred site of entry by first instars, and a majority of larvae in germ were fourth instars after 21 d, whereas those in brush end and endosperm were third instars. Consequently, larvae developing in the germ were significantly heavier, caused more weight loss, and facilitated earlier adult emergence than those developing in endosperm

and brush end. Damage to kernels increased infestation by first instars on artificially damaged kernels by 6.8- to 7.5-fold when compared to that in sound kernels.

2.2. 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). In the grain mass, 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). Larvae hatching from eggs (first instars) are 0.78 mm long, with a head capsule width of 0.13 mm, and campodeiform in shape (Potter, 1935). 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).

There are a few studies on wheat kernel infestation by *R. dominica* first instars. *R. dominica* first instars can successfully infest an undamaged wheat kernel (Crombie, 1945; Stemley, 1962) or sound durum kernels (Limonta et. al., 2011). They can establish and attack sound maize grains according to Potter (1935). Osuji (1982) found that 24% (12 out of 50) 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 sorghum kernels; germ is the first point of entry on sound kernels. Stemley (1962) reported 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, 1945a, 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, 1969) 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°C and 75% r.h. 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*. On maize grains, 100% of *R. dominica* first instars were able to enter into mechanically damaged germ (Osuji, 1982). He observed larvae developing in the germ develop to mature larvae and prepupae within 17-19 d as opposed to 40-43 d for those developing in the endosperm. He further reported that 100% (50 out of 50) larval entry was achieved by larvae when it entered a transversely-cut maize (germ portion) versus 48% (24 out of 50) establishment at the other half (crown end portion) of maize. Similarly, longitudinally-split maize revealed a 94% (47 out of 50) larval establishment in the germ portions versus 82% (41 out of 50) in the endosperm. Similar type of work has not been reported for first instars of *R. dominica* on wheat kernels.

The objectives of the study were: 1) to determine the probability of *R. dominica* first instar infestation of sound hard red winter (HRW) wheat kernels by different wheat kernel-to-first instar ratios; 2) to determine infestation of artificially-damaged HRW wheat kernels by first instars at 1:1 ratio; and 3) to determine preferred site of kernel entry by first instar.

2.3. Materials and Methods

2.3.1. Experiment 1 - Probability of infestation by *R. dominica* first instars on different sound kernel-to-first instar ratios of hard red winter (HRW) wheat

2.3.1.1 Screening of sound HRW wheat kernels

Organic hard red winter (HRW) wheat samples were obtained from Heartland Mills, Marienthal, KS, USA. The wheat was stored in the laboratory freezer (-13°C) for a couple of weeks to kill any live insects present. Approximately 600 g were placed in glass jars and held in a 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 was 11.3 ± 0.54% (mean ± SD), which was determined using the Single Kernel Characterization System (SKCS) unit (SKCS 4100 Model, Perten Instruments, Hagersten, Sweden). A stereoscopic microscope (Nikon SMZ 1000 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 research 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 in chamber prior to first instar infestation.

2.3.1.2 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 wire mesh screens to prevent insect escape. Unsexed adults of mixed ages of *R. dominica* were collected from the laboratory culture jars (insects reared on organic HRW wheat). 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. One hundred screened *R. dominica* adults were counted and 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 middle sieve and all of the flour passed through it and collected in the bottom pan. Eggs collected from each container were placed in 9-cm diameter glass Petri dishes and held at the same chamber and observed for egg hatchability. Egg hatchability was observed after 5 d and first instars that hatched from eggs were used in tests.

2.3.1.3 Infestation by different ratios of first instar on sound HRW wheat kernels

Five hundred screened sound kernels were retrieved from the growth chamber and each was weighed and transferred in glass vial (4 ml capacity) and labeled. The 500 vials were subdivided into five groups of 100 vials each representing treatment sets of 1:1, 1:2, 1:3, 1:4, and 1:5 kernel-to-first instar ratio combinations and labeled accordingly. Each glass vial with 1 sound kernel was infested with different ratios of *R. dominica* first instars that hatched within less than 24 h. Individual first instars were carefully transferred with a camel's hair brush, and placed directly on sound kernels. Each vial was sealed with pre-cut 2.5 cm^2 sized parafilm wax paper (American National Can™, Chicago, IL, USA), and 12 holes with a pin were made for air ventilation. 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.

2.3.1.4 Calibration of stereoscopic microscope

Calibration of stereoscopic microscope was done before dissection of infested kernels on the 21st day. An ocular eyepiece micrometer was inserted into the scope and calibrated with an objective micrometer mounted on glass slide and set on scope stage. The numbered scales of the ocular eyepiece micrometer had 10 major scale lines and further subdivided into 10 minor scale lines. On the other hand, the glass slide-mounted objective micrometer had a minimum scale line of 0.01 mm or 2/200. First, the left eyepiece of the stereomicroscope was removed and its lock tube opened. The circular ocular eyepiece micrometer was inserted into the hollow cylinder tube with the objective lens. The micrometer numbers were right side up and eyepiece lock tube was securely closed after insertion. The left eyepiece was placed back to the stereomicroscope and adjusted to the right magnification until numbered lines were clear when viewed through the scope. Calibration was done by placing the glass slide-mounted objective micrometer on the microscope stage and set at 8X magnification. The ocular eyepiece micrometer scale line was aligned precisely with the objective micrometer. Viewed from the scope lens, the alignment showed that 4 scale lines on the ocular eyepiece micrometer were equivalent to 5 scale lines on the objective micrometer. Each scale line of the objective micrometer was equivalent to 0.01 mm. Hence, 4 scale lines in ocular eyepiece micrometer is equivalent to 0.05 mm on glass slide-mounted objective micrometer.

2.3.1.5 Kernel dissection and larval head capsule width measurements

The Table of Random Numbers was used prior to the thorough inspection and dissection of infested kernels 21 days after infestation. Fifty vials were randomly picked for each 1:1 to 1:5

wheat kernel-to-larval ratios. The remaining 250 vials were used to monitor the growth and development of *R. dominica* first instars until adult emergence from day 27 to 50.

At 21 d after infestation, vials with different kernel-to-first instar ratios were thoroughly inspected under a stereomicroscope 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 orange-colored adhesive mounting putty (Elmer's Products, Inc., High Point, NC, USA) for head capsule width measurement. One drop of 95% ethyl alcohol (Decon Laboratories, Inc., King of Prussia, PA) was applied to immobilize the larva. The head was measured at the widest part of the capsule just past the eyes with the 8X magnification of the scope used. Classification of *R. dominica* instars at 21 d 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 (Table 2.1). Differences in mean head capsule width among instars were determined by subjecting data to one-way analysis of variance (ANOVA) followed by Ryan-Einot-Gabriel-Welsch (REGWQ) multiple comparison test at $\alpha = 0.05$ (SAS Institute, 2008). Individual weights of larvae were also determined using an analytical balance (Mettler Toledo Model AB204, Switzerland) before head capsule width was measured.

2.3.1.6 Monitoring of duration to adult emergence

On the 27th day, each vial was checked under the same stereomicroscope. Each kernel in the vial was removed and thoroughly inspected with holes at the dorsal, lateral, and ventral portions to locate specific site of entry. Indication of successful first instar infestation was determined by the presence of brown and white powdery or floury materials observed adhering at the entrance hole, near or around the base of the kernel as a result of larval tunneling. Those entrance holes determined the specific site of entry by first instar of a particular kernel either at the germ, endosperm, or near the brush end portions. Non-infested kernels were discarded. Only infested kernels were maintained and observed daily for adult emergence from 27th day until the 50th day and data were recorded accordingly. In this particular experiment, only adults that came out of the kernel were considered as emerged. This was without considering the size of the exit hole created by adult feeding. This monitoring strategy might have affected the result of analysis by overestimating the adult days of emergence as influenced by various treatments and sites of entry, because adults tend to stay inside the kernel before emerging out of the kernels.

2.3.1.7 Determination of 50-day kernel weight loss

Kernel weight loss was determined from first instar to adult feeding, covering a period of 50 days. Estimation of percentage weight loss of each kernel was calculated by subtracting the weight of the undamaged kernel with the weight of damaged kernel. The difference was divided by the weight of undamaged kernel and multiplied by 100 to get the percent weight loss:

$$\frac{Initial\ weight\ of\ kernel - Final\ weight\ of\ kernel}{Initial\ weight\ of\ kernel}\ x\ 100$$

2.3.2. Experiment 2 - Probability of infestation by *R. dominica* first instars on sound and artificially-damaged kernels of HRW wheat

The same materials and procedures in screening sound kernels, unsexed *R. dominica* adult of mixed ages for oviposition, collection of eggs, infestation of kernels by first instars, calibration of steromicroscope, random sampling of kernels for dissection, individual weighing of larva, monitoring duration to adult emergence, and 21-day and 50-day kernel weight loss were followed as previously stated. However, in this second study the kernel-to-first instar infestation ratio was 1:1. This was based on the first experiment where infestation rates among various kernel-to-larval ratios were found to be not significant (see Results).

2.3.2.1 Micro-drilling of individual sound HRW wheat kernels

A total of 400 individual HRW wheat sound kernels were used for this study. Three-hundred kernels were selected and 100 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 100 sound kernels were used as the control treatment. All 300 artificially-damaged and 100 sound kernels were individually weighed using an analytical balance (Mettler Toledo Model AB204, Switzerland), recorded, transferred in each glass vial (4 ml cap.), and labeled accordingly.

2.3.2.2 First instar infestation, 21-day kernel dissection, and 50-day monitoring of duration of adult emergence and weight loss

In the control treatment, 100 glass vials each with a sound kernel were directly and carefully infested with first instar of *R. dominica*. The same infestation procedure was used on a for all other 300 artificially-drilled kernels at brush end, endosperm, and germ portion treatments. The vials were individually covered with parafilm paper and poked with a pin 12 times to provide ventilation. These vials were then inserted into cell well plates to prevent them from tipping, and placed on plastic trays and held at 28ºC and 65% r.h. in a growth chamber. At 21 d, 50 vials for each kernel treatment (200 vials for 4 treatments) were randomly picked using the Table of Random Numbers and observed for the number of infestated kernels and site of entry. Larvae were carefully extricated, weighed, and the head capsule width measured. The remaining 200 sound and artificially-drilled kernels were used to monitor larval development of first instar to adulthood by examing kernels from the 27th to 50th day, after which kernel weight loss was measured as described previously.

2.3.3. Data Analysis

All experiments used the completely randomized design (CRD). Data for the two experiments were analyzed using SAS software versions 9.2 and 9.4 (SAS Institute, 2008; 2012). In experiment 1, data on probability of infestation by different kernel-to-first instar ratios and head capsule width measurements were analyzed using the PROC GLIMMIX procedure and means separated using the Least Squares Means test. Data on preferred sites of entry were analyzed using the PROC FREQ procedure followed by a Chi-square Test. Larval weight data were analyzed using a PROC GLM (SAS Institute, 2015) and means separated using Bonferroni *t*-tests. Adult emergence used the PROC GLIMMIX and PROC MIXED and means separated by Tukey-Kramer multiple comparison test. Percentage kernel weight loss recorded on day 50 d was analyzed by the PROC MIXED procedure. In experiment 2 data on probability of infestation of sound and artificially-damaged were analyzed the PROC GLIMMIX procedure. The differences of means was further analyzed using the Bonferroni multiple comparisons adjustment test, because data were not normally distributed. Association of kernel damaged location and preferred sites of entry data was analyzed using the Chi-square test. Since association was established, the 95% multinomial confidence intervals were calculated using the "R" statistical program (version 3.2.1, 2015). Head capsule width data were compared using the Least Squares Means test. Larval weight and kernel weight loss data were analyzed using the PROC GLMMIX procedure, and mean differences among treatments were separated using the Tukey-Kramer adjustment. All statistical tests were considered significant at the $\alpha = 0.05$ level.

2.4. Results

2.4.1. Experiment 1 - Probability of infestation by *R. dominica* first instars on different sound kernel-to-first instar ratios of hard red winter (HRW) wheat

2.4.1.1 Probability of infestation of *R. dominica* first instars 21 d post-infestation

The 1:1 kernel-to-first instar ratio resulted in only 12% of sound kernels being infested. At the 1:2 and 1:4 kernel-to-first instar ratios, 20 and 26% of the sound kernels were infested, respectively. At both the 1:3 and 1:5 kernel-to-first instar ratios, 34% of kernels were infested (Table 2.2). However, the probability of infestation among the five treatments were not significant ($F = 2.23$; df = 4, 245; $P = 0 .0662$).

2.4.1.2 Influence of site of entry on infestation across all HRW sound kernel-to-first instar ratios 21 d post-infestation

Across all sound kernel-to-first instar ratios, germ was found to be the preferred entry point (41% infested), followed by the brush end (29%) and endosperm (27%). There were only 2 kernels with 2 first instars that entered at both the germ and brush end portions (1:3 and 1:4 kernel-to-first instar ratios) with only 3% of kernels being infested (Table 2.3). Significant differences were noted among the preferred sites of entry ($\chi^2 = 19.0952$; df = 3; $P = 0.0003$) across all sound kernel-to-first instar ratios. Pairwise comparison among preferred sites of entry showed that significant differences only existed between germ and germ plus brush end entry points.

2.4.1.3 Influence of site of entry on first instar head capsule width across all HRW sound kernel-to-first instar ratios 21 d post-infestation

Table 2.4 shows that at the various sound kernel-to-first instar ratios tested there was a wide range of head capsule widths measured (see Table 2.1). The measurements varied based on site of entry of larvae. It indicated so much variability on first instar's choice of entry points on various anatomical portions of the sound wheat kernel. Despite these differences in head capsule widths, there were no differences among the various kernel-to-fist-instar ratios (*F* = 2.09; df = 4, 33; *P* = 0.1042). Head capsule widths also did not var based on site of entry (*F* = 1.16; df = 2, 33; *P* = 0.3254), and interaction between ratios and sites was not significant (*F* = 1.08; df = 8, 33; *P* = 0.4035).

2.4.1.4 Influence on larval weight of different HRW sound kernel-to-first instar ratios 21 d post-infestation

Table 2.5 showed the influence of different kernel-to-first instar ratios on larval weight at 21 d. At the 1:1 ratio, larval weight was 0.55 ± 0.01 mg (mean ± SE). At the 1:2, 1:4, and 1:5 ratios, larval weight was 0.69 ± 0.2 mg, 0.77 ± 0.1 mg, and 0.85 ± 0.1 mg, respectively. The heaviest mean larval weight registered was 0.92 ± 0.1 mg at the 1:3 ratio. Despite these differences, larval weight among the kernel-tofirst instar ratios was not significant (*F* = 0.73; df = 4; *P* = 0.5773). Table 2.6 showed that the lowest mean larval weight was observed (0.50 ± 0.1 mg) when 2 larvae entered the same kernel at the germ and brush end portions. The mean larval weight was 0.56 ± 0.08 and 0.88 ± 0.1 mg mg for larvae found in the brush end and endosperm, respectively. The heaviest mean weight was recorded for larvae in the germ (0.89 ± 0.1 mg). Despite these numerical differences, larval weights did not differ significantly differences among sites of entry (*F* = 1.92; df = 3; *P* = 0 .1396) pooled across first instar to kernel-to-first instar ratios.

2.4.1.5 Adult emergence of *R. dominica* on HRW sound kernel-to-first instar ratios

Table 2.7 showed that the subsequent percent growth and development of first instars to adult emergence ranged from 12-44% for the different kernel-to-larval ratios. The 1:1 and 1:2 treatments registered the lowest adult emergence at 12% (6 out of 50). In contrast, the 1:4 ratio registered the highest emergence rate of 44% (22 out of 50). The average duration to adult emergence (mean ± SE) was noted to be shortest at 1:5 ratio with 39.50 ± 1.4 d and longest at 1:4 with 43.76 ± 0.8 d. However, these differences in duration of adult emergence was not different among kernel-to-larval ratios (*F* = 2.30; df = 4, 56; *P* > 0.0703). Across all sound kernel to first instar ratios (Table 2.8), the influence of site of entry on percent adult emergence was highest at the germ portion with 54%, while emergence was only about 23% both for brush end and endosperm portions. Adult emergence was faster and almost identical at 41 d for both germ and brush end entry points while emergence of adults from larvae in the endosperm was 44 d. The duration of adult emergence was not influenced (*F* = 1.67; df = 2, 58; *P* > 0.1979) by site of entry.

2.4.1.6 Kernel weight loss after adult emergence on HRW wheat at different sound kernel-to-first instar ratios 50 d post-infestation

The 1:2 and 1:3 kernel-to-first instar ratio treatments had the lowest average weight loss of sound kernels (28%) followed by the 1:1 ratio (29%) and 1:5 ratio (31%) (Table 2.9). The 1:4 kernel-to-larval ratio treatment had the highest weight loss at 33%. However, weight loss among the different kernel-to-larval ratios was not significant (*F* = 0.43; df = 4, 16; *P* = 0.7857). Weight loss was 31% in kernels where larvae entered through the germ and endosperm, and 27% in kernels were larvae entered through the brush end, but there were no significant differences in weight loss among kernels where larvae entered through the three sites (*F* = 0.52; df = 2, 16; *P* =0.6063). The interaction between kernel-to-larval ratios and site of entry also was not significant (*F* = 1.54; df = 6, 16; *P* =0.2274).

2.4.2. Experiment 2 - Probability of infestation by *R. dominica* first instars on sound and artificially-damaged (AD) HRW wheat kernels

2.4.2.1 Probability of infestation of HRW sound and AD-kernels 21 d post-infestation

Only 12% of sound kernels were infested as opposed to 82 to 90% for AD-kernels (Table 2.11). There were significant differences in probability of infestation among sound and artificially damaged germ, endosperm, and brush end treatments (*F* = 20.27; df = 3, 196; *P* < .0001). However, infestation rates in AD-kernels at the germ, endosperm, and brush end were not significantly different from one another (*P* > 0.05), but were significantly different (*P* < 0.05) from infestation observed on sound kernels (Table 2.12).

2.4.2.2 Effect of site of entry on HRW sound and AD-kernels 21 d post-infestation

There was a significant deviation in observed and expected counts of larvae in the three anatomical parts of wheat kernels among sound and AD kernels (χ^2 = 251.366; df = 6; *P* < 0.0001). The low number of sound kernels infested infested by first instars made this test unreliable. Therefore, sound kernel site of entry data were removed and the data were reanalyzed. There was still a significant deviation in observed and expected counts among kernel entry sites in AD kernels (χ^2 = 248.01; df = 4; *P*-value = < 0.0001) (Table 2.13). The overlapping 95% multinomial confidence intervals for infestation in germ, endosperm, and brush end of AD kernels indicated that the percentage infestation did not vary amnong the three sites of entry. However, these percentages were significantly higher than percentage infestation observed in sound kernels based on non-overlapping (% confidence intervals). The likelihood of a sound kernel being entered by first instar through endosperm and germ sites was low (0-14%).

2.4.2.3 Effect of site of entry on head capsule width of *R. dominica* first instars 21 d post-infestation

Our data on site of entry seemed to indicate a positive effect on larval head capsule width at 21 days (Table 2.14). These data showed that 45 out of the total 136 larvae entered via germ portion of the kernels. Out of these 45 larvae, about 98% (44/45) were already 4th instars and only 2% (1/45) were 3rd instars. In AD kernels, 41/136 (30.1%) larvae entered through AD endosperm portion. Larvae in endosperm were in three different development stages: 78% (37/41) were 4th instars, 17% (7/41) were 3rd instars, and 5% (2/41) were 1st instars. In AD danmaged brush end kernels, 37/44 larvae (84%) weer 4th instars while 7/44 (16) were 3rd instars. In sound kernels, larvae entered via germ and endosperm portions in equal numbers, that is, 3 for each anatomical part. This observation further revealed that 50% (3) and 34% (2) out of the total five larvae were 4th instars when they entered via germ and endosperm portions, respectively. In the remaining one out of six sound kernels (16%), larva entered via the endosperm was a 3rd instar. Least squares means test (Table 2.15) revealed that head capsule width for larvae in the germ was significantly different than those that entered via the AD brush end (t = -4.79; df = 125; Adj. P <0.0001) and AD endosperm (t = -3.60; df = 125; Adj. P = 0.0014). Head capcule widths of larvae in AD brush end and AD endosperm were not different from one another (t = 0.25; df = 125; Adj. P = 1.0000). The sound kernel treatment was excluded from the least squares means test to only six larvae infesting the kernel.

2.4.2.4 Effect of HRW sound and AD kernels on larval weight 21 d post-infestation

Larvae that were initially developing in the AD germ were the heaviest (mean ± SE; 1.32 ± 0.03 mg) compared with those that were developing in the AD brush end (0.87 ± 0.06 mg), AD endosperm (0.92 ± 0.07 mg), and in sound kernels (0.93 ± 0.08 mg) (Table 2.16). The weight of larvae developing in AD germ was significantly greater than those developing in AD brush end (t = -5.74; df = 126; Adj. P < 0.0001) and AD endosperm (t = -4.85; df = 126; Adj. P < 0.0001). The rest of the pair-wise comparison of differences between treatments showed insignificant differences as noted in Table 2.17. In particular, it is worth mentioning that the sound kernel treatment had only a total of four larvae that were weighed out of 6 due to unavoidable circumstances. These include accidentally injuring the larvae when extricating them from kernels.

2.4.2.5 Mean kernel weight loss on HRW sound and AD kernels 21 d post-infestation

In Table 2.18, mean percent weight loss due to larval feeding for 21 days was recorded, and lowest weight loss occurred in sound kernels at (mean ± SE; 4.07 ± 1.8 mg), while the highest weight loss was found when larvae were developing in AD germ (10.35 ± 0.5 mg). There were significant differences in weight loss among among treatments (F value = 5.01; df = 3, 128; P = 0.0026). Significant differences in weight loss were found between kernels AD at the germ and sound kernels (t = 2.77; df = 128; Adj. P = 0.0327) and AD kernels damaged at the brush end (t = -3.06; df = 128; Adj. P = 0.0141) (Table 2.19). Other pair-wise comparisons of treatments were not significantly different from one another.

2.4.2.6 Adult emergence of *R. dominica* on HRW sound and AD kernels 50 d post-infestation

Sound kernels had a low probability of first instar infestation at 14% (7 out of 50) when observed 27 d post-infesattion (Table 2.20). Subsequent monitoring of adult emergence 50 d post-infestation showed a 2% reduction, with only 6 out of 7 larvae fully developing into adulthood. In stark contrast, AD kernels at the germ, endosperm, and brush end portions registered 96%, 86%, and 84% infestation, respectively, and this pattern was sustained until adult emergence. The shortest mean adult emergence was noted in the AD germ treatment (31 d), followed by AD endosperm (33 d), and AD brush end (34 d). In the sound kernel treatment, adult emergence on average was 40 d. The duration of adult emergence was significantly among the sound and AD kernel treatment ($F = 13.22$; df = 3, 135; $P < 0.0001$) (Table 2.21). The duration of adult emergence was significantly greater in the sound kernel treatment compared with emergence in kernels that were AD at the germ (t value = -5.73; df = 135; Adj. $P < 0.0001$), endosperm (t value = -4.16; df = 135; Adj. $P = 0.0003$), and brush end (t value = -3.90; df = 135; Adj. $P = 0.0009$). Emergence was also different between AD germ treatment and AD brush end treatment (t value = 3.68; df = 135; Adj. $P = 0.0019$) and AD endosperm treatment (t value = 3.18; df = 135; Adj. $P = 0.0099$). The adult emergence duration between AD brush end and AD endosperm treatments were essentially similar (t value = 0.51; df = 135; Adj. $P = 0.9555$).

2.4.2.7 Mean kernel weight loss after adult emergence on HRW sound and AD kernels 50 d post-infestation

The average percentage of weight loss when larvae developed from first instars to the adult stage among treatments is shown in Table 2.22. Weight loss was highest for AD germ kernels (mean ± SE; 14.78 ± 0.6%), followed by AD brush end kernels (13.73 ± 0.8%), and AD endosperm kernels (13.38 ± 0.9%). The lowest kernel weight loss was observed in the sound kernel treatment (13.22 ± 2.0%). However differences among treatments were nto significant (*F* = 0.66; df = 3, 135; *P* = 0.5783).

2.5. Discussion

Sound HRW wheat kernels exposed to varying kernel-to-first instar ratios of *R. dominica* showed that larvae were abel to successfully infest 12-34% of the kernels. Similarly for the second experiment, involving sound kernels, at 1 kernel to 1 first instar ration, only 12% of the kernels were infested. Of these, 3 kernels had larvae entering through the germ and 3 through the endosperm. This is probably due to the absence of defects on the surface of sound kernels that may have deterred first instars from successfully entering the kernels. Another factor to consider is hardness of the grain which is one of the physical properties associated with resistance or susceptibility to insect attack (Hoseney and Faubion, 1992; Toews et al., 2000) and an important parameter in classifying the functional properties of each wheat class (Pomeranz et al., 1988). For this study, the HRW wheat hardness index average value was noted to be 73.38 ± 16.14 (mean ± SE). Watts and Dunkel (2003) findings showed positive correlation between hardness and percentage of sound kernels.

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 1 larva that entered through the germ, all 40 larvae entered through the damaged endosperm. Similarly, in brush end damaged kernels 43 larvae entered and established at this site compared to 1 larva that entered through the endosperm. These results suggested that kernel damage facilitated successful larval establishment. Several authors have reported damage to kernel surface to facilitate successful larval entry and establishment of *R. dominica* (Schwardt, 1933; Birch, 1945a, b; Pedersen, 1992; Semple, 1992). Another observation worth mentioning is that 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, 1969). Starving larvae may have apparently weakened (Breese, 1960) to gainfully enter a sound kernel. Stemley (1962) stated that an average of 30-35 hours is required for newly hatched first instar to successfully enter a wheat kernel and 5 to 8 hours to bore it. Further observation shows that the consequence of first instars' unsuccessful entry as late as two days results in death (Stemley, 1962) or larvae tend to complete development outside the grain if broken kernels and plenty of dust are present (Pedersen, 1992).

Across all kernel-to-first instar ratios and artificially-damaged experiments, there is preference for germ over other anatomical portions of the wheat seed. This may be due to germ being nutritionally rich because of proteins, oild, sugars, vitamins B and E, and minerals (Pomeranz, 1987; Hoseney and Faubion, 1992; Cornell and Hoveling, 1998; Hoseney, 1998; Serna-Saldivar, 2010). The nutrients may have helped in the faster development of larvae. Compared with endosperm and brush end, where majority of its nutritive content is from carbohydrates, primarily pentosans, hemicelluloses, beta-glucans (Hoseney, 1998; Serna-Saldivar, 2010), and starch granules embedded in a protein matrix (Pomeranz, 1987). Another plausible explanation is the inner structural arrangements of the aleurone layer along the germ and endosperm portions of the wheat grain. Hoseney (1994) stated that the aleurone layer completely envelopes the kernel from the germ to the endosperm portions. However, the aleurone layer is irregular in thickness (Pomeranz, 1987) or its cells are modified and thinner at the germ portion compared to the other remaining portions of the kernel (Hoseney, 1994). This can confer vulnerability for germ portion to be easily damaged by the powerful mandibles of first instars. In relation to this, Stemley (1962) observed that first instars of *R. dominica* had little difficulty in boring into the germ portion of the kernels.

The seminal work on head capsule width measurement to determine the specific larval stages of insects is done on molts of lepidepterous larvae by Dyar (1890). The primary idea is anchored on investigations made that the head width of a larva at each stage follow a regular geometric progression. Based on this, it is suggested that the width of the head of each stage be measured because it is that part of the insect that does not grow in between each successive molt and its width as the most appropriate measure to obtain. Based on results of our experiments, *R. dominica* larval head capsule at 21 d was generally wider for larvae developing at the germ portion. This observation also suggests that there is faster growth associated with germ as entry point. Compared to some young larvae that entered through endosperm and brush end, there is wider variation of larval stage classification ranging from the 1st to 4th instars. Based on this wide variation of larval stages on different kernel-to-first instar ratios and insignificant differences among entry points, it indicates that measuring head capsule width was not able to capture the developmental rates of *R. dominica*. On the other hand, the observance of 1st instar at 1:4 and 2nd instar at 1:3 densities at the endosperm and brush end portions, respectively, could have been due to the delayed entry of young larvae inside sound wheat kernels rather than slow growth. It is possible that larvae entered the germ faster than those entering the endosperm or brush end, and as a result were already 4th instar on day 21. Additional studies are needed to determine if first instars enter the germ much sooner than they do other kernel portions.

R. dominica larval weight determination is one attribute to measure its successful feeding and eventual development to adulthood. The study did not find evidence of statistically significant differences on larval weight as affected by different larval ratios and sites of entry.

This may be due to exposure of first instars to sound kernel condition (without defects or abrasions on kernel surface) that make it difficult to infest and bore holes especially at the endosperm portion but not at the germ end of the kernels (Stemley, 1962). The delayed entry and establishment inside the kernel, more dead first instars were noted on all treatment densities and entry sites. Another important observation was that double entry of larvae in 1:3 and 1:4 kernel-to-larval ratios lead to differences in larval weight as affected by site of entry. Larvae entering through the germ end were bigger in size and heavier in weight, while larvae that entered via brush end portions were smaller. Also, their weights seemed lighter because it cannot be detected by the analytical balance due to its 0.1 mg readability limit. The same observations were noted in the second experiment with sound and AD kernels. Results indicated that germ end as entry point for first instars promote heavier weight of larvae compared to brush end and endosperm sites of entry. From a nutritional perspective, the germ portion contains plenty of growth enhancing proteins, lipids, vitamins, and minerals in the form of ash (Pomeranz, 1987; Hoseney and Faubion, 1992). Specifically, these protein in the germ are classified as water-soluble albumins and salt-soluble globulins (Pomeranz, 1987; Hoseney and Faubion, 1992) which are considered to be of best quality due to the high quantities of lysine and other essential amino acids and are readily digested (Cornell and Hoveling, 1998; Serna-Saldivar, 2010). Hence, this must have contributed to the faster growth of larvae and eventual increase in weight. In contrast the endosperm and brush end portions, which contain largely of starch granules enclosed in protein matrix (Pomeranz, 1987), or 70-80% of the total protein due to its anatomical size (Cornell and Hoveling, 1998; Serna-Saldivar, 2010). These storage protein fractions are called prolamins (alcohol-soluble) and glutelins (acid- and alkali-soluble) (Pomeranz, 1987; Serna-Saldivar, 2010). The former is considered nutritionally deficient due to the presence of non-essential amino acids like glycine, glutamic acid, and aspartic acid. Glutelins are located in the protein matrix that envelops the endosperm starch granules and nutritionally better compared to prolamins (Serna-Saldivar, 2010).

Kernel weight loss is a direct damage caused by insect feeding either during the larval stage or adult stage. Internal infesting insect species like *R. dominica* feed predominantly on the endosperm portion of the grain (Pedersen, 1992). Overall, results of the study showed that AD kernels had a mean weight loss of 9% at 21 d, which is almost similar to the 9.5% loss at 20 d of reported by Rao and Wilbur (1972). In particular, 21 d larval feeding significantly affected kernel weight loss in AD germ (10.35%) compared with sound grains (4.07%), but not with other AD portions of the kernels. It was observed in the two experiments conducted, that young larvae are opportunistic in behavior in the presence of any cracks or opening in any anatomical parts of the grain.

The subsequent adult emergence from first instar infestation of sound HRW wheat kernels were not significantly influenced by the different kernel-to-first instar ratios and initial sites of entry. This suggests that larval ratios or sites of entry are not determinant factors that affect the ability of adult *R. dominica* to emerge. Probably its emergence is dictated by their feeding behavior, sexual maturity, or chemical substancess that still remains inside the grain. It was observed by Schwardt (1933) that teneral adults tended to remain inside the kernel 3-5 days before starting to feed and exit the grain. This author also observed similar adult behaviors. Once it has emerged, adults have the habit of going in and out of the same damaged wheat kernel. It is also likely that the adult is not yet sexually mature and prefers to remain inside the kernel until such time it is ready to find a mate. In the second experiment, the shorter average duration to adult emergence (32-33 d) from all AD kernel treatments compared with 40 d over sound kernels was expected. A previous study showed that the first instar always entered through the damaged surface of grain (Birch, 1945a) or the surface roughness of the food medium helps larvae to successfully enter kernels (Schwardt, 1933) compared with sound grains. This feeding behavior of selecting a suitable entry point provides good establishment into the kernel and ensures the successful growth and development into adulthood. Likewise, the initial site of entry chosen by first instar dictated its assured development to adult stage as shown in this experiment. However, AD germ is the preferred site of entry over other artificially-damaged portions of the kernel. Even for sound kernel treatment, germ is the favored site of entry against brush end and endosperm portions. Osuji (1982) found that on artificially-drilled corn grain, adult emergence of the *R. dominica* was shorter (38 d) compared with emergence from the endosperm (58 d). Similarly, Khare and Mills (1968) reported that there is faster development by the Angoumois grain moth, *Sitotroga cerealella* (Olivier), when they fed early in the germ portion compared to the endosperm of the wheat kernel.

The average percent weight loss obtained due to feeding by first instar that successively developed to adult stage was also found to be insignificantly affected by various kernel-to-larval ratios and sites of entry. This negative finding can probably be attributed to several reasons. First, the small population size used was only 25 sound kernel samples. Probably due to this small sample size, it was difficult to capture the effect on kernel weight loss. The second reason is related to the daily monitoring strategy decision to score when an adult successfully emerged from the damaged kernel. For this specific experiment, an adult was scored as successfully emerged if it physically exited the damaged kernel or was seen roaming in the vials. The monitoring strategy applied is a problematic one because it is not done on a 24 h basis. It is possible that adults might have already tunneled out of the wheat kernel within a 24 h period and this was not captured during non-monitoring hours at night. Also, it is worth noting to mention that based on daily feeding habit observations, adults tend to go in and out and linger inside the previously damaged kernel. This adult habit was observed to result in continuous feeding until nothing is left of the kernel. Such behavior was reported in experiments conducted by Rao and Wilbur (1972) and Campbell and Sinha (1976). In effect, the adopted monitoring strategy could have affected the precision of estimating the computed weight loss as shown by the high standard error values both for the different treatments and sites of entry. The second experiment showed a similar result where insignificant weight loss among sound and AD kernels tested were found. This might have been due to extended observance of adult emergence exiting from the grain that might have resulted in overestimation of reduction in weight due to feeding. For this experiment, the average weight loss of 14% found from AD germ feeding was about 3X less than the 49% (at 48 d) or 4X less than the 52% (at 52 d) reported by Rao and Wilbur (1972).

In conclusion, infestation rates were similar across all kernel-first instar ratios. Germ portion was the preferred site of entry across all first instar ratios. The second experiment findings showed that probability of infestation of damaged kernels were significantly greater than on sound kernels. It also indicated and confirmed that germ was the favored site of entry. Entering via the germ portion facilitated faster growth of larvae. It also promoted heavier weight of larvae. Entry site through germ shortened adult emergence. Percentage kernel weight loss at 21 d was higher for damaged versus sound kernels. However, 50 d kernel weight loss was similar across all treatments and entry sites. Finally, undamaged wheat grains prolonged adult emergence compared with damaged kernels.

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Table 2.1. Head capsule width measurements of *Rhyzopertha dominica* instars.

Instar[1]	Number of larvae	Mean ± SE (range) of head capsule width (mm)[2]	Development time in days at 28°C and 70% r.h.[3]
1	116	0.139 ± 0.003 (0.108 - 0.188)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] Data reanalyzed from Stemley (1962).

[2] Means followed by different letters are significantly different from one another ($P < 0.05$; by Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test).

[3] *Source*: Howe (1950).

Table 2.2. Probability of infestation by *R. dominica* first instars on different HRW wheat sound kernel-to-first instar ratios at 21 days.

Treatment	Number of infested kernels	Number of un-infested kernels	Infestation (%)^{a,*}
1:1	6	44	12
1:2	10	40	20
1:3	17	33	34
1:4	13	37	26
1:5	17	33	34

[a] $n = 50$ kernels/treatment.

*There were no significant differences among treatments ($F = 2.23$; df = 4, 245; $P = 0.0662$; PROC GLIMMIX, fixed effects; Type III SS).

Table 2.3. Influence of site of entry on infestation across all HRW wheat sound kernel-to-first instar ratios at 21 days.

Site of entry	Number of infested kernels[a]	% Infestation (Multinomial 95% CI)*
Brush end	18	28.6 (15.9 – 41.5)
Endosperm	17	26.9 (14.3 – 39.9)
Germ	26	41.3 (28.6 – 54.2)
Germ + brush end	2	3.2 (0.00 – 16.1)
Total	63	

[a] Percentage infestation is based on a total of 63 kernels.

*Percentage infestation observed deviates from the expected 25% for each site of entry (χ^2 = 19.0952; df = 3; P = 0.0003).

Table 2.4. Influence of site of entry on first instar head capsule width across all HRW wheat sound kernel-to-first instar ratios at 21 days.

Treatment	Site of entry	Instar				Mean ± SE
		1	2	3	4	Head capsule width (mm)*
1:1	Brush end	-	-	-	1	0.43
1:1	Endosperm	-	-	3	-	0.30 ± 0.04
1:1	Germ	-	-	1	1	0.37 ± 0.05
1:2	Brush end	-	-	2	-	0.27 ± 0.09
1:2	Endosperm	-	-	-	2	0.45 ± 0.08
1:2	Germ	-	-	3	2	0.34 ± 0.04
1:3	Brush end	-	-	3	-	0.30 ± 0.04
1:3	Endosperm	-	1	2	5	0.30 ± 0.04
1:3	Germ	-	-	2	4	0.39 ± 0.03
1:4	Brush end	1	-	-	3	0.31 ± 0.06
1:4	Endosperm	-	-	-	1	0.44
1:4	Germ	-	-	-	7	0.43 ± 0.04
1:5	Brush end	-	-	1	7	0.40 ± 0.03
1:5	Endosperm	-	-	-	3	0.43 ± 0.04
1:5	Germ	-	-	-	6	0.44 ± 0.04

*There were no significant differences among treatments ($F = 2.09$; df = 4, 33; $P = 0.1042$; PROC GLIMMIX, fixed effects; Type III SS); There were no significant differences among sites of entry ($F = 1.16$; df = 2, 33; $P = 0.3254$; PROC GLIMMIX, fixed effects; Type III SS);There were no significant differences between treatments and sites of entry ($F = 1.08$; df = 8, 33; $P = 0.4035$; PROC GLIMMIX, fixed effects; Type III SS).

Table 2.5. Mean larval weight as influenced by different HRW wheat sound kernel-to-first instar ratios at 21 days.

Treatment	Number of infested kernels	Mean ± SE larval weight (mg)*
1:1	4	0.55 ± 0.1
1:2	7	0.69 ± 0.2
1:3	13	0.92 ± 0.1
1:4	10	0.77 ± 0.1
1:5	15	0.85 ± 0.1

*There were no significant differences in larval weight among treatments (F = 0.73; df = 4; P = 0.5773; PROC GLM, fixed effects; Type III SS).

Table 2.6. Mean larval weight as influenced by sites of entry across all HRW wheat sound-to-first instar ratios at 21 days.

Site of entry	Number of infested kernels	Mean ± SE larval weight (mg)*
Brush end	10	0.56 ± 0.08
Endosperm	13	0.88 ± 0.1
Germ	24	0.89 ± 0.1
Germ + brush end	2	0.50 ± 0.1

*There were no significant differences among preferred sites of entry (*F* = 1.92; df = 3; *P* = 0.1396; PROC GLM, fixed effects; Type III SS).

Table 2.7. Adult emergence of *R. dominica* on HRW wheat sound kernel-to-first instar ratios.

Treatment	Number of infested kernels[a]	Infestation (%)[b]	Number of adults emerged	Emergence (%)[b]	Mean ± SE duration to adult emergence (days)*
1:1	7	14	6	12	42.33 ± 1.9
1:2	8	16	6	12	43.57 ± 1.3
1:3	16	32	13	26	42.76 ± 1.2
1:4	23	46	22	44	43.76 ± 0.8
1:5	14	28	14	28	39.50 ± 1.4

[a] Based on observing kernels for boring hole/dust on day 27 under a stereomicroscope.

[b] Percentages based on 50 kernels.

*There were no significant differences among treatments ($F = 2.30$; df = 4, 56; $P = 0.0703$; PROC GLIMMIX; Type III SS).

Table 2.8. Adult emergence of *R dominica* as influenced by site of entry across all HRW wheat sound kernel-to-first instar ratios.

Site of entry	Number of adults emerged	Emergence (%)[a]	Mean ± SE duration to adult emergence (days)*
Brush end	14	22.95	41.64 ± 1.2
Endosperm	14	22.95	44.28± 1.3
Germ	33	54.09	41.93 ± 0.7
Total	61		

[a] Percentages based on a total of 61 adults.

*There were no significant differences in emergence among sites of entry (F = 1.67; df = 2, 136; P = 0.1979; PROC GLM; Type III SS).

Table 2.9. Mean kernel weight loss at 50 days after adult emergence on HRW wheat sound kernel-to-first instar ratios.

Treatment	Number of kernels[a]	Mean ± SE kernel weight loss (%)*
1:1	3	29.35 ± 10.5
1:2	7	28.08 ± 2.7
1:3	10	28.15 ± 1.7
1:4	9	33.48 ± 2.911
1:5	4	30.80 ± 3.037

[1] *n* = 25 sound kernels per larval ratio.

*There were no significant differences among treatments (*F* = 0.43; df = 4, 16; *P* = 0.7857; PROC MIXED; Type III SS).

Table 2.10. Mean kernel weight loss at 50 days after adult emergence pooled across all HRW wheat sound kernel-to-first instar ratios.

Site of entry	Number of kernels	Mean ± SE kernel weight loss (%)*
Brush end	7	26.91 ± 2.4
Endosperm	9	31.58 ± 2.5
Germ	13	31.09 ± 2.1

*There were no significant differences among sites of entry across all treatment ratios (*F* = 0.52; df = 2, 16; *P* = 0.6063; PROC MIXED; Type III SS).

Table 2.11. Probability of infestation of HRW wheat sound and artificially-damaged (AD) kernels 21 day post-infestation.

Treatment	Number of infested kernels	Number of un-infested kernels	Infestation (%)[a, *]
Sound kernels	6	44	12
AD-brush end	44	6	88
AD-endosperm	41	9	82
AD-germ	45	5	90

[a] n = 50 kernels/treatment.

*There were significant differences among treatments (F = 20.27; df = 3, 196; P < 0.0001; PROC GLIMMIX, fixed effects; Type III SS).

Table 2.12. Results of least squares means test with Bonferroni adjustment comparing pair-wise probability of infestation shown in Table 2.11.

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 kernel	6.16	< 0.0001*
AD-brush end vs. Sound kernel	6.47	< 0.0001*
AD-germ vs. Sound kernel	6.53	< 0.0001*

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 2.13. Effect of site of entry on HRW wheat sound and AD kernels 21d post-infestation.

Treatment	Number of infested kernels*				% Infestation
	Brush end	Endosperm	Germ	Total	(Multinomial 95% CI)[a]
AD-brush end	43	1	0	44	32.4 (23.5 – 41.5)
AD-endosperm	0	40	1	41	30.1 (21.3 – 39.3)
AD-germ	0	0	45	45	33.1 (24.2 – 42.2)
Sound kernels	0	3	3	6	4.4 (0.00 – 13.6)
Total				136	

[a] Percentage infestation is based on a total of 136 kernels.

*Observed counts deviate from expected counts ($\chi^2 = 251.366$; df = 6; $P < 0.0001$). With sound kernels data removed counts deviate from expected counts; $n = 130$ ($\chi^2 = 248.096$; df = 4; $P < 0.0001$).

Table 2.14. Effect of site of entry on head capsule width of *R. dominica* first instars 21 d post-infestation.

Site of entry	Instar				Number of infested kernels	Mean ± SE head capsule width (mm)
	1	2	3	4		
Sound kernels	0	0	1	5	6	0.40 ± 0.02
AD-brush end	0	0	7	37	44	0.39 ± 0.01
AD-endosperm	2	0	7	32	41	0.39 ± 0.01
AD-germ	0	0	1	44	45	0.43 ± 0.00

Tabldfe 2.15. Results of least squares means differences comparing head capsule widths between sites of entry across all treatments shown in Table 2.14.

Treatments compared	*t*-value (df = 125)	Adjusted *P*-value
Brush end vs. endosperm	0.25	1.0000
Brush end vs. germ	-4.79	< 0.0001*
Endosperm vs. germ	-3.60	0.0014*

*Significant ($P < 0.05$; by least squares means test).

Table 2.16. Mean larval weight on HRW wheat sound and AD kernels 21 d post-infestation.

Treatment	Number of infested kernels	Mean ± SE larval weight (mg)*
Sound kernels	4	0.93 ± 0.08
AD-brush end	44	0.87 ± 0.06
AD-endosperm	38	0.92 ± 0.07
AD-germ	44	1.32 ± 0.03

*There were significant differences among treatments (*F*-value = 13.00; df = 3, 126; $P < 0.0001$; PROC GLIMMIX, fixed effects; Type III SS).

Table 2.17. Results of differences of least squares means with Tukey-Kramer adjustment test comparing pair-wise larval weight shown in Table 2.16.

Treatments compared	*t*-value (df = 126)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-0.67	0.9069
AD-brush end vs. AD-germ	-5.74	< 0.0001*
AD-brush end vs. Sound kernel	-0.31	0.9900
AD-endosperm vs. AD-germ	-4.85	< 0.0001*
AD-endosperm vs. Sound kernel	-0.02	1.0000
AD-germ vs. Sound kernel	2.04	0.1801

*Significant ($P < 0.05$; by Tukey-Kramer multiple comparisons adjustment test).

Table 2.18. Mean kernel weight loss of HRW wheat sound and AD kernels at 21 d post-infestation.

Treatment	Number of infested kernels	Mean ± SE kernel weight loss (%)*
Sound kernels	6	4.07 ± 1.8
AD-brush end	43	6.92 ± 0.9
AD-endosperm	39	9.50 ± 1.0
AD-germ	44	10.35 ± 0.5

*There were significant differences among treatments (*F*-value = 5.01; df = 3, 128; *P* = 0.0026; PROC GLIMMIX, fixed effects; Type III SS).

Table 2.19. Results of differences of least squares means with Tukey-Kramer adjustment test comparing pair-wise percent kernel weight loss shown in Table 2.18.

Treatments compared	*t*-value (df = 128)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-2.23	0.1207
AD-brush end vs. AD-germ	-3.06	0.0141*
AD-brush end vs. Sound kernel	1.25	0.5934
AD-endosperm vs. AD-germ	-0.74	0.8789
AD-endosperm vs. Sound kernel	2.37	0.0879
AD-germ vs. Sound kernel	2.77	0.0327*

*Significant ($P < 0.05$; by Tukey-Kramer multiple comparisons adjustment test).

Table 2.20. Adult emergence of *R.domnica* first instars on HRW wheat sound and AD kernels.

Treatment	Number of infested kernels[a]	Infestation (%)[b]	Number of adults emerged	Emergence (%)[b]	Mean ± SE duration to adult emergence (days)*
Sound kernels	7	14	6	12	40.00 ± 1.2
AD-brush end	42	84	42	84	34.23 ± 0.6
AD-endosperm	43	86	43	86	33.86 ± 0.5
AD-germ	48	96	48	96	31.60 ± 0.3

[a] Based on observing kernels for boring/dust on day 27 under a stereomicroscope.

[b] Percentages based on 50 kernels.

*There were significant differences among treatments (F = 13.22; df = 3, 135; P < 0.0001; PROC GLIMMIX; Type III SS).

Table 2.21. Results of differences of least squares means with Tukey-Kramer multiple adjustment test comparing pair-wise percent adut emergence shown in Table 2.20.

Treatments compared	*t*-value (df = 135)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	0.51	0.9555
AD-brush end vs. AD-germ	3.68	0.0019*
AD-brush end vs. Sound kernel	-3.90	0.0009*
AD-endosperm vs. AD-germ	3.18	0.0099*
AD-endosperm vs. Sound kernel	-4.16	0.0003*
AD-germ vs. Sound kernel	-5.73	< 0.0001*

*Significant ($P < 0.05$; by Tukey-Kramer multiple comparisons adjustment test).

Table 2.22. Mean kernel weight loss at 50 days after adult emergence on HRW wheat sound and AD kernels.

Treatment	Number of infested kernels	Mean ± SE kernel weight loss (%)*
Sound kernels	7	13.22 ± 2.0
AD-brush end	42	13.73 ± 0.8
AD-endosperm	43	13.38 ± 0.9
AD-germ	48	14.78 ± 0.6

*There were no significant differences among treatments (*F* = 0.66; df = 3, 135; *P* = 0.5783; PROC GLIMMIX; Type III SS).

Chapter 3

Monitoring development of *Rhyzopertha dominica* larvae by head capsule width measurement on artificially-damaged hard red winter wheat kernels

3.1. Abstract

Rhyzopertha dominica (F.) (Coleoptera: Bostrichidae), also known as the lesser grain borer, undergoes complete metamorphosis with four major life stages of egg, larva, pupa, and adult. It is classified as an internal feeder with immature stages developing inside the grain kernels. The first instars from newly-hatched eggs of *R. dominica* has the capability to bore inside hard grains and complete its development until an adult emerges from the infested kernel. Experiments were performed to determine the speed of first instar development on different artificially-damaged hard red winter wheat kernels by head capsule width measurement. Three-hundred sound individual kernels artificially-drilled with a microdrill at the brush end, endosperm, and germ 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. The development of first instars entering through three different artificially-drilled anatomical portions of hard red winter wheat kernels was monitored by dissection and 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. The 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 be resistant to *R. dominica* infestation.

3.2. Introduction

Rhyzopertha dominica (F.) (Coleoptera: Bostrichidae) or commonly known as the lesser grain borer (Hagstrum and Subramanyam, 2013; Reed, 2006) originally comes from the family of wood borers (Reed, 2006) that attacks dead wood or fallen logs (Potter, 1935) in the forest. Its current status and presence in post-production systems of infesting more than 115 commodities (Hagstrum and Subarmanyam, 2013) can be regarded as a secondary adaptation (Potter, 1935). *R. dominica* belongs to the group of holometabolous insects. This is a group of insects that go through complete metamorphosis; meaning, with four major life stages such as egg, larva, pupa, and adult (Potter, 1935; Reed, 2006). Further, it is also classified as an internal feeder on the basis of its development inside the grain kernels (Birch, 1945b; Howe, 1950; Pedersen, 1992). First instars from newly-hatched eggs of *R. dominica* have the capability to bore inside hard grain kernels or pellets and complete its development until an adult emerges from the kernel (Reed, 2006). In general, there are four instars (Schwardt, 1933; Birch 1945b; Campbell and Sinha, 1978; Pedersen, 1992; Elek, 1994; Reed, 2006) if fed on whole grain, but, in exceptional cases a fifth stage occurs if the form of food material is ground like whole meal flour (Potter, 1935; Howe, 1950). Potter (1935) made an exhaustive study from egg to adult stage of the lesser grain borer in maize and synthetic whole meal flour comprised of equal proportions of wheat germ, bran and semolina at environmental conditions of 26°C and 65% r.h. The first instar is an active campodeiform larva capable of active locomotion as noted by Potter (1935). It has three pairs of small legs attached after the head (Pedersen, 1992) that moves in a looping motion (Potter, 1935). It has a soft body and round rigid head capsule (Reed, 2006) partially retracted into the thorax (Pedersen, 1992) with an average width of 0.132 mm and body length of 0.783 mm (Potter, 1935). Another distinctive identifier for first instar is the presence of a terminal median spine that is curved and located at the last abdominal segment (Potter, 1935; Howe, 1950). The second instar is fairly similar in appearance with the first instar and still actively mobile. The average head capsule width is 0.165 mm and body length of 1.075 mm. The third instar is obviously different from the preceding two stages with its head retracted, body is recurved, immobile on a flat surface, thorax is larger than the abdomen, and the last abdominal segment has a well-distinct anal furrow. It has an average of 0.264 mm head capsule width and 2.037 mm body length. The fourth instar is fairly similar to third instar but larger and all other physical features are somewhat accentuated. Its average head capsule width measures 0.413 mm. and body length of 3.069 mm (Potter, 1935).

One method to determine and identify *R. dominica*'s four larval stages is to measure its head capsule width (Howe, 1950; Campbell and Sinha, 1978) using an ocular micrometer (Potter, 1935; Mayer and Babers, 1944; Soderstrom, 1959; Stemley, 1962). The seminal work on head capsule width measurement which is also known as the Dyar's rule is started by H.G. Dyar (1890) using Lepidepterous larvae as test insects. This rule has been used by Potter (1935); Mayer and Babers (1944); Soderstrom (1959); Stemley (1962); Bailey and Chada (1968); and Beck (1971) in their experiments. Dyar's rule stated that the widths of larval head follow a regular geometrical progression in its successive stages of development. It is that anatomical part of the larva that is not affected in between each successive stage growth and its width as the most suitable measurement to obtain (Mayer and Babers, 1944). Measurement of the head capsule of each larva was made across the widest part of its dorsal surface past the eyes (Stemley, 1962). A powerful microscope with a detachable eyepiece ocular micrometer (Mayer and Babers, 1944; Soderstrom, 1959) is calibrated with an objective or slide micrometer placed on stage prior to head measurement (Stemley, 1962).

There are several factors that can affect larval growth. One is nutrition, which is an internal plant factor that influences larval growth (Zalucki et al., 2002). Among larval stages, it is reported that younger instars normally have higher growth rates (Scriber and Slansky, 1981) or production which is equivalent to larval biomass per unit time and reproduction through egg production (Campbell and Sinha, 1978). Other positive nutritional effects on early instar growth include faster metabolic rates through respiration and efficient assimilation of nutrient (Zalucki et al., 2002) which is used during development (Campbell and Sinha, 1978) but then again it is less efficient in converting digested food than older instars (Scriber and Slansky, 1981). Another factor is higher consumption rates by younger instars with respect to its body weight. According to Bailey and Chada (1968), larval weights are slightly greater when reared on natural food compared with an artificial diet. Also, head capsule width is positively correlated with larval weight as mentioned by Beck (1971). Second factor in larval growth can be the quality of food that larvae consumes. Scriber and Slansky (1981) stated that better food quality promotes more rapid growth for immature insects. Thirdly, the type of diet, either whole grain or ground form, offered to insects contributed to its growth and development. Howe (1950) reported that larval development times are slower in whole meal flour compared to whole wheat grain both at temperatures of 25°C and 28°C and same 70% r.h. conditions. Lastly, the site of initial larval entry greatly affects growth rate as observed by Osuji (1982) using maize that is artificially-drilled near germ and endosperm portions. He noted that larva turned into pupae 60% faster if it entered through the germ portion compared to endosperm portion of maize grain. Hence, this research seeks to validate the speed of larval development on different anatomical sites in artificially-damaged hard red winter wheat kernels by tracking its growth and development over a period of 30 days.

3.3. Materials and Methods

3.3.1. Screening and micro-drilling of sound kernels, first instar infestation, and larval head capsule width measurements

Again, this experiment used HRW wheat samples that were organically-grown and sourced from Heartland Mills, Marienthal, KS, USA. The same disinfestation process in the freezer, temporary storage in growth chamber at 28°C and 65% r.h. for moisture content equilibration were done prior to the screening of sound wheat kernels using stereoscopic microscope. The moisture content of the grain samples was 10.2 ± 0.98% (mean ± SD) which was determined using the same SKCS unit in our milling laboratory facility.

Three-hundred (300) individual HRW wheat sound kernels were retrieved from the chamber. As previously done in experiment 2, Chapter 2, 100 of each were artificially-drilled at the same spot and an approximate depth of 1.0 mm using the 0.24 mm diameter micro-drill on endosperm, germ, and the brush end portions. Each artificially-drilled kernel was placed and stabilized firmly inside labelled glass vials using washable glue stick. The glued kernels were allowed to dry for 24 hours. Each glass vial was inserted into a 24 cell well plates and were placed on plastic trays and held at 28ºC and 65% r.h. before first instar infestation.

Egg collection of *R. dominica* was done on sifted bleached flour contained in plastic cups. One hundred unsexed *R. dominica* adults of mixed ages were counted and introduced into each plastic cups and held in the same growth chamber conditions. After 3 days, the bleached flour was sifted, eggs collected, and were placed in glass Petri dishes and held at the same chamber. Egg hatchability was observed after 5 days and first instar that hatched from eggs were used in tests.

Overall, there were 300 artificially-damaged kernels with 3 major treatments for this experiment. Daily, for three consecutive days, 100 vials of each 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 first instars were carefully transferred with a camel's hair brush, and placed directly on artificially-damaged kernels. Each vial was sealed with pre-cut 2.5 cm^2 sized parafilm wax and 12 pin holes were made for air circulation. The infested kernels in glass vials were held in the same chamber and controlled conditions.

Each larval head capsule width was measured using the same calibrated microscope. Calibration procedure was done the same way as described in experiment 1, Chapter 2 of this paper. Again, the Table of Random Numbers was used before kernel dissection was done. A random number list was made that randomly picked 10 kernels per day (3, 6, 9, 12, 15, 18, 21, 24, 27, and 30) per treatment to avoid sampling biases. Every 3 days for 30 days (10 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. Under the same microscope and at 8x magnification, each of the 10 kernels was thoroughly inspected at the dorsal, lateral, and ventral portions for first instar kernel establishment and sites of entry. The dorsal side (crease side on top view under microscope) was mounted on orange-colored mounting putty fixed in petri dish for stabilization. Removal of larva from each infested kernel was done through dissection by holding a kernel with forceps and a scalpel to cut it open. Dissection was always carefully started at the crease side due to its natural depression that provided ease of cutting aside from its relatively softer portion compared to the dorsal side. The number of cutting motions was adjusted based on the toughness of the kernel and to avoid the chance of damaging the larva. Larva was carefully extricated from the cut kernel with a slight twisting motion of the scalpel. Upon extrication and using insect brush, it was carefully placed at the surface of the clay dough for head capsule measurement. The larva was rested on its dorsal side using a dissecting needle and pinned down at its ventral-central abdominal portion. This achieved a curled position with its head protruding on the clay dough surface. A few drops of 100% ethyl alcohol was applied to immobilize the larva before measurement. The head was measured across the widest part of the capsule just past the eyes with the scope at 8x magnification. Classification of *R. dominica* instar at 21 days was based on the range of head capsule width measurements as shown in Table 2.1.

3.3.2. Data Analysis

The experiment was conducted on CRD structure. Consolidated data was analyzed on a non-linear regressions fit to head capsule width over time. It used the pair-wise comparison of regression model that were made from a pooled model. Differences in the speed of larval development by site of kernel entry were determined by pair-wise comparisons of regression model using the model comparison procedure ($\alpha = 0.05$) by Draper and Smith (1981).

3.4. Results

Overall, the proportion of larval survival by first instars on all AD-kernels were noted to be in the range of 51% to 53% over a 30-day monitoring period as shown in Table 3.1. The AD-brush end treatment registered the highest survival rate at about 53% while the AD-germ treatment had the lowest at approximately 51%. It is interesting to note that for AD-germ treatment, as early as 12th day, 20% of the larvae were at 3rd instar while 90% and 50% were at 1st instar for AD-brush end and AD-endosperm, respectively. On the 15th day, 20% were at 4th instar for AD-germ treatment but AD-brush end had 30% at 2nd instar while AD-endosperm had 40% of larvae at 3rd instar. Starting on the 24th day, the number of larval survival at AD-germ had tapered off at 30% and 70% had turn into pupal stage. On the other hand, AD-brush end and AD-endosperm treatments still had 50% at 3rd and 4th instars for the former and 1st and 4th instars for the latter on 24th day.

Given in Table 3.2 was the mean head capsule width of the three AD-kernel treatments. Based on instar classification on Table 2.1, those larvae that enetered through the AD-germ had an early larval development with 2nd instars observed as early as the 9th day. Third instars were observed starting at 12th and 15th day and 4th instar at 18th day. Compared with AD-brush end, 2nd instars were observed only on the 15th day, 3rd instars on the 18th to 21st day, and 4th instar on the 24th day. On the other hand, for AD-endosperm, no 2nd instars were observed on all days monitored. Third instars started to appear on only the 15th day until 21st day and 4th instar was noted to appear on the 24th day similar to AD-brush end treatment.

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: AD-germ vs AD-brush end ($F = 10.92$; df = 2, 90; $P = 0.00006$) and AD-germ vs AD-endosperm ($F = 4.48$; df = 2, 89; $P = 0.01397$) except for AD-brush end vs AD-endosperm ($F = 1.44$; df = 2, 91; $P = 0.24215$).

3.5. Discussion

The experiment results showed a low range of larval survival (51-53%) for the three artificially-damaged treatments. Dead larvae were observed stuck on glue underneath kernels during monitoring and data gathering. This can be attributed to the glue used to stabilize the position of the grain inside the vial which may not have fully dried within 24 hours after the first instar infestation was introduced. Other causes of the remaining failed infestation is natural death where larvae are found away from kernels and some unaccounted or missing larvae.

The graphical representation presented in Figure 3.1 clearly sums up the influence of first instar's kernel site of entry on its head capsule width with regard to speed of development. Although the fitted line showed an upward projection, data showed that at germ entry point, 70% of the larvae are turning into pupal stage on 24th day, which is 6 days earlier from the termination of 30-day monitoring period. Compared on the same day where 50% were still in the 1st and 4th instars for endosperm and 3rd and 4th instars for brush end sites. Further, it was only at the 27th day when pupae were noted for endosperm and brush end treatments. Consequently, adults showed up 3 days early or on the 27th day via germ entry while it is only until the last day of 30-day monitoring period for endosperm and brush end sites of entry.

Clearly, these findings indicated 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 noted from previous studies that first instars generally enter and establish in the germ of wheat kernels (Birch, 1945a, 1945b; Stemley, 1962) or early instars of Angoumois grain moth which is also classified as an internal feeder (Mills, 1965; Khare and Mills, 1968). In a similar experiment conducted by Osuji (1982) on artificially-drilled maize near the germ and endosperm portions, he noted that lesser grain borer larvae entering via the germ area developed faster with an abundance of frass surrounding the entrance hole. He further stated that pupae detection is observed earlier at 26 days after larval entry at the germ compared with 47 days after entry through endosperm portion. The reasons for faster development of larvae developing in the germ may be related to availability of nutrients (Potter, 1935; Osuji, 1982; Hoseney and Faubion, 1992; Cornell and Hoveling, 1998; Serna-Saldivar, 2010). In support of results of this study, MacMasters et al. (1964) found differences in nutrient structure and composition of wheat kernel. Their findings showed that germ is rich in sterols rather than in starchy endosperm. Thiamin or Vitamin B1 is completely confined in scultellum of the germ area while only small amounts are in the endosperm portion.

It is clear from the data generated by this particular experiment 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 be resistant to *R. dominica* infestation.

3.6. References

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Table 3.1. Proportion of *R. dominica* larval survival on artificially-damaged HRW wheat kernels over a period of 30 days.

Day	AD-brush end (*n* = 10)		AD-endosperm (*n* = 10)		AD-germ (*n* = 10)	
	Survival (%)	Instar (% of larva)	Survival (%)	Instar (% of larva)	Survival (%)	Instar (% of larva)
0	100	1 (100)	100	1 (100)	100	1 (100)
3	50	1 (50)	40	1 (40)	90	1 (90)
6	50	1 (50)	60	1 (60)	50	1 (50)
9	40	1 (40)	60	1 (60)	50	1 (30) 2 (20)
12	100	1 (90) 2 (10)	50	1 (50)	50	1 (30) 3 (20)
15	50	1 (20) 2 (30)	60	1 (20) 3 (40)	50	1 (10) 3 (20) 4 (20)
18	30	1 (10) 3 (20)	60	1 (20) 3 (30) 4 (10)	60	3 (20) 4 (40)
21	90	1 (30) 3 (50) 4 (10)	60	1 (20) 3 (20) 4 (20)	70	1 (10) 4 (60)
24	50	3 (10) 4 (40)	50	1 (10) 4 (40)	30	3 (10) 4 (20)
27	0*	0*	20	4 (20)	10	3 (10)
30	20	4 (20)	10	4 (10)	0*	0*

*All larvae were dead.

Table 3.2. Mean head capsule widths of larvae on artificially-damaged (AD) HRW wheat kernels.

Day	Treatment					
	AD-brush end		AD-endosperm		AD-germ	
	n	Mean ± SE Head capsule width (mm)	*n*	Mean ± SE Head capsule width (mm)	*n*	Mean ± SE Head capsule width (mm)
0	10	0.129 ± 0.002	10	0.130 ± 0.000	10	0.133 ± 0.001
3	5	0.136 ± 0.002	4	0.132 ± 0.002	9	0.135 ± 0.001
6	5	0.136 ± 0.004	6	0.138 ± 0.004	5	0.132 ± 0.002
9	4	0.137 ± 0.002	5	0.162 ± 0.012	4	0.197 ± 0.004
12	6	0.188 ± 0.003	3	0.176 ± 0.008	4	0.242 ± 0.030
15	5	0.202 ± 0.007	6	0.260 ± 0.022	4	0.355 ± 0.037
18	2	0.290 ± 0.000	4	0.317 ± 0.038	6	0.415 ± 0.011
21	6	0.311 ± 0.023	4	0.362 ± 0.041	6	0.418 ± 0.008
24	4	0.407 ± 0.002	4	0.427 ± 0.006	2	0.405 ± 0.005
27	0*	0*	2	0.410 ± 0.000	1	0.263
30	2	0.425 ± 0.015	1	0.430	0*	0*

*All dead larvae.

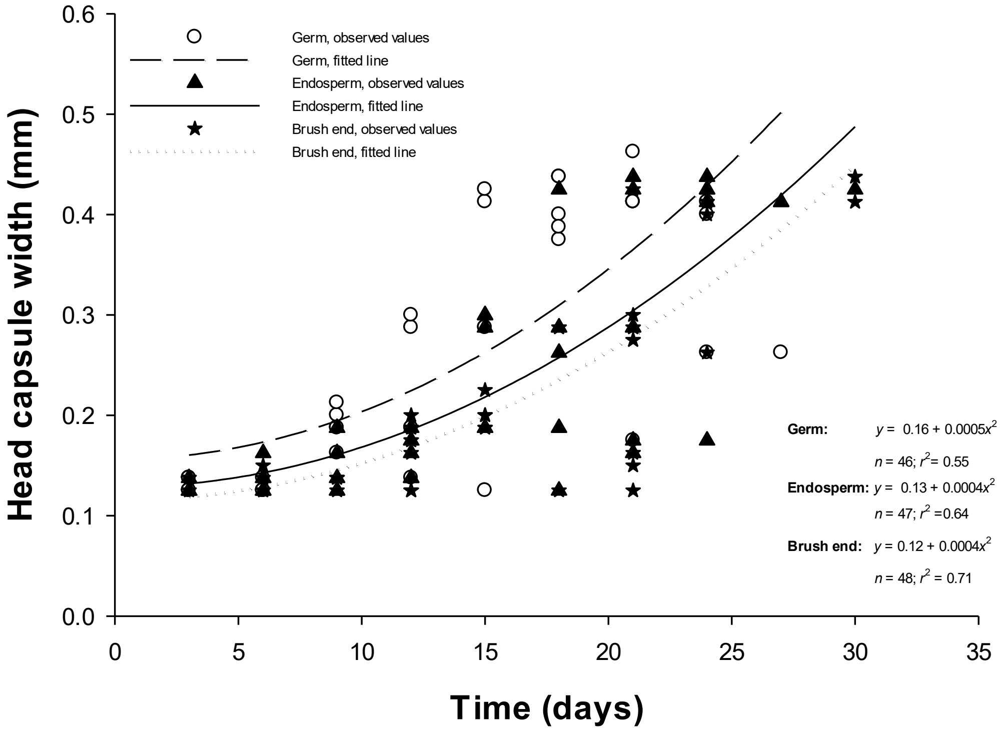

Figure 3.1. Observed and fitted lines showing relationship between head capsule widths of *R. dominica* larvae by site of entry as first instars.

Chapter 4

Short-term feeding by adults of *Rhyzopertha dominica* on successful first instar infestation of hard red winter wheat sound kernels

4.1. Abstract

Wheat, *Triticum aestivum* (L), is one of the principal grain foods attacked by one of the most destructive insect pests in storage, *Rhyzopertha dominica* (F.), also known as lesser grain borer. This study aimed to test if short-term feeding exposure by *R. dominica* adults influenced the successful infestation of first instars on wheat kernels. Two laboratory tests were conducted exposing sound hard red winter wheat grains to *R. dominica* adults and first instars feeding in successive periods under single and grouped kernel densities. Signs of feeding were monitored on the first 7 days, 14th, and 21st day on single kernels while 1, 3, 5, and 7 days for grouped grains. Data were analyzed with the McNemar statistical test to determine consistency in response between 2 life stages with 4 probable contrasted response feeding on sound wheat kernels. Results showed that single kernel 21-day exposure to short-term feeding by adults registered a higher and wider probability ranging from 27-67% compared with first instar feeding response at 10-23%. Grouped kernel findings registered a narrower range of 26-40% adult feeding while only 8-10% for first instar response. Both life stages were significantly associated in terms of feeding on these 2 types of kernel densities tested. Feeding sites on germ and brush end were strongly connected between life stages but not on endosperm portion. Perfect match up on specific feeding sites by both life stages that can contribute to successful wheat infestation were 2% for single kernels compared to 15% on grouped kernels. In conclusion, this study has established that adult short-term feeding either on sound single and grouped hard red

winter wheat kernels paved the way for first instar’s successful infestation albeit at low probability.

4.2. Introduction

Wheat, *Triticum aestivum* (L), is one of the principal grain foods attacked by one of the most destructive insect pests in storage, *Rhyzopertha dominica* (F.), also known as lesser grain borer (Cotton, 1954). In addition to wheat and other cereals, *R. dominica* have been reported feeding on peanut, kidney bean, cowpea, soybean, lentil, mung bean (Hagstrum et al., 2013), and ground dog biscuits (Cline, 1973).

It has been observed that both adult and larval feeding habits involve complete consumption of cereal kernels so that only the external shell remains (Potter, 1935; Cotton, 1954; Pajni and Shobha, 1979; USDA, 1986). Adult lesser grain borers do not feed uniformly prior to oviposition but they produce a big amount of frass or debris and increased uniformly with time (Golebiowska, 1969). Each adult female lays 300 to 500 eggs (Golebiowska, 1969) and prefers cracks and crevices sites (Stemley, 1962). It is deposited in grain mass either singly or in cluster (Schwardt, 1933; Potter, 1935; Golebiowska, 1969; Pajni and Shobha, 1979; USDA, 1986). Immature stages develop inside the kernel and emerge as an adult (Schwardt, 1933; Potter, 1935; Stemley, 1962; USDA, 1986; Reed, 2006). In sound wheat kernels, adults normally attack the germ portion (Stemley, 1962; Haines, 1991) and make tunnels into the grain. But other kernels are commonly damaged at sites other than germ as observed by Stemley (1962). In a paper by Bashir et al (2003), they have measured boring activity of an adult lesser grain borer by the amount of the dust created. Their findings showed that heavier adult males produced more dusts indicating they were more active borers.

On the other hand, first instar feeding behavior after coming out of the egg is observed to be quite active (Schwardt, 1933) and wander about freely on grain of mass before entering the grain (Birch, 1945b). Similarly, Stemley (1962) observed that immediately after emergence from egg, the larva begins to search for food. It nibbles on bits of flour or on grain debris that remained from adult feeding (Stemley, 1962; Golebiowska, 1969). It starts boring into a broken piece of wheat grain (Pajni and Shobha, 1979) or tunnel into the kernels of grain by way of abrasions made by adult beetles (Schwardt, 1933). If flour is not present, it crawls on kernels and attempt to feed on hard surfaces with little success (Stemley, 1962) and it is only after the second ecdysis that they are able to bite into the undamaged grain (Golebiowska, 1969). It has little difficulty boring into the kernel when it chooses germ as entry point. Observations show that it takes 30-35 hours to successfully enter into the kernel from egg hatching; boring time is 5-8 hours 2 days after hatching, and if not, they either die or complete development outside (Stemley, 1962). On sorghum commodity, it was reported that upon emergence from egg, first instar begins to look for food and nibble at farinaceous matter produced by adult feeding. When no damage or broken sorghum is available, first instar tried to bore the grain and it is dependent on its moisture content. If moisture content is greater than 8%, after 3 days, *R. dominica* larvae can enter into the grain sorghum consistently. Boring starts at the germ and average time required for successful entry is 20 to 40 hours (Thomson, 1966).

However, little is known about the plausible role of *R. dominica* adults in paving the way for first instars to successfully colonize a certain grain such as wheat in storage. Behavioral feeding studies earlier mentioned are either done involving egg to adult life stages, or it starts as first instar until adult emergence, or tested on feeding damage by adults alone. In this paper, two laboratory experiments were conducted wherein sound HRW wheat were first exposed to *R. dominica* adult feeding and successively exposed the same wheat class to first instar infestation and assessed its successful establishment into the kernels. Specifically, the objective was to investigate the role of *R. domnica* short-term adult feeding on probability of successful infestation by first instars on single and grouped sound wheat kernels.

4.3. Materials and Methods

4.3.1. Single sound kernel experiment

4.3.1.1 Wheat samples

Sound kernels of organic hard red winter (HRW) wheat were used for this experiment. Single wheat kernels were screened under a stereomicroscpe (Nikon SMZ 1000 Model, Nikon Instruments, Inc., Melville, NY, USA) to assure that only those without any defects or abrasions on all its surfaces were used. Individual sound organic HRW wheat kernel was placed in each of the consecutively-numbered 270 glass vials (4 ml cap.). Each glass vial was inserted into a 24-cell well plates (Corning Glass Works, Corning, NY, USA) to prevent them from rolling.

4.3.1.2 Bioassay of *R. dominica* adults on single sound kernels

Unsexed adults of mixed ages of *R. dominica* were sourced from the Stored-Product Entomology Education Research and Laboratory (SPEREL) at Manhattan, KS, USA. Adults were retrieved from bottled insect cultures, sieved, and placed in aluminum bottom pan. Using an insect brush, one adult was carefully transferred in each of the 270 individual glass vials containing 1 sound kernel at 1:1 density. Vials were covered with parafilm wax, poked with pin-sized holes for aeration purposes, and placed in the growth chamber under 28°C and 65% relative humidity (r.h.) conditions.

Adult feeding was evaluated for a total of 21 days. Evaluation was done daily for the first 7 days, followed up on the 14th day, and lastly on 21st day. Using the Table of Random Numbers, thirty (30) vials were randomly picked per day for 7 days, on the 14th, and 21st day. Each *R. dominica* adult was removed from the vial and the kernel thoroughly inspected for signs of adult feeding at the dorsal, lateral, ventral portions, and specific site of entry whether at the brush end, endosperm, or germ. Kernels with signs of adult feeding were individually marked using permanent marker. Each damaged and undamaged kernel was returned in the same numbered vial for succeeding bioassay of first instar establishment test.

4.3.1.3 Bioassay of *R. dominica* first instars on single sound kernels previously exposed to adult feeding

Prior to a short-term adult feeding bioassay tests, collection of eggs and first instars were prepared. From the same insect culture bottles sourced at SPEREL laboratory, 3-100 adults were counted and transferred on three 150-ml plastic cups containing 20 grams sifted bleached flour and placed in the same growth chamber. After 3 days, eggs were collected and placed in 3 glass petri dishes and replaced back in growth chamber. After 5 days, first instars were harvested.

At 1:1 density, on the same numbered vial, each first instar was transferred carefully to kernels previously-exposed to adult feeding that were formerly evaluated and scored. Again for 7 consecutive days, on the 14th day, and 21st day, each kernel was inspected thoroughly for larval establishment and specific site of entry.

4.3.2. Grouped sound kernel experiment

4.3.2.1 Wheat samples

The same organic HRW wheat class was used for this experiment. Likewise, the same procedure in screening sound wheat kernels was followed. After screening, ten (10) sound kernels were placed in 40 individual glass vials of 4- ml capacity. These 40 vials were further sub-divided into four 10 vials representing 4 separate days of observation period at day 1, 3, 5, and 7. Each glass vial was numbered consecutively and inserted in the same 24-cell well plates that prevented them from tipping. The vials were set aside inside the same growth chamber conditions used previously.

4.3.2.2 Bioassay of *R. dominica* adults on grouped sound kernels

The same source and procedure of preparing mixed unsexed adults of lesser grain borer were used for this test. Using an insect brush, two (2) adults were carefully transferred to infest 10 kernels per vial and repeated on each of the 40 glass vials previously prepared. The vials were covered with the same parafilm wax, poked with holes for aeration, re-inserted in 24-cell well plates, and replaced back in the same growth chamber. On day 1, using the Table of Random Numbers, 10 vials were randomly picked and adults removed from the wheat kernels. Each of the 10 kernels was thoroughly inspected on all anatomical surfaces for signs and sites of feeding and recorded. Damaged kernels were marked with permanent marker. Both undamaged and damaged kernels were replaced back inside the vial and returned to the same growth chamber for larval infestation test. The same procedure was repeated and followed for day 3, day 5, and day 7 observation periods.

4.3.2.3 Bioassay of *R. dominica* first instars on grouped sound kernels previously exposed to adult feeding

Approximately ten days before first instar infestation of adult-fed grouped sound kernels, sifted bleached flour as food medium, 100-counted adults for egg oviposition, egg collection, days of hatchability, and first instar harvesting were prepared. Again, with the use of an insect brush, 2 first instars were carefully transferred to vials of 10 wheat kernels previously exposed and infested with lesser grain borer adults. These vials were again returned to the growth chamber. On the same observation period schedule and at 10 vials per day, each of the 10 kernel per vial was inspected thoroughly for signs and sites of feeding and recorded.

4.3.3. Data analysis

The two short-term feeding studies were structured as a completely randomized design (CRD). Data for percent probability of kernel feeding by each life stage were compared and plotted using the Sigma Plot version 12.5 (Systat Software Inc., CA, USA). Daily monitoring of kernel infestation and verification of site of feeding during *R. dominica* adult exposure and after first instar feeding were assessed through significant differences ($P < 0.05$) and were determined by the McNemar Test (SAS Institute, 2015). Specifically, it was testing for consistency in responses by first instars on previously infested sound kernels by adults. Each life stage was scored as “0” if a given day or site had no signs of kernel feeding, or as “1” if a given day or site had signs of kernel feeding. Hence, for each life stage, there are one of four possible outcomes: (0,0), (1,1), (0,1), or (1,0). For a given day or site, each life stage in a given vial was contrasted in this manner.

4.4. Results

For these 2 experiments, the McNemar test statistic was applied. This test determines consistency in responses among two variables, that is, *R. dominica* adults and first instars exposed on a short-term feeding activity on separate time periods to sound kernels of HRW wheat in single and grouped units. We aimed to test whether the signs of short-term feeding exposure of adults influenced the successful infestation of first instar on HRW wheat kernels.

4.4.1. Single sound kernel experiment

Figure 4.1 showed the graphical representation of probability of short-term feeding by *R. dominca* adults and first instars on sound HRW wheat individual kernels for 21 days. Monitoring of adult feeding showed a range of 27-67% and it also indicated variability in consumption within 7 days but reflected an upward trend on the 14th and 21st days. Comparatively, the likelihood of first instar feeding on sound kernels registered a lower range of about 10-23% for the same 21-day period. Likewise, wide variability in kernel consumption within a 7-day period was observed but reflected an opposite trend on 14th and 21st days compared with adult feeding.

There were 4 possible contrasted feeding responses by each life stage on sound HRW wheat single kernel for 21 days using PROC Frequency statistic (Table 4.1). In general, about 50% (134 out of 270) of both life stages showed no signs of feeding while around 12% (33 out of 270) of them fed on the same kernels. Comparatively, there was a higher 33% (90 out of 270) chance for adult feeding than first instars while only almost 5% (13 out of 270) of the larvae fed on the kernels previously offered for consumption to adults.

The overall McNemar test statistic result (Table 4.2.) showed a highly significant association across the two life stages (df = 1; McNemar's test = 57.5631; $P < 0.0001$) upon feeding on sound HRW wheat individual kernels. Further, detailed analysis as reported in Table 4.3 revealed that majority of days monitored (6 out of 9 days) showed existence of significant association between adult and first instar infesting the kernels. Exceptions were for day 1 (df = 1; McNemar's test = 0.3.5714; $P = 0.0588$), day 3 (df = 1; McNemar's test = 0.4000; $P = 0.5271$), and day 5 (df = 1; McNemar's test = 2.2727; $P = 0.1317$) where no association across feeding by the life stages were found at $P > 0.05$ significance level.

Table 4.4 showed us the summarized contrasted responses by adults and first instars on the probability of choosing a site of feeding on sound individual kernels. About 87% (937 out of 1,080) of adults and first instars did not show any signs of feeding at all while only 2% (23 out of 1,080) of both stages showed kernel consumption on the same grain and site. Further, this differentiated responses showed 9% of adults (101 out of 1,080) preferred to feed on a specific anatomical site of the kernel compared with first instars' lack of feeding at all. On the other hand, only about 2% (19 out of 1,080) of first instars chose to feed on a specific anatomical portion of the sound individual kernel where adult showed no signs of kernel feeding at all.

Across all short-term feeding on specific sites by both life stages, Table 4.5 shows a highly significant association between stages (df = 1; McNemar's test = 56.0333; $P < 0.0001$) at 0.05 level of significance. Specific statistical results of analysis revealed that adult and first instar's choice of brush end (df = 1; McNemar's test = 12.5652; $P = 0.0004$) and germ (df = 1; McNemar's test = 38.2439; $P = < 0.0001$) portions for feeding were significant. However, no link was observed for endosperm site of feeding (df = 1; McNemar's test = 0.5000; $P = 0.4795$) for both life stages of the lesser grain borer (Table 4.6). On the precise match up on site of feeding by both adults and first instars (Table 4.7), only 23 kernels out of 270 had signs of feeding on specific anatomical sites. Specifically, about 8% (21 out of 23) of both life stages had perfect math up on germ as site of feeding, a very low of less than 1% (2 out of 23) chose brush end, and none for endosperm as feeding site.

4.4.2. Grouped sound kernel experiment

Figure 4.2 showed the general trend of percent infestation of successive short-term feeding by *R. dominica* adults and first instars exposed to sound HRW wheat grouped kernels. For seven days of monitoring, signs of adult kernel feeding registered between 26-40%. Meanwhile, after adult feeding exposures, first instars registered a low of 8-10% range of probability of feeding on the same HRW wheat kernels.

Table 4.8 showed the probability of differentiated insect responses observed when HRW sound kernels were grouped and exposed successively to 2 lesser grain borer adults and 2 first instars. Both life stages showed no signs of kernel feeding and it registered as high as 65% (258 out of 400). On the other hand, the chances of both life stages fed on same kernel registered a low of almost 8% (30 out of 400). The likelihood of adults not feeding while first instars did registered a low of 1.25% (5 out of 400) while a reversed scenario obtained a higher 27% (107 out of 400) signs of feeding. Based on this wide-ranging probability of feeding responses, McNemar test statistics obtained a significant link (df = 1; McNemar test = 92.8929; $P < 0.0001$) across all days the two life stages were exposed to feed (Table 4.9). Further analysis as shown in Table 4.10 indicated that each day of monitoring (1, 3, 5, and 7 d) was all significantly connected in terms of successive response feeding by *R. dominica* adults and first instars.

Meanwhile, taking into consideration the site of feeding as a factor on the probability of insect response, 58% of both adults and first instars (93 out of 160) did not show signs of feeding on grouped sound kernels as shown on Table 4.11. Also, 15% (24 out of 160) of both stages showed signs of feeding on the same kernel but not necessarily on all same anatomical site of feeding. Site of feeding scenarios where adults did not feed but first instars fed showed a low probability of almost 2% (3 out of 160) but when adults fed but first instar did not feed revealed a high of 25% (40 out of 160) probability of feeding scenario. The foregoing scenarios revealed an overall significant association among sites of feeding by both life stages (df = 1; McNemar test = 31.8372; $P < 0.0001$) as shown in Table 4.12. Succeeding detailed analysis (Table 4.13) showed endosperm as choice of feeding site by both adults and first instars were not significantly linked (df = 1; McNemar test = 2.2727; $P = 0.1317$) with each other. However, both brush end (df = 1; McNemar test = 13.0000; $P = 0.0003$) and germ (df = 1; McNemar test = 17.0000; $P < 0.0001$) portions sites of feeding were found to have significant association from the successive feeding activities of adults and first instars. Overall data obtained (Table 4.14) showed a high of 60% (24 out of 40) probability when both adults and first instars fed on the same grain and perfectly matched up on feeding site. Again, germ as site of feeding obtained a very high 55%

matching probability for both life stages. Both brush end and endosperm were tied at 2.5% probability.

4.5. Discussion

Consistently and majority of *R. dominica* adult feeding and first instar infestation responses were significantly associated on sound kernels of HRW wheat whether in single or grouped units. However, daily exposure of individual sound kernels to short-term feeding by adults and subsequent exposure to first instar infestation had a wide variation of associated responses on the first week of monitoring. In particular, it was observed that days 1, 3, and 5 had an insignificant association between 2 life's stages feeding action versus other days monitored. This can be due to several factors like day 1 transfer of first instars to vials with previously exposed kernels to adult feeding resulted to its disorientation of new surroundings. Day 3 and 5 insignificant feeding connection between life stages can be due to satiety of first instars on these days and led them to temporarily stop feeding on kernels. After day 5 and as the days of feeding exposure continued, there is now the sustained need to support its increased size and weight so the rest of the monitoring days (2, 4, 6, 7, 14, and 21 d) resulted in a significant link on feeding responses on exposed kernels between life stages. On the other hand, these observations were not noted on grouped kernels. Monitored days of 1, 3, 5, and 7 were found to be consistently associated in the feeding action between 2 life stages. This may be due to the fact that there were more kernels available for them to feed on within the contained vial. Also, this grouped experiment more or less simulates the actual scenario of storing grain in the field, silos and bins, and warehouse facilities.

Across all single kernel samples monitored, the germ portion was the preferred choice of site of feeding by both adults and first instars. These findings indicated a strong association between life stages. Similar results were obtained on the grouped kernel experiment. First instars indicated a higher percent infestation affinity to germ portions after adult feeding exposure. This may be due to feedings initiated by adults by way of scratches or small openings that paved the way for its easy entry. The relative softness of germ compared with other anatomical parts of the wheat kernel is another factor that can be considered. Endosperm as site of feeding for both life stages and kernel unit experiments suggested some disconnect in feeding action. This may be attributed to the hardness of endosperm compared with the germ portion of the kernel.

Precise or perfect match-up of feeding sites by adults and first instars on a given kernel is anchored on a simple understanding that it leads to successful infestation of the grain and hence, better perpetuation of insect colony. Also, this is in a way saves time and effort for first instars in expending energy by boring into kernels. Instead, searching for feeding sites that are already available for them through abrasions and openings will be more beneficial to speed up growth and development within the infested kernel. Based on these premises, results showed that there were only 2% (23 kernels) of precise match-up found in single kernel experiment out of the total 1,080 kernels with contrasted responses. Although the data indicated a small percent of perfect match-up of feeding sites by both life stages on single kernels, it can still be a substantial result in affirming our hypothesis that *R. dominica* adults provide an opportunity for first instars to successfully establish an infestation in sound kernels of HRW wheat. Comparatively, across all our grouped kernel experiment of 160 samples, a 15% (24 kernels) perfect match-up was obtained. Of these, 55% (22 kernels) had perfect match up with germ portion while only 2.5% (1 kernel) for both brush end and endosperm were found. One implication for the results on grouped kernel study was that it gave us a rough estimate and possibly better scenario in our field storage facilities of a higher probability of infestation if adult feeding on kernel precisely matches-up with the response feeding of first instars on wheat grains.

In conclusion, this study has established that adult short-term feeding either on sound single or grouped HRW wheat kernels provided a successful infestation opportunity for first instar even though at low probability.

4.6. References

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Table 4.1. 21-day probability of contrasted responses by *R. dominica* adult feeding and first instar infestation on sound HRW wheat single kernels.

R. dominica short-term feeding/infestation[1]		Count	Percent (%)
Adults	First instars		
0	0	134	49.63
0	1	13	4.82
1	0	90	33.33
1	1	33	12.22
Total		**270**	**100.00**

[1] "0" - without feeding signs on kernels; "1" - with feeding signs on kernels.

Table 4.2. Overall McNemar test results on contrasted responses of *R. dominica* adult feeding and first instar infestation shown in Table 4.1.

Total days of observations	*R. dominica* short-term feeding/infestation (%)		McNemar test statistic	df	*P*- value*
	Adults	First instars			
9 days	82.96	17.04	57.5631	1	< 0.0001

[*] There were significant association between short-term adult feeding and first instar infestation on sound HRW wheat single kernels.

Table 4.3. McNemar test results on daily short-term feeding by *R. dominica* adults and first instar infestation on sound HRW wheat single kernels.

Day[a]	*R. dominica* short-term feeding/infestation (%)		McNemar test statistic	df	*P*- value*
	Adults	First instars			
1	90	10	3.5714	1	0.0588
2	83.33	16.67	6.4000	1	0.0114
3	73.33	26.67	0.4000	1	0.5271
4	90	10	6.2308	1	0.0126
5	80	20	2.2727	1	0.1317
6	80	20	10.2857	1	0.0013
7	83.33	16.67	8.0000	1	0.0047
14	80	20	10.2857	1	0.0013
21	86.67	13.33	16.0000	1	< 0.0001

[a] $n = 30$ vials per day; 1 sound kernel with 1 adult or first instar per vial.

*There were significant association between short-term adult feeding and first instar infestation on sound HRW single kernels at $P < 0.05$ level of significance.

Table 4.4. Probability of contrasted responses on specific site of feeding by *R. dominica* adult and first instar infestation on sound HRW wheat single kernels.

R. dominica short-term feeding/infestation[1]		Count	Percent (%)
Adults	First instars		
0	0	937	86.76
0	1	19	1.76
1	0	101	9.35
1	1	23	2.13
Total		**1,080**	**100.00**

[1] “0” - without feeding signs on kernels; “1” - with feeding signs on kernels.

Table 4.5. Overall McNemar test results on contrasted responses on specific site of feeding by *R. dominica* adults and first instar infestation shown in Table 4.4.

Total number of sites observed	*R. dominica* short-term feeding/infestation (%)		McNemar test statistic	df	*P*- value[1]
	Adults	First instars			
4 sites	96.11	3.89	56.0333	1	< 0.0001

[1] There were significant response between adult feeding and first instar infestation on site of feeding on sound HRW wheat single kernels.

Table 4.6. McNemar test results on specific site of feeding by *R. dominica* adults and first instar infestation on sound HRW wheat single kernels.

Site of kernel feeding[1]	*R. dominica* short-term feeding/infestation (%)		McNemar's test statistics	df	*P*- value[2]
	Adults	First instars			
Brush end	98.15	1.85	12.5652	1	0.0004
Endosperm	98.89	1.11	0.5000	1	0.4795
Germ	87.41	12.59	38.2439	1	< 0.0001
Others	-	-	-	-	-

[1] n = total of 1080 samples.

[2] There were significant association on the choice of feeding site by adults and first instars on sound HRW wheat single kernels at $P < 0.05$ level of significance.

Table 4.7. Probability of precise match up of *R. dominica* adult feeding and first instar infestation specific site of feeding on sound HRW wheat single kernels.

Site of feeding	Kernel feeding/infestation (count)		Percent (%)
	Adults	First instars	
Brush end	2	2	0.74
Endosperm	0	0	0
Germ	21	21	7.78

Table 4.8. 7-day probability of contrasted responses by *R. dominica* adult feeding and first instar infestation on sound HRW wheat grouped kernels.

R. dominica short-term feeding/infestation[1]		Count	Percent (%)
Adults	First instars		
0	0	258	64.50
0	1	5	1.25
1	0	107	26.75
1	1	30	7.50
Total		**400**	**100.00**

[1] "0" - without feeding signs on kernels; "1" - with feeding signs on kernels.

Table 4.9. Overall McNemar test results on contrasted responses of *R. dominica* adult feeding and first instar infestation shown in Table 4.8.

Total days of observations	*R. dominica* short-term feeding/infestation (%)		McNemar test statistic	df	*P*- value[1]
	Adults	First instar			
4 days	91.25	8.75	92.8929	1	< 0.0001

[1] There were significant association between adult feeding and first instar infestation on short-term feeding on sound HRW wheat grouped kernels.

Table 4.10. McNemar test results on short-term feeding by *R. dominica* adults and first instar infestation on sound HRW wheat grouped kernels.

Day[1]	*R. dominica* short-term feeding/infestation (%)		McNemar test statistic	df	*P* – value[2]
	Adults	First instars			
1	92	8	13.5000	1	0.0002
3	92	8	28.1250	1	< 0.0001
5	90	10	28.1250	1	< 0.0001
7	91	9	24.0000	1	< 0.0001

[1] *n* = 10 vials per day; 10 sound kernels with 2 adults and/or first instars per vial.

[2] There were significant association between adult feeding and first instar infestation short-term feeding on kernels at $P < 0.05$ level of significance.

Table 4.11. 7-day probability of contrasted responses on specific site of feeding by *R. dominica* adult feeding and first instar infestation on sound HRW wheat grouped kernels.

R. dominica short-term feeding/infestation[1]		Count	Percent (%)
Adults	First instars		
0	0	93	58.13
0	1	3	1.88
1	0	40	25.00
1	1	24	15.00
Total		**160**	**100.00**

[1] “0” - without feeding signs on kernels; “1” - with feeding signs on kernels.

Table 4.12. Overall McNemar test results on contrasted responses on specific site of feeding by *R. dominica* adults and first instar infestation shown in Table 4.11.

Total sites of feeding	*R. dominica* short-term feeding/infestation (%)		McNemar test statistic	df	*P*- value[1]
	Adults	First instars			
4 sites	83.13	16.88	31.8372	1	< 0.0001

[1] There were significant association between adults and first instars on site of feeding choices on sound HRW wheat grouped kernels.

Table 4.13. McNemar test results on specific site of feeding by *R. dominica* adults and first instar infestation on sound HRW wheat grouped kernels.

Site of kernel feeding[1]	*R. dominica* short-term feeding/infestation (%)		McNemar test statistic	df	*P*- value[2]
	Adults	First instars			
Brush end	97.50	2.50	13.0000	1	0.0003
Endosperm	90	10	2.2727	1	0.1317
Germ	45	55	17.0000	1	< 0.0001
Others	-	-	-	-	-

[1] *n* = total of 160 samples.

[2] There were significant association on the choice of feeding site by adults and first instar infestation on sound HRW wheat grouped kernels at $P < 0.05$ level of significance.

Table 4.14. Probability of precise match up of *R. dominica* adult feeding and first instar infestation specific site of feeding on sound HRW wheat grouped kernels.

Site of feeding	Kernel feeding/infestation (count)		Percent (%)
	Adults	First instars	
Brush end	1	1	2.5
Endosperm	1	1	2.5
Germ	22	22	55.0

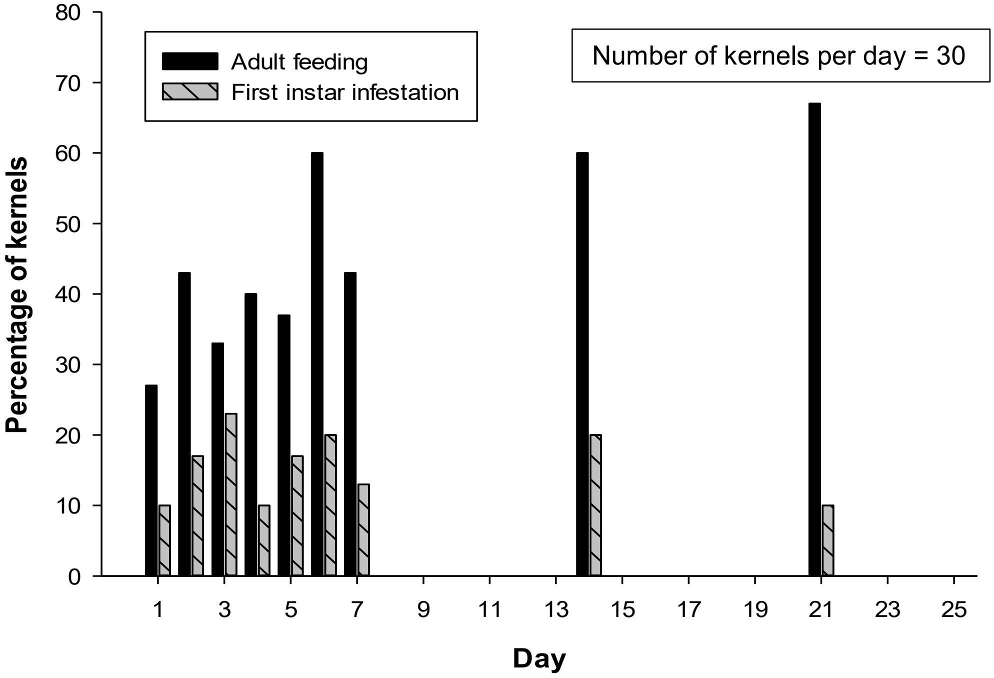

Figure 4.1. Percentage of kernels successfully fed by *R. dominica* adults and infested by first instars on sound hard red winter wheat single kernels.

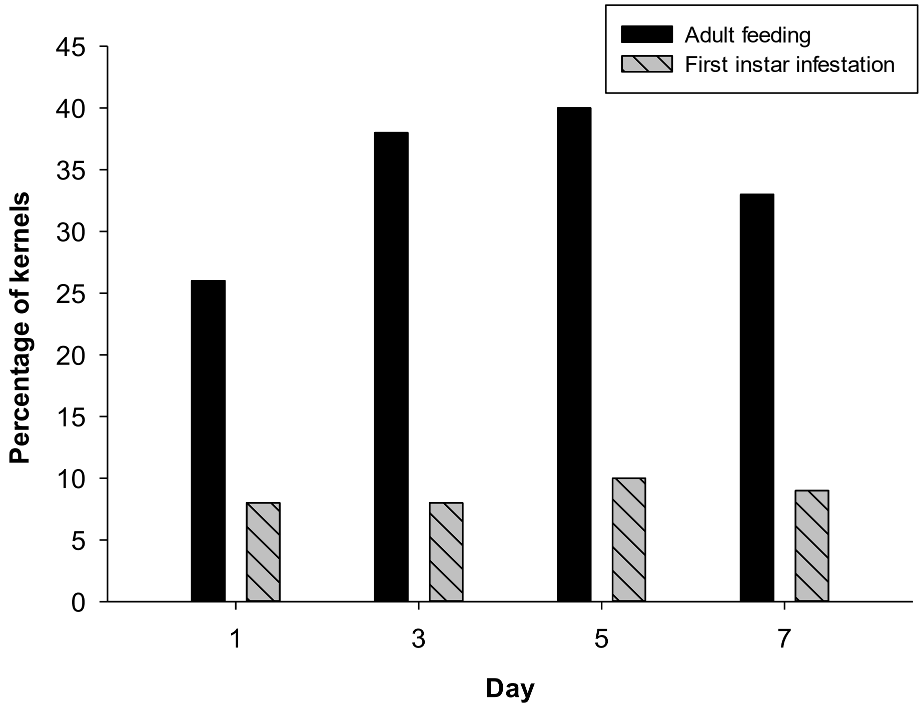

Figure 4.2. Percentage of kernels successfully fed by *R. dominica* adults and infested by first instars on sound hard red winter wheat grouped kernels.

Chapter 5

Probability of infestation of sound and artificially-damaged kernels by *Rhyzopertha dominica* first instars on six classes of wheat

5.1. Abstract

Rhyzopertha dominica (F.) is a primary insect pest of stored grain worldwide. Both larvae and adults are serious pests of wheat and a wide range of other commodities. Our experiment goal was to compare successful *R. dominica* first instar infestation and track its subsequent development into adults on six classes of wheat. Specifically, we investigated its probability of infestation, larval development by head capsule width, larval weight, kernel weight loss, and adult emergence as influenced by site of entry. Four treatments of 100 individual sound and artificially-damaged kernels from each wheat class were used in these laboratory experiments. Larval successful infestation was assessed by dissecting kernels at 21st day. Tracking of *R. dominica* first instar duration to adult emergence was monitored from 27th to 50th day. Findings revealed that sound kernels of all six wheat classes had 2- to 11-fold lower probability of first instar infestation than in artificially-damaged kernels. Statistically, all wheat classes that were artificially-damaged at germ, brush end, and endosperm sites were equally important on first instar manner of infestation. First instar's germ preference for site of entry on all wheat classes showed faster larval development, heavier larval weight, and higher 21 d kernel weight loss. However, 50 d kernel weight loss had wider variability on other wheat classes. At 50 d monitoring, 5 out of 6 wheat classes showed shorter duration to adult emergence at 34 days if first instars entered via germ compared with 42 d on other sites. However, the soft white wheat class experiment showed non-emergence of adults on all treatments tested. Hence, all six wheat

classes tested showed that sound kernels prevent the success of first instar infestation. Germ as entry site conferred positive influence on insect larval growth and weight, 21d kernel weight loss, and adult emergence.

5.2. Introduction

Economically, *Rhyzopertha dominica* (F.) is one of the most important and destructive pests infesting stored-grains (Limonta et al., 2011) in the United States (Christensen and Kaufmann, 1969) and worldwide (Özkaya et al., 2009). It is considered to be cosmopolitan (Potter, 1935; Hagstrum and Subramanyam, 2009) or worldwide in distribution (Krischik and Burkholder, 1991; Hagstrum et al., 2013). It can infest a wide range of commodities (USDA, 1986; Haines, 1991) or damage 115 types of food products (Hagstrum and Subramanyam, 2009; Hagstrum et al., 2013). Both the adults and larvae are voracious feeders (Potter, 1935; Golebiowska, 1969; Toews et al., 2000; Hagstrum et al., 2013). It is considered a primary insect because its manner of infestation is to bore into hard grain materials (Reed, 2006) and subsequent development is spent inside it (Stemley, 1962; Osuji, 1982) until adult emergence. On average, its minimum life cycle is 25 days at 34ºC (Hagstrum et al., 2013). Adults are 2-3 mm long, reddish brown in color, cylindrical or bullet-shaped in appearance, and slow moving with the tendency to remain in a specific area (Haines, 1991; Reed, 2006). Its head is hidden under the thorax and has club-shaped antennae on its last 3 segments (Hagstrum et al., 2013). Females lay their eggs either singly or in the form of a raft (Potter, 1935) in a mass of grain or frass produced through insect feeding (Edde, 2012). The first instar that hatches from egg are elongated in shape and actively moves about the grains. It is white in color, slightly yellowish towards the head, mouth parts brownish, the head has very short antennae (Potter, 1935) and has soft body and a stiff head capsule (Reed, 2006). This head capsule is the anatomical part of the larva that is used to measure and determine developmental rates of its 4 larval stages inside a kernel (Stemley, 1962). Upon eclosion, the larva searches for food and start to feed through morsels of flour left by adult feeding or tries the hard surface of wheat and usually succeeds to enter through its germ portion (Stemley, 1962). It completes its growth within the grain, transforms into pupa (USDA, 1986), and immature adult usually prefers to remain inside until it is ready to move out of the infested grain to feed (Schwardt, 1933) and mate shortly after emergence (Haines, 1991). A round hole is created by emerging adults (Haines, 1991; Reed, 2006). This exit hole causes the kernel to be considered an insect-damaged kernel (IDK) under the US grain grading system (Reed, 2006), hence, it is a basis for rejection of the wheat grain being traded in the market.

In the 2015 US census, 433,105 thousand metric tons (15.9 million bushels) of HW wheat were harvested from 440,000 acres of land. Meanwhile, SW wheat had 4.5 million metric tons (168 million bushels) harvested in almost 3 million acres all over United States (www.nass.usda.gov; NAMA, 2016). Hard white wheat is used in pan bread and can also be used in making hamburger buns, steamed bread, pita bread, and tortillas (Chang et al., 1995). Soft white wheat is used in a wide variety of baked products such as cookies, biscuits, cakes, oriental noodles, steamed breads, pastries, (Nemeth et al., 1994) wafers, pretzels, and crackers (Hoseney et al., 1988). Durum (*Triticum durum*) and the SRW wheat crops are among the classes of wheat produced and traded here in the US. Durum was introduced in North America around year 1900 from Russia (Dick and Matsuo, 1988). Currently, it is produced in the northern part of the US like North Dakota and Montana (www.nass.usda.gov). For the period covering 2015 US survey of crops, durum wheat was harvested in 767,284 thousand hectares (1,896,000 million acres) producing 2.2 billion metric tons (82,484,000 bushels) (www.nass.usda.gov). Durum wheat products include pasta such as spaghetti, lasagna, elbow macaroni, vermicelli, couscous, bulgur, etc. (Hoseney et al., 1988). Soft red winter wheat crops are grown within the vast agricultural lands of the US such as the Central states (Indiana, Michigan, Ohio, West Virginia, and

Wisconsin), Mid-Atlantic states (Delaware, Maryland, New Jersey, New York, and Pennsylvania), Midwest (Illinois, Kentucky, and Missouri), Southeast states (North Carolina, South Carolina, and Virginia), and South/Delta/Southwest states (Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, Oklahoma, Tennessee, and Texas) (McGaughey et al., 1990; NAMA, 2016). SRW wheat was harvested in 2,717,464 million hectares (6,715,000 million acres) producing 9.7 billion metric tons (359,055,000 bushels) according to the 2015 crop survey (www.nass.usda.gov; NAMA, 2016). Its manufactured product includes crackers, soup mixes, and gravy thickeners (Hoseney et al., 1988). The 2016 USDA-National Agricultural Statistics Service (NASS) reported that US total production of hard red spring (HRS) wheat class reached 13.4 million metric tons (MMT) (493.1 million bushels). It is grown in the states of Idaho, Minnesota, Utah, South Dakota, Colorado, Washington, Montana, North Dakota, and Oregon. According to US Wheat Associates (2016) statistics, HRS wheat is exported worldwide and a total of 8.0 MMT was sold for year 2016. It is considered an aristocrat among wheat classes because it is labeled as “designer” wheat such as hearth breads, croissants, rolls, bagels, and pizza crust. Meanwhile, HWS wheat class’ total reported production amounted to only 205.2 thousand metric tons (7.5 million bushels) and is grown in less number of states such as Idaho, Utah, Colorado, Oregon, Nevada, and Washington (USDA-NASS, 2016). It is processed into Asian-style noodles, whole wheat while flour, tortillas, pan and flat breads (US Wheat Associates, 2016).

According to Semple (1992), wheat is a highly infestible grain commodity. As mentioned earlier, it is one of the 115 various commodities infested by *R. dominica* as enumerated by Hagstrum and Subramanyam (2009) and Hagstrum et al. (2013). There are several factors in the successful infestation of LGB first instars and adults to a stored-product commodity like wheat.

One of the factors is the physical or morphological make-up of the wheat grain. The presence of defects in the outer layers of the whole wheat grain provides efficiency and strongly influence the penetration of infesting larvae. Therefore, wheat grains that possess strong, intact outer layers will exhibit a certain level of resistance to *R. dominica* first instar (Semple, 1992). He further stated that there is a reduction in the oviposition frequency to about 12% of the normal rate if there is the absence of broken grain on which adult lesser grain borer can feed on. Rice, a related cereal commodity, was observed to have a fast multiplication of LGB population in paddy form. It is dependent on certain varietal characteristics such as the failure of the husk to close properly around the kernel. This morphological defect makes a small quantity of the loose stock of paddy susceptible to *R. dominica* colonization (Semple, 1992). Seed hardness is another physical factor that can be considered in the successful infestation and multiplication of LGB first instar and adult stages. Currently, hardness of wheat grain is measured using the device called Single Kernel Characterization System or SKCS. It measures grain hardness by crushing each kernel one at a time and recording the force required. After which it averages the force for crushing the kernels in terms of a unit-less measurement score termed as hardness index (HI) (Posner and Hibbs, 2005). Generally, a wheat with an HI score of 75 is classified as hard and 25 HI score is a soft wheat (McGaughey et al., 1990). Durum as a class has a horny tough endosperm and is the hardest species of wheat known (Irvine, 1964). The harder the seed, the less the pericarp is vulnerable to penetration by larvae of *R. dominica* (Semple, 1992). In an experiment conducted in Turkey by Özkaya et al. (2009), they concluded that there is a larger number of population of *R. dominica* recorded in the soft wheat versus hard wheat varieties. But, their study distinguished soft and hard wheat based on two commonly grown bread wheat varieties of ‘Gerek-79’ as soft wheat (particle size index 29) and ‘Gün-91’ as hard wheat (particle size index 14). Another factor cited by Semple (1992) is the nutritional component of a seed or grain. In a paper by Limonta et al. (2011), lesser grain borer first instar had difficulty and needed a longer time in boring sound kernels of durum. However, when semolina or debris is added, drilling of kernels is shortened. Similarly, first larvae that emerged into adults from colonizing sound durum kernels is at 32 days compared with only 27 days of adult emerging when first instar is reared on semolina diet. The last factor to consider would be varietal characteristics within classes of wheat. Colonization or susceptibility of various classes of wheat to the LGB is documented by McGaughey et al. (1990; Sinha et al., 1988). Their findings showed that the progeny of LGB is more on durum wheat compared with soft red winter. For the soft red winter wheat class, 7 varieties were evaluated on LGB susceptibility. 'McNair 1003" variety posted the highest number of LGB progeny over other 6 varieties. But, this progeny differences can only be significantly attributed with the lowest ranked varieties such as 'Arthur 71', 'Pike', and Florida 302'. In this research, we have sought to understand and determine the effect of sound and artifically-damaged kernels of six classes of wheat on probability of infestation and site of entry preference by *R. dominica* first instars

5.3. Materials and Methods

The materials used and procedures followed on this particular experiment were all the same as discussed in experiment 2 of Chapter 2. This includes the screening of sound kernels of six wheat classes, *R. dominica* adult oviposition and collection of eggs, micro-drilling of sound kernels, infestation of first instars into the kernels at 1:1 kernel-to-first instar infestation ratio, calibration of steromicroscope, random sampling of kernels for dissection contained in 24-cell well plates (Corning Glass Works, Corning, NY, USA), individual weighing of larva, monitoring of duration to adult emergence, and 21 d and 50 d kernel weight loss. The difference was the use of a 24-cell well plates where kernels of all six wheat classes were contained compared with the use of glass vials for the HRW expeiments as mentioned in Chapter 2. Another difference was the determination of duration to adult emergence. For these six experiments, an adult was considered to have emerged if its body width (0.8-1.0 mm) is equal to or greater than the diameter of the exit holes. This strategy was used as an improvement on experiment 1 and 2 of Chapter 2 on HRW wheat kernels. Also, it was a way to address the limitation of the study where monitoring of individual kernels was not done on a 24-hour basis. Kernel weight loss estimation as expressed in percentage was determined after adult had emerged from the kernel and weighed individually in the analytical balance. The same formula of % kernel weight loss due to 21d and 50 d feeding periods was similarly applied.

5.3.1. Profiles of quality characteristics of six wheat classes

5.3.1.1 Hard White wheat and Soft White wheat samples

The HW wheat samples were obtained from the United States Department of Agriculture-Agricultural Research Services, Center for Grain and Animal Health Research (USDA-ARS-CGAHR) based in Manhattan, KS, USA. It was originally sourced from Oklahoma (coded OK08707W) but no information was available if treatments were applied on this wheat class sample. The SW wheat samples were acquired in November 2014 from the milling laboratory storage room of the Department of Grain Science and Industry, Manhattan, KS, USA. It was originally sourced from the Agronomy North Farm and Shop – Kansas Foundation Seed Project of the Department of Agronomy, Kimball Avenue, Manhattan, KS, USA. The history of seed treatments was unknown. The 2 wheat class samples were stored in the laboratory freezer (-13°C) for two weeks to kill any residual live insects present. After freezing, about 600 g were placed in 2 separate labeled glass jars and placed in the growth chamber at 28°C and 65% r.h. for a week to equilibrate the moisture content. The average (mean ± SD) moisture content of the grain samples were 14.0 ± 0.46% for HW wheat and 13.2 ± 0.50% for SW wheat. Also verified for each wheat class was the average (mean ± SD) hardness index (HI) values of 69.72 ± 8.11 for HW wheat and 30.72 ± 10.72 for SW wheat. These measurements were all determined using the SKCS machine.

5.3.1.2 Durum wheat and Soft red winter wheat samples

The durum wheat samples were sourced in November 2014 from the GRSCI milling laboratory storage room. It was acquired from the Agronomy North Farm – Kansas Foundation Seed Project of the Department of Agronomy, Kimball Avenue, Manhattan, KS, USA. The history of seed treatments were unknown. The SRW wheat samples were secured from the USDA-ARS-CGAHR laboratory. It was originally sourced from Labette County, KS with a variety code of Pioneer 25R39 but no information was available as far as seed treatments were concerned on this wheat class sample. Prior to a week of equilibration of moisture content in the same growth chamber conditions, these 2 wheat samples were also stored in the same laboratory freezer for two weeks to kill any live insects present. The measurements for moisture contents and hardness index were all determined using the same SKCS apparatus. Durum had an average (mean ± SD) moisture content of 11.7 ± 0.80% and 12.4 ± 0.42% for SRW. The average (mean ± SD) hardness index value of durum was 86.40 ± 12.86 and 18.39 ± 22.44 for SRW wheat.

5.3.1.3 Hard red spring and Hard white spring samples

Both HRS and HWS wheat class samples were sourced from the GRSCI milling laboratory storage room. It was originally obtained from the Agronomy Research North Farm, Manhattan KS, USA. The seed treatment history of the samples were unknown at the rime of acquisition from the milling laboratory store room. In order to disinfest any residual insects present, the wheats were stored in the same laboratory freezer for couple of weeks. After disinfestation, about 800 g were placed in glass jars and held in the same growth chamber conditions for a week to equilibrate the moisture content. The moisture content of HRS samples was 12.5 ± 0.53% (mean ± SD) and the HWS samples was 12.0 ± 0.3%. The average hardness index (HI) of 76.94 ± 15.58 was obtained for HRS wheat class while 88.92 ± 13.71 HI for HWS samples. All these 2 wheat quality parameters were determined using the SKCS apparatus at the milling labaoratory of GRSCI department.

5.3.2. Data Analysis

The same CRD was used for the six experiments conducted. All data for six wheat classes on probability of infestation, measured head capsule width of larvae, larval weight, kernel weight loss, and adult emergence used the SAS Generalized Linear Mixed Model (PROC GLIMMIX) (SAS Institute, 2012; 2015). The least squares means was used to determine differences of treatments and further subjected to adjustment for multiple comparisons using the Pairwise Bonferroni test method. In order to determine existence of association on preference of first instar on kernel site of entry, we have used SAS PROC FREQ computing for Chi-square test. All tests results were compared with $\alpha = 0.05$ level of significance.

5.4. Results

5.4.1. Hard White (HW) wheat class at 21-day *R. dominica* first instar infestation

Table 5.1 showed there was a higher range of probability of infestation by first instar for artificially-damaged (AD) kernels at the brush end, endosperm, or germ at 66-82% compared with 10% for sound kernels at 21 days. Statistics showed significant infestation differences (F = 14.19; df = 3, 196; P = < 0.0001) among treatments using the PROC GLIMMIX, Type III tests of fixed effects. Further analysis using t-test with Bonferroni adjustment (Table 5.2), we obtained adjusted *p*-values showing sound kernels were all significantly resistant compared with AD-brush end (*t* = 5.54; *P* = < 0.0001), with AD-endosperm (*t* = 5.13; *P* = < 0.0001), and AD-germ (*t* = 6.21; *P* = < 0.0001). No significant differences in infestation were found between AD-brush end vs. AD-endosperm (*t* = 0.65; *P* = 1.0000); AD-brush end vs. AD-germ (*t* = -1.18; *P* = 1.0000); and AD-endosperm vs. AD-germ (*t*-value = -1.80; *P* = 1.0000).

The preferred site of entry of first instar at 21 days on all AD kernel treatments (Table 5.3) was the germ portion (36%), followed by brush end (31%), and endosperm (29%). The sound treatment had 4 out of 5 kernels (3.5%) entered via germ portion. Chi-square test showed significant (χ^2 = 27.2957; df = 3; *P* = < 0.0001) association among treatments. Results of "R" statistics at 95% confidence interval detected significant differences between sound kernel and all AD-kernel treatments due to non-overlapping of confidence intervals but not among AD-wheat grains.

As shown in Table 5.4, both sound kernels and AD-germ entry points registered the biggest mean head capsule width with both 0.43 mm measurements. This was further confirmed by instar classification, wherein 85% of larvae were already 4th instara and almost 10% were in pupal stage after they entered via AD-germ portion. Whereas for sound kernel treatment, 4 out of 5 entry points were at the germ portion (see Table **5.3**) and also noted to be at the 4th instar. On the other hand, AD-endosperm entry point registered the smallest head capsule width at 0.30 mm. Also, only 24% were in 4th instar, 54% still at the 3rd instar, 3% at 2nd instar, and 18% at 1st instar. Our statistical analysis results indicated that head capsule width of first instars at 21 days were significantly influenced by sites of entry (*F* = 28.28; df = 3, 107; *P* = <0.0001. Results of the least squares means using *t*-test with Bonferroni adjustment, showed significant differences among treatments of AD-germ versus: AD-brush end (*t* = -6.79; *P* = < 0.0001) and AD-endosperm (*t* = -8.22; *P* = < 0.0001) and sound kernels versus: AD-brush end (*t* = -3.43; *P* = 0.0052) and AD-endosperm (*t* = -4.20; *P* = 0.0003) (Table 5.5).

Shown in Table 5.6 is the average larval weight of different treatments having significant differences (*F* = 43.54; df = 3, 79; *P* = < 0.0001). Bonferroni adjustment test as presented in Table 5.7 revealed that AD-brush end treatment was significantly lighter in larval weight over AD-endosperm (*t* = -2.74; *P* = 0.0456), AD-germ (*t* = -10.85; *P* = < 0.0001), and sound kernels (*t* = -5.76; *P* = < 0.0001). Also, larvae from AD-endosperm treatment were significantly lighter in weight compared with AD-germ (*t* = -5.58; *P* = < 0.0001) and sound kernels (*t* = -3.57; *P* = 0.0037). But, larval weights from AD-germ and sound kernel treatments were found to be equally heavier from each other (*t* = -0.17; *P* = 1.0000).

The mean % weight loss of kernels due to larval feeding for 21 days on all treatments was found to be significantly different with each other (*F* = 35.80; df = 3, 111; *P* = < 0.0001) utilizing the PROC GLIMMIX procedure (Table 5.8). The Bonferroni Multiple Comparison Adjustment Test (Table 5.9) showed that the 3.61% kernel weight loss for AD-endosperm treatment was significantly lowest compared with AD-brush end (*t* = 3.45; *P* = 0.0048), AD-germ (*t* = -9.00; *P* = < 0.0001), and sound kernels (*t*-value = -6.84; *P* = < 0.0001). Likewise, AD-brush end's 7.21% kernel weight loss versus sound kernel (*t* = -5.14; *P* = < 0.0001) and AD-germ (*t* = -5.57; *P* = < 0.0001) were significantly different. The 2 highest % kernel weight loss that were obtained between sound kernels and AD-germ treatment were found to be insignificantly different (*t* = -2.49; *P* = 0.0862) from each other.

5.4.2. Hard White (HW) wheat class at 50-day monitoring of *R. dominica* adult emergence

Overall, the probability of successful infestation of LGB first instar on AD-HW wheat and sound kernels from day 1 ranged from 66 - 84% and 8%, respectively. However, successful adult emergence up to day 50 decreased to 56 - 76% on all AD-kernels and 6% for sound kernels (Table 5.10). In terms of average adult emergence (days) for different treatments, the sound kernel treatment posted an odd shortest adult emergence at 33 days. This was due to the actual observation that the 3 out of 4 larvae had entered through the germ portion as origin of site of entry. AD-germ kernels posted the second shortest emergence at 34 days, followed by 40 days for AD-brush end, and 43 days for AD-endosperm treatment. Results of PROC GLIMMIX statistics showed significant differences ($F = 36.83$; df = 3, 103; $P = < .0001$) on mean adult emergence among four treatments. Table 5.11 results of *t*-test with Bonferroni adjusted *p*-values further revealed strong evidence of differences on adult emergence between AD-brush end and AD-germ ($t = 8.07$; $P = < 0.0001$) and sound kernels ($t = 3.51$; $P = 0.0040$); AD-endosperm versus AD-germ ($t = 9.22$; $P = < 0.0001$) and sound kernels ($t = 4.20$; $P = 0.0003$). No significant differences on duration to adult emergence between kernel treatments of AD-brush end and AD-endosperm ($t = 3.45$; $P = 0.4631$) as well as between AD-germ and sound kernels ($t = 0.42$; $P = 1.0000$) were obtained.

The range of 50 d mean % kernel weight loss due to first instar to adult feeding was observed to be 18 - 24% on all treatments examined (Table 5.12). Both AD-brush end and sound kernel treatments were found to have registered the 2 highest % kernel weight losses at about 25% and 24%, respectively. AD-endosperm treatment obtained the lowest % weight loss at almost 19% while AD-germ's % value loss was almost 1% higher than AD-endosperm treatment. PROC GLIMMIX statistical results showed that there were significant weight losses among treatments (*F* = 7.03; df = 3, 103; *P* = 0.0002). Further analysis through multiple comparisons *t*-test with Bonferroni adjustments showed significant weight losses between AD-brush end and AD-endosperm (*t* = 3.97; *P* = 0.0008) and AD-germ (*t* = 3.79; *P* = 0.0015) treatments. All other treatment comparisons showed equal mean % kernel weight losses as shown in Table 5.13.

5.4.3. Soft White (SW) wheat class at 21-day *R. dominica* first instar infestation

Table 5.14 showed that the chances of infestation on artificially-damaged SW wheat kernels by first instar were higher at 76-96% range while undamaged kernels had 56%. Statistical results on 4 treatments using PROC GLIMMIX were noted to be significantly different on infestation rates with each other (*F* = 8.51; df = 3, 196; *P* < 0.0001). The probability of rate of infestation on sound kernels was significantly lower versus both AD-brush end and AD-endosperm (*t* = 3.79; *P* = 0.0012) but equally the same with AD-germ treatment (*t* = 2.09; *P* = 0.2293). Likewise, multiple comparisons of other treatments showed equal chances of infestation as presented in Table 5.15 – Bonferroni adjustment test results.

Presented in Table 5.16 was the preferred site of entry for all 4 treatments of SW wheat kernels. Results indicated that the brush end and endosperm portions were the preferred site with 30% or 48 kernels infested. This was followed by germ portion at 23% or 38 kernels. Preference for the sound kernel treatment had mixed results with 10% (16 kernels) entering via endosperm, 6% (10 kernels) at germ, and 1% (2 kernels) at brush end portion. However, Chi-square test result showed similar preferences (χ^2 = 6.7901; df = 3; *P* = 0.0789) across treatments examined at the α = 0.05 level.

Table 5.17 showed that the influence of site of entry on head capsule width of *R. dominica* at 21 days was very evident with AD-germ treatment registering the biggest average head width at 0.42 mm. The smallest average head width at 0.38 mm was obtained from larvae entering via AD-endosperm treatment. Further, 92% of those that entered via AD-germ were already at the 4th instar while only 60% were on the same larval stage via AD-endosperm kernels. Also worth noting on endosperm portion entry was that 35% were still on 3rd instar while only about 8% for germ entry point. The former had 2% of larvae both at 2nd and 1st instars while the latter had nothing of the same larval stage. Using PROC GLIMMIX, significant differences were detected on the 4 treatments tested ($F = 4.21$; df = 3, 158; P-value = 0.0068). However, significantly different head capsule width was only found between AD-endosperm and AD-germ treatments ($t = -3.34$; $P = 0.0064$) using Bonferroni multiple comparison adjustment test as shown in Table 5.18. The rest of the pair-wise treatment comparison tested show insignificant effect of site of entry on head capsule width of larvae at 21 days.

The average larval weight (Table 5.19) among treatments was found heaviest on AD-germ at 0.84 mg. Sound kernel treatment with 0.70 mg ranked second and third for AD-brush end with 0.62 mg. The lightest of all larval weight of 0.61 mg was noted on AD-endosperm treatment. These differences in larval weights were found to be significantly different with each other using the PROC GLIMMIX statistical procedure ($F = 4.82$; df = 3, 155; $P = 0.0031$). But the larval weight with significant differences were only found between AD-germ and AD-brush end ($t = -3.26$; $P = 0.0082$) and AD-endosperm ($t = -3.40.10$; $P = 0.0052$). The rest of the treatment's larval weight comparisons were found to be statistically similar as shown in Table 5.20 on the Bonferroni adjustment test conducted.

The highest average % kernel weight loss due to larval feeding (Table 5.21) was noted on sound kernel treatment at 12.59%, followed by AD-germ at 10.45%, and 7.29% for AD-brush end. The lowest % kernel weight loss was noted for AD-endosperm treatment at 5.94%. These % weight loss differences were found to be significant ($F = 13.67$; df = 3, 155; $P = < 0.0001$) among treatments when statistically analyzed with PROC GLIMMIX. Further analysis through Bonferroni adjustment test obtained positively higher % weight loss for sound kernels against AD-brush end ($t = -4.54$; $P = < 0.0001$) and AD-endosperm ($t = -5.67$; $P = < 0.0001$) but not with AD-germ ($t = -1.75$; $P = 0.4883$) treatment (Table 5.22). Similarly, AD-germ % kernel weight loss was significantly higher compared with AD-brush end ($t = -2.96$; $P = 0.0212$) and AD-endosperm ($t = -4.21$; $P = 0.0003$) treatments. Percent kernel weight loss differences between AD-brush end and AD-endosperm were found to be similarSof White ($t = 1.33$; $P =$ 1.0000).

5.4.4. Soft White (SW) wheat class at 50-day monitoring of *R. dominica* adult emergence

Table 5.23 indicated that there was successful infestation at day 1 of first instars on 4 different treatments tested. It showed a 46-100% range of infestation rates, with the sound kernel treatment registering the lowest and AD-brush end the highest, respectively. However, no adults emerged on all 4 treatments tested at the end of monitoring period at day 50.

5.4.5. Durum wheat class at 21-day *R. dominica* first instar infestation

Durum sound kernels had 42% probability of first instar infestation as compared to 84 – 94% on artificially-damaged kernels at the brush end, endosperm, and germ portions as shown in Table 5.24. PROC GLIMMIX statistical results showed significant differences among treatment means (df = 3, 196; F-value = 12.25; $P = < 0.0001$). Sound kernel treatment was confirmed to have significantly low infestation probability against all other three treatments (AD-brush end, *t* = 4.65; *P* = < 0.0001; AD-endosperm, *t* = 4.12; *P* = 0.0003; and AD-germ, *t* = 4.29; *P* = 0.0002) as presented in Table 5.25 and revealed by the Bonferroni adjustment test. Other paired comparisons made on 3 AD-kernels showed similar infestation responses.

Among all kernel treatments, durum's brush end was the preferred site by first instars with 31% chance of infestation. Least preferred was endosperm portion at 27% probability. While for the sound kernel treatment, germ was the preferred site of entry with 19 out of 21 larvae/pupa infesting it. Chi-square analysis results showed that site preference was significant (χ^2 = 10.7386; df = 3; *P*-value = 0.0132) in theinfestation action of lesser grain borer first instar on durum wheat kernels (Table 5.26). The only significant difference established among all treatments compared using the "R" statistics on 95% multinomial confidence interval was between brush end and sound kernels as evidenced by non-overlapping of confidence intervals.

Head capsule width measurement of larva was one way of indicating the specific larval stage of an insect with regards to its growth and development. Our data on Table 5.27 clearly showed that entry via AD-germ kernels showed about 91% (39/43) were at 4th larval stage, 5% (2/43) at 3rd instar, and both at 2% (1/43) were at 2nd instar and pupal stage, respectively, using instar classification on Table 2.1. On the other hand, AD-brush end larval entry site showed that there were only 70% of them at 4th instar and 30% still at 3rd instar. AD-endosperm still had 17% of larvae at 3rd instar. Also, it was interesting to note that sound kernel treatment had 95% (20/21) of its larvae at the 4th instar and 5% (1/21) at pupal stage of which 19 out of 21 entered via the germ portion (see Table **5.26**). PROC GLIMMIX statistical procedure showed that treatments positively affected head capsule width of 21-day old larvae (*F* = 5.49; df = 3, 147; *P* = 0.0013). Further, pair-wise comparison between treatments using Bonferroni adjustment test confirmed that AD-germ and AD-brush end were significantly different in larval development (t = -3.97; P = 0.0007) but not with other paired treatments compared (Table 5.28).

Heavier larvae were noted for AD-germ treatment at 1.13 mg followed by sound kernel at 1.03 mg. Weights of larvae for both AD-brush end and AD-endosperm treatments were between 0.56 and 0.59 mg, respectively. These significant differences in larval weights among treatments tested (F = 33.72; df = 3, 147; P < 0.0001) were confirmed through PROC GLIMMIX procedure as seen in Table 5.29. It was further established by the results of least squares means test with Bonferroni adjustment, that both AD-germ and sound kernels were significantly heavier in larval weights versus AD-brush end and AD-endosperm. But, AD-brush end vs. AD-endosperm and AD-germ vs. sound kernel treatment comparisons showed that larval weights were not different from each other (Table 5.30).

Mean kernel weight loss due to 21-day larval feeding was found to be higher on sound kernel (12.46%) and AD-germ (11.50%) treatments. It was almost doubly lower % weight loss for both AD-brush end (7.44%) and AD-endosperm (7.82%) compared with the other 2 treatments. These differences in grain weight loss was found to be significant (F = 10.60; df = 3, 148; P < 0.0001) among treatment means as shown in Table 5.31. Results of pair-wise treatment comparison through Bonferroni adjustment test showed that significantly higher weight loss was registered on both sound kernels and AD-germ versus AD-brush end and AD-endosperm treatments. However, % kernel weight loss were all found to be similar between AD-brush end vs. AD-endosperm and AD-germ vs. sound kernel treatments (Table 5.32).

5.4.6. Durum wheat class at 50-day monitoring of *R. dominica* adult emergence

Although durum AD-brush end treatment had the highest % probability of first instar infestation (96%) among treatments tested, it was the only treatment that had reduction in adult emergence of about 14% (Table 5.33). Meanwhile other AD-kernels were able to sustain the subsequent adult emergence of LGB from a range of 84 to 88%. The sound kernel treatment registered the lowest at 36%. In terms of days of duration to emergence, both sound kernel and AD-germ treatments registered the earliest emergence at 34.17 and 34.52 days, respectively. Adult emergence on both AD-brush end and AD-endosperm registered a delay of 4 days later compared with the 2 other treatments mentioned earlier. These differences in days of adult emergence were found to be significant as per PROC GLIMMIX statistical procedure used. Further analysis by Bonferroni adjustment test (Table 5.34) confirmed that the days of emergence on sound kernels and AD-germ treatments were significantly earlier compared with AD-brush end and AD-endosperm. Other paired-wise comparison treatment tests showed no differences in duration to adult emergence of lesser grain borer.

Higher % kernel weight losses, both in the 20% range, were noted for sound kernel and AD-brush end treatments due to first instar to adult feeding. Comparatively, AD-endosperm treatment registered the lowest % weight loss at 17.64% while almost 19% for AD-germ as shown in Table 5.35. These numerical differences in % kernel weight losses were found to be significantly different with each other ($F = 3.24$; df = 3, 140; $P = 0.0242$) using the PROC GLIMMIX statistical test. However, comparison among treatments all showed insignificant differences in % kernel weight losses except between AD-brush end and AD-endosperm treatments as presented in Table 5.36.

5.4.7. SRW wheat class at 21-day *R. dominica* first instar infestation

Table 5.37 showed that sound kernels of SRW wheat class were more resistant (26%) to LGB first instar's infestation compared with the other 3 AD-kernels (82 - 84%) observed at 21 days. PROC GLIMMIX statistical test showed significant differences ($F = 15.08$; df = 3, 196; P

< 0.0001) in probability of infestation. These differences were validated through pair-wise comparison test (Bonferroni adjustment) indicating that sound kernels were significantly lower in chances of infestation versus the 3 other artificially-damaged kernels (AD-brush end - t = 5.24; $P < 0.0001$; AD-endosperm – t = 5.24; $P < 0.0001$; AD-germ – t = 5.38; P = <0.0001) of SRW wheat as shown in Table 5.38. The rest of the pair-wise AD-treatment compared were noted to have equal chances of infestation from the LGB first instars.

Germ portion was the favored site of entry at 31%, followed by both brush end and endosperm at 30%. Site of entry preference for sound kernel was endosperm at 69% (9/13 kernels), and both brush end and germ portions had about 15.5% (2 kernels each/13) preferences (Table 5.39). However, larvae entering via germ on sound treatment had 1 out of 2 kernels already at the pupal stage. Similarly, 5 pupae were noted for AD-germ preferred entry point. PROC FREQ Chi-square test result showed that these numerical differences for site of entry preference at 21-day infestation were significantly different with each other ($\chi 2 = 17.5985$; df = 3; $P = 0.0005$) at $\alpha = 0.05$ level of significance. Sound kernel treatment was found to be significantly different compared with the other 3 AD-kernel treatments based on the non-overlapping of the 95% multinomial confidence intervals using the "R" statistical results.

Table 5.40 showed that first instar infestation of SRW wheat via germ entry influenced head capsule width measurements. Eighty-eight percent (88%) were already at the 4th instar and 12% were on pupal stage both at 21st day. On the other hand, both AD-brush end and endosperm treatments showed wider range of head capsule classification from 2nd to 4th larval stages. Sound kernel treatment had a different and diverse infestation scenario. Its larval classifications revealed that 15% (2/13) were at 3rd instar; ~ 77% (10/13) at 4th instar and ~8% (1/13) at pupal stage. Significant differences were found between treatments tested ($F = 4.03$; df = 3, 126; P =

0.0090) using the PROC GLIMMIX tests. Results of pair-wise comparison among treatments using the Bonferroni adjustment test revealed that only AD-germ entry was significantly contributing to a bigger head capsule width of larvae over AD-brush end (t = -3.20; P = 0.0103) and AD-endosperm (t = -2.75; P =0.0408) treatments but not with other treatments compared (Table 5.41).

The weight of larvae at 21 days was heavier when they infested AD-germ kernels (1.34 mg) as well as the germ portions of the sound kernel (1.07 mg) treatment. The lightest weight of 21-day old larvae was obtained from AD-brush end treatment at 0.85 mg as noted in Table 5.42. These numerical differences of 21-day old larval weight among treatments were found to be significantly different (F = 12.96; df = 3, 119; P < 0.0001) from one another. Further analysis confirmed that AD-germ treatment registered significantly heavier larval weight over AD-brush end (t = -5.70; P = < 0.0001) and AD-endosperm (t = -5.01; P = < 0.0001) using the pair-wise treatment comparison with Bonferroni adjustment test. All other treatment comparisons obtained insignificant differences in 21-day old larval weight (Table 5.43).

The lowest % kernel weight loss due to 21-day old larval feeding was obtained from AD-endosperm treatment at about 16% as shown in Table 5.44. Comparatively, both AD-brush end and AD-germ treatments obtained the highest % kernel weight loss in the range of approximately 21%. Statistical results through PROC GLIMMIX found significant differences (F = 6.74; df = 3, 132; P = 0.0003) among treatments tested. Supplementary analysis showed that AD-endosperm was significantly lower in % kernel weight loss compared with AD-brush end (t = 3.50; P = 0.0038) and AD-germ (t = -3.80; P = 0.0013) treatments at α =0.05 level of significance. All other treatment comparisons yielded insignificant differences in % kernel weight loss based on Bonferroni adjustment test results (Table 5.45).

5.4.8. SRW wheat class at 50-day monitoring of *R. dominica* adult emergence

The % adult emergence for the sound kernel treatment registered the lowest at 16% compared with AD-kernels that ranged from 76 – 90%. However, in terms of number of days, sound kernels had the earliest adult emergence at 33 days followed by AD-germ at 34 d and 35 d for AD-endosperm treatment. It took 38 days for adult to emerge when they have entered via AD-brush end (Table 5.46). There were significant differences ($F = 13.74$; df = 3, 131; $P < 0.0001$) found among treatment means on days of adult emergence. But these significant differences in emergence were only found between AD-brush end versus AD-endosperm $t = 4.06$; $P = 0.0005$); AD-germ $t = 5.89$; $P = < 0.0001$); and sound kernel $t = 3.79$; $P = 0.0014$) treatments tested. All other comparisons between treatments were found to be insignificantly different from each other on their adult emergence (Table 5.47).

Percent kernel weight loss due to feeding of first instar until adult emergence was highest on AD-germ treatment at 26%. This was followed by both sound kernel and AD-brush end at 24.79% and 24.09%, respectively. The lowest % kernel weight loss was obtained from AD-endosperm treatment at 23% (Table 5.48). However, PROC GLIMMIX statistical test result showed that % kernel weight loss were not different ($F = 1.92$; df = 3, 128; $P = 0.1295$) among treatments tested.

5.4.9. HRS wheat class at 21-day *R. dominica* first instar infestation

Table 5.49 showed that the first instar's probability of infestation was lower for hard red spring sound kernel (8%) treatment compared with artificially-damaged wheat (88-94%). It was established through PROC GLIMMIX statistical analysis that the differences were highly significant ($F = 21.13$; df = 3, 196; $P < 0.0001$) at alpha = 0.05. Further analysis through the Bonferroni pairwise multiple comparison adjustment test (Table 5.50), confirmed that sound kernel treatment had significantly lower chances of infestation compared with AD-brush end (t = 6.56; df = 196; $P < 0.0001$), AD-endosperm (t = 6.53; df = 196; $P < 0.0001$), and AD-germ (t = 6.60; df = 196; $P < 0.0001$). However, *R. dominica* first instar ability in establishing infestation on all AD-kernel treatments were found to be similar at 21 days.

Numerically, 34% preferred AD-brush end as entry site for infestation followed by 32% at AD-germ and 31% at AD-endosperm as the least preferred portion among artificially-damaged kernels. For sound kernel treatment, first instars preferred germ portion at 3 out of 4 kernels while 1 out of 4 kernels preferred brush end portion. Further, the tabulated results showed that the various anatomical portions of wheat grain indicated significant association (($\chi^2 = 36.7429$; df = 3; $P = < 0.0001$) using the Chi-Square test. But this significant association was only strongly established between sound and all AD-kernels as evidenced by non-overlapping of confidence intervals (CI) and not among artificially-damaged grains based on the "R" statistics on multinomial 95% CI test (Table 5.51).

Table 5.52 indicated that the choice of entry site by first instar had a positive impact on head capsule width of larvae at 21 days. Germ entry showed advanced life stage where about 98% (44 out of 45) were at 4th instar and 2% (1 out of 45) at pupal stage. Also, the average head capsule width registered the biggest at 0.43 mm at 21 days and confirmed that majority were at its last larval stage based on Table 2.1. The same was observed with sound kernel treatment wherein 75% (3 out of 4) were at 4th instar that entered via the germ site while 25% (1 larva) still at 3rd instar entering via brush end portion. It also registered the second biggest head capsule width at 0.39 mm. On the other hand, first instars that entered via AD-brush end and AD-endosperm were observed to have delayed life stages covering 1st, 3rd, and 4th instars. For AD-brush entry, 15% were still at 1st instar, about 48% and 37% were at 3rd and 4th instars, respectively, and had the second smallest mean head capsule width at 0.33 mm. The smallest average head capsule width of 0.30 mm was noted for AD-endosperm entry point and 20% were still at 1st instar, about 57% and 23% were at 3rd and 4th instars, respectively. PROC GLIMMIX statistical results showed that there were significant differences noted among treatments (F = 21.94; df = 3, 134; $P < 0.0001$). Further statistical analysis through Bonferroni adjustment test (Table 5.53) confirmed that AD-germ entry site had significantly bigger head capsule width compared with AD-brush end (t = -6.10; P = < 0.0001) and AD-endosperm (t = -7.60; P = < 0.0001) sites of entry. Meanwhile, other pair wise treatment comparisons tested showed similar sizes in head capsule width of larvae at 21 days.

Larvae at 21 days entering via AD-germ showed heavier average weight at 1.22 mg. Similar observation was noted for sound kernel treatment where it was noted to have the second heaviest average weight of 0.90 mg. This was due to the noted observation that 3 out of 4 first instars have entered through the germ portion of the sound kernel. Both AD-brush end and AD-endosperm entry points registered the lightest average weight at 0.46 mg and 0.48 mg, respectively (Table 5.54). Results of statistical analysis (PROC GLIMMIX) showed significant differences in the average weight of larvae at 21 days (F = 34.63; df = 3, 74; $P < 0.0001$) among treatments tested. Since significant differences on larval weight were noted, results were subjected to a post hoc test via the Bonferroni multiple comparisons adjustment test. Final results, as shown in Table 5.55, revealed that indeed larval weight was significantly heavier if entered via AD-germ compared with AD-brush end (t = -9.28; P = < 0.0001) and AD-endosperm (t = -6.36; P = < 0.0001) anatomical portions. Other pair wise comparisons tested showed no significant differences in larval weight at 21 days.

Table 5.56 showed that AD-germ treatment registered the highest % kernel weight loss at 10.5% due to larval feeding for 21 days. This was followed by sound kernel treatment at 5.6% where 3 larvae entered at the germ portion and 1 larva at the brush end part of the kernel. The lowest % kernel weight loss at 1.62% was noted for AD-endosperm treatment. These kernel weight losses were found to be significantly different with each other ($F = 74.39$; df = 3, 115; $P < 0.0001$) based on results of PROC GLIMMIX analysis. Further tests as shown in Table 5.57 confirmed that AD-germ entry point had significantly higher % kernel weight loss over the 3 other treatments compared such as AD-brush end (t value = -12.36; $P = < 0.0001$), AD-endosperm (t value = -13.00; $P = < 0.0001$), and sound kernels (t value = 3.27; $P = 0.0084$). All other treatment comparisons were found to be insignificantly different from each other.

5.4.10. HRS wheat class at 50-day monitoring of *R. dominica* adult emergence

The number of adults emerging from first instar infestation was noted to have been reduced to as much as 10% for those that entered through AD-endosperm as shown in Table 5.58. A similar trend was observed with AD-brush end where as much as a 6% reduction was noted. Likewise, the mean duration to adults emerging for both sites of entry were noted to be late at 40 days. On the other hand, adult emergence remained constant from the time the first instars chose AD-germ as entry site. It also registered the earliest days of emerging adults at almost 34 days. The sound kernel treatment showed a 2% decrease in adult emergence from original infestation rate (12%) and was recorded to have the second earliest adult emergence at 36 days. All these numerical data, when subjected to PROC GLIMMIX analysis, revealed significant differences in duration to adult emergence ($F = 19.18$; df = 3, 129; $P < 0.0001$). A post hoc tests by pair wise multiple comparison tests showed AD-germ treatment were significantly earlier in adult emergence compared with AD-brush end (t value = 6.68; $P <$

0.0001) and AD-endosperm (*t* value = 6.31; *P* = < 0.0001), but not with sound kernel (*t* value = -1.14; *P* = 1.0000). All other treatment comparisons showed no significant differences in adult emergence at 50 days as shown in Table 5.59.

Mean % kernel weight loss covering 50-day period and due to first instar to adult feeding, showed almost similar results for AD-germ (17.53%), AD-endosperm (17.43%), and sound kernel (17.10%) treatments. AD-brush end registered the lowest mean % kernel weight loss at 15.82%. However, all these % weight losses tested between treatments were found to be not significantly different with each other upon application of the PROC GLIMMIX statistical analysis (Table 5.60).

5.4.11. HWS wheat class at 21-day *R. dominica* first instar infestation

Artificially-damaged HWS wheat kernels at various anatomical portions were found to be highly susceptible to first instar infestation, which ranged from 86 – 92%. Comparatively, the sound kernels were more resistant at a low rate of 10%. These infestation rate differences were found to be significant (*F* = 20.54; df = 3, 196; *P* < 0.0001) (Table 5.61). Pair-wise multiple comparisons by Bonferroni adjustment test confirmed that sound kernels were highly resistant to first instar establishment versus all AD-kernels at brush end (*t* = 6.44; df = 196; *P* < 0.0001), at endosperm (*t* =6.44; df = 196; *P* < 0.0001), and at germ (*t* = 6.60; df = 196; *P* < 0.0001) portions. Other pair-wise comparisons showed similar establishment rates by first instars on HWS wheat class (Table 5.62).

Approximately 34% of the AD-germ was the preferred site of entry by *R. dominica* first instars compared with 31% preference for both AD-brush end and AD-endosperm sites. On sound kernel treatment, it showed that both germ and endosperm were the preferred sites with 4 out of 5 kernels entering through these 2 portions while those that entered through the brush end was 1 out of 5 kernels. Chi-Square test results showed these site preferences were significantly different among treatments ($\chi^2 = 33.4818$; df = 3; $P < 0.0001$). Further analysis by "R" statistical program on 95% multinomial confidence interval (CI) found that sound kernel treatment had significantly lower probability in preferential entry versus all other AD-kernels based on non-overlapping of CI as shown in Table 5.63.

Head capsule width measurement is a method of determining larval stages of insects. The data on AD-germ treatment as shown in Table 5.64 indicated that advanced life phases were observed with about 70% at 4th instar, 21% at pupal stage, while only 9% at 3rd instar. On average, germ entry produced the biggest head capsule width at 0.42 mm which was equivalent to 4th instar (see **Error! Reference source not found.**). Comparatively, the smallest average h ead capsule width of 0.32 mm was obtained from AD-endosperm entry site. It further showed that all larvae head measured were classified from 1st to 4th instar range and 60% of them were at 3rd instar. The choice of entry site had a significant influence on the head capsule width based on PROC GLIMMIX statistical results ($F = 18.44$; df = 3, 123; $P < 0.0001$). Table 5.65 showed that AD-endosperm had significantly smaller head capsule width versus all other AD-kernels such as AD-brush end ($t = 4.80$; $P < 0.0001$), AD-germ ($t = -7.19$; $P < 0.0001$), and sound grains ($t = -2.85$; $P = 0.0306$). Other pair wise comparisons conducted by Bonferroni adjustment test showed insignificant differences in head capsule widths measured.

Based on our data on mean larval weight, those that entered via AD-germ were heavier (1.13 mg) compared with AD-brush end (0.89 mg) and AD-endosperm (0.90 mg) treatments. Results of PROC GLIMMIX analysis found significant differences among treatments ($F = 4.55$; df = 2, 51; $P = 0.0151$) except for the sound kernel treatment which was excluded from said analysis due to small sample size obtained and it weakened the test analysis (Table 5.66). Post hoc tests by Bonferroni multiple comparisons test on larval weight confirmed that AD-germ entry was indeed heavier over AD-brush end kernels (t = -2.74; P = 0.0255), but not those that chose AD-endosperm as entry point (t = -1.99; P = 0.1551). AD-brush end and AD-endosperm treatment comparison were found to be similar (t = -0.09; P = 1.000) in larval weight response (Table 5.67).

The data obtained due to larval feeding for 21 days at AD-germ kernel, indicated a higher % kernel weight loss at about 7%. The lowest % weight loss of about 3% was observed from AD-endosperm. The 4% kernel weight loss registered on sound kernel treatment was a combination of larval feeding at the germ, endosperm, and brush end portions. These weight losses were found to be significantly different from each other (F = 16.64; df = 3, 119; P <0.0001) as per PROC GLIMMIX procedure shown in Table 5.68. Pair wise comparisons between treatments by Bonferroni test confirmed that AD-germ entry point had significantly higher % weight loss compared with AD-brush end (t = -5.66; P < 0.0001) and AD- endosperm (t = -6.27; P < 0.0001), but not with sound kernel treatment (t = 2.11; df = 119; Adj. P = 0.2219). Likewise, sound kernel treatment versus AD-brush end and AD-endosperm as well as between AD-brush end and AD-endosperm were found to be not significantly different in % kernel weight loss response due to larval feeding for 21 days (Table 5.69).

5.4.12. HWS wheat class at 50-day monitoring of *R. dominica* adult emergence

The data shown in Table 5.70 indicated that sound kernel and AD-germ treatments were able to sustain the successful first instar establishment to subsequent adult emergence of the lesser grain borer. However, the opposite was observed with the AD-brush end and AD-endosperm treatments. Percent adult emergence dipped from 88% to 84% for the former while 94% to 88% for the latter treatment. In terms of days of duration to adult emergence, both AD-germ and control treatments had established 33 shorter days compared with AD-brush end of 4 days delay and 6 days for AD-endosperm treatment. All these data differences were found to be significant ($F = 22.01$; df = 3, 145; $P < 0.0001$) based on the PROC GLIMMIX statistical procedure. Further post hoc tests by Bonferroni multiple comparisons adjustment procedure (Table 5.71) showed that both AD-germ and sound kernel treatments were confirmed to be significantly shorter in days of adult emergence versus AD-brush end ($t = 5.14$; $P <0.0001$; $t = 3.81$; $P = 0.0012$, respectively), and AD-endosperm ($t = 7.16$; $P < 0.0001$; $t = 5.16$; $P < 0.0001$, respectively). All other pair-wise comparisons tested were found to be similar in duration in days of adult emergence.

The percent kernel weight loss for 50-day period due to first instar to adult feeding as shown in Table 5.72 suggested that AD-endosperm registering the highest at more than 13%. This was followed by AD-brush end and AD-germ treatments with almost similar % weight loss at around 12%. The lowest weight loss at 11% was noted on the control treatment. But further analysis by PROC GLIMMIX procedure showed that these feeding differences between treatments were found to be insignificant from each other ($F = 1.19$; df = 3, 145; $P = 0.3141$).

5.5. Discussion

5.5.1. HW and SW wheat classes at 21-day *R. dominica* first instar infestation

Significant differences in probability of infestation for both experiments on HW and SW wheats can be probably attributed to the effect of kernel endosperm texture (hard or soft) and presence of physical damage. Larvae of the lesser grain borer are considered voracious feeders (Golebiowska, 1969). Upon emerging from hatched eggs, first instars start to seek for source of food and if remnants of flour from adult feeding is present it becomes their initial source of food, but if wheat kernels are available, they crawl on its hard surface but find little success in colonizing it as observed by Stemley (1962). This could have been the same possible scenario based on the findings of the experiment where sound kernels had lower probability of infestation at 21 days compared with AD-kernels of HW and SW wheats. This experiment had earlier mentioned that the HW wheat samples had an average hardness index (HI) value of 69.72 ± 8.11 while SW wheat sound kernel had 30.72 ± 10.72 HI value. Based on this textural property differences, the latter had 5x more infestation probability compared with the former wheat class. It seems to indicate that hardness is a factor in resisting the successful insect infestation of first instar of lesser grain borer. Relatively, Watts and Dunkel (2003) reported in their correlation analysis study on postharvest resistance in wheat that hardness is positively associated with percentage of sound kernels. Secondly, for AD-kernels of these 2 wheat classes, due to the presence of defects in the outer layers (Schwardt, 1933; Birch, 1944a, 1944b; Pedersen, 1992; Semple, 1992) of the grain it strongly influenced *R. dominica* first instar's efficiency of penetration in physically-damaged whole grains of wheat. Similar result was found by Birch (1944a) on wheat damaged with deep scalpel cuts versus sound wheat where first instar larvae had higher survival and establishment from the former at temperature ranges of 26 - 36°C and moisture content ranges of 10 -14%. He further stated that mortality on sound wheat grains most usually happens in first instar due to longer period of exposure to severe environmental conditions. This apparently weakened the larvae considerably (Breese, 1960) and hampering it to gainfully enter the sound kernel. For this experiment, one additional probable cause of high mortality for first instars in the sound kernel treatment was the absence of grain debris and dusts available for first instars as initial source of food while trying to feed on undamaged grain. Secondly, it will take first instars more time to chew or break entry into the sound grain and this requires more energy thus leading to exhaustion and death.

Across all types of kernel treatments tested, first instars preferred germ as entry site for HW wheat samples while it was the endosperm portion for SW wheat experiment. In these 2 experiments conducted, it was found that preferential site of entry was of equal importance in the manner of infesting grains. It suggests that first instars are opportunists in their infestation behavior because it will exploit whatever defects were present at the time of sourcing food resources. This is probably for survival purposes and it will save them time and energy in successfully infesting a damaged kernel against an undamaged one. Similarly, Thomson (1966) reported that LGB first instar usually enter the grain by boring through the germ portion of the whole milo grain. Also, the result is in close similarity with the findings of Birch (1944a) stating that 80% of the 400 first instars observed have entered at the germ end of wheat at 30°C and 34°C at 10% moisture content. In addition, it cannot be discounted that the differences in site of entry preference by first instars may have been the effect of moisture content and textural property of the 2 wheat classes observed. As mentioned earlier on this paper, HW wheat is 0.8% higher in moisture content and more than 2X harder versus SW wheat samples.

It was very evident that the choice of first instars' entry point positively produced bigger head capsule width of larvae. This is when they entered via the AD-germ treatment and germ as the overall preferred site of entry across all treatments on both HW and SW wheat experiments. In a way, this also strengthened the notion that first instars entering through the germ enabled them faster growth and development. The data of these experiments showed that majority, if not all, were already at the 4th larval and final stage. The results suggest that the specific choice by the first instar on the anatomical site of the grain, either germ, brush end, or endosperm, had a great impact on its development. In fact even on sound kernel treatments, first instars preferred to enter through the germ portion and showed significantly bigger head capsule width compared with brush end and endosperm treatments and sites. Wheat germ, with its nutritious composition of proteins, vitamins, lipids, and minerals can contribute to faster growth rate. Comparatively, wheat endosperm and brush-end portions are mainly comprised of starch granules providing only the energy for the growing first instar inside the grain. In a similar study by Osuji (1982) with artificially-damaged corn, he observed that LGB first instars that entered via the germ portion developed faster compared with those that entered through the crown end of the corn.

Again, AD-kernels at the germ portion proved to be a better choice of point of entry by first instars because it conferred heavier weight within 21 days of infestation. Similar observation was noted for first instars entering via germ portion of sound kernel treatment. The HW wheat data showed that it was almost 3x heavier compared with those that entered brush end while 2x lighter via endosperm portion of the wheat grain. Similarly, SW wheat data showed the same results where larvae that entered via AD-germ kernel weighs significantly heavier versus AD-brush end and AD-endosperm treatments. It is probable that these results can be tied up with the nutritive composition of the germ particularly that of protein compounds. MacMasters et al. (1964), Nawrot et al. (1985), and Serna-Saldivar (2010) mentioned that wheat germ is mainly composed of albumin and globulin proteins. Nutritionally, these proteins are considered to have the best quality, balance, and presence of essential amino acids and is thoroughly digested when consumed (Serna-Saldivar, 2010). Hence, this suggests that there is better assimilation of these digested protein within the larval body that contributes to its heavier weight. On the contrary, endosperm portion with the starch granules encased in protein bodies (Hoseney, 1994) are mostly prolamin proteins which are nutritionally poor in terms of the essential amino acid and balance as further stated by Serna-Saldivar (2010).

The sound kernel treatments on HW wheat and SW wheat experiments both showed consistently and significantly higher percent weight loss due to larval feeding at 21 days. However, in the case of HW wheat, there was higher standard error due to lesser number of grains infested (5 out of 50) compared with SW wheat's lower SE value (28 out of 50). For the AD-kernel treatments, similar observation were noted on HW and SW wheat experiments for AD-germ kernels where it showed the higher vulnerability of damaged grains to first instar infestation. Probably because according to Serna-Saldivar (2010), more than 80% of total lipids are mainly located in the germ portion. Once germ is damaged, these lipids are synthesized into volatile forms of fatty acids and prone to oxidation process and turns rancid that gives off off-odor and flavor. This condition can attract larvae thereby preferring to enter via germ portion of the wheat grain. Since it is a nutrition-rich portion of the kernel, it promoted rapid development that can lead to higher consumption rate, hence, obtaining a higher % kernel weight loss compared to AD-brush end and AD-endosperm treatments. Also, it seemed possible that the more the presence of grain damage, the higher the weight losses it can incur due to easiness and efficiency in establishing larval infestation. Based on Golebiowska's (1969) study, the larval stage of *R. dominica* is a voracious feeder. It can consume an average of 12 mg (9-16 mg) of wheat grain for over a 30-day period.

5.5.2. HW and SW wheat classes at 50-day monitoring of *R. dominica* adult emergence

The successful development of first instar into adult LGB in infesting sound and AD-kernels is shown in our experiment on HW wheat class (Table 5.10). But, it was clear that there was reduction in the number of emerged adult on all treatments tested. The sound kernel treatment showed the least number in terms of infestation and adult emergence. Likewise, sound kernel treatment had registered the shortest adult emergence at 33 days followed by AD-germ at

34 days, 40 days for AD-brush end, and the longest at 43 days for AD-endosperm treatment. The sound kernel treatment's recorded shortest day of emergence was probably due to the effect of site of entry where 3 out of the 4 successful first instar infestation have entered through the germ portion. All 3 larvae successfully emerged while the 1 larva that did not emerge have entered via endosperm portion. This result suggested that the initial site of entry by first instars significantly influences the speed of adult emergence. In a related study by Mills (1965), on Angoumois grain moth, *Sitotroga cerealella* (Olivier) which is similar in infestation behavior with *R. dominica*, he found that early entry into the germ area of wheat grain successfully shortened its growth and development. The same observation was noted on adult emergence on AD-germ treatment and it all showed significantly shorter days of emergence versus AD-brush end and Ad-endosperm. A previous study showed that the first instar always enter in damaged surface of damaged grain (Birch, 1945a) compared with sound grains. This feeding behavior of selecting a suitable entry point provides good establishment into the kernel and ensures the successful growth and development into adulthood.

On the contrary, experiment on SW wheat class completely showed a different scenario on adult emergence from the 27th to 50th day monitoring period. Initially on the 27th day, there were successful infestation of first instars ranging from 46 to 100%. However, up until the last 50th day, non-emergence of adults were obtained on all AD-kernel treatments including sound kernels serving as control check. Results of individual kernel dissection on the 50th day revealed that for sound kernel treatment, 10 were in 4th larval stage while 13 in pupal stage; AD-brush-end had 18 4th larval stage and 32 pupal stage; AD-endosperm had 12 larvae at 4th stage and 36 at pupal stage with 2 shriveled pupae; and AD-germ had 14 larvae at 4th stage plus 1 dead and 30 pupae and 1 in shriveled condition. These results seemed to indicate a delay in the emergence of adults of *R. dominica* considering that there was telltale signs of advanced immature stages inside kernels after dissection. Probably, had it been monitored beyond the 50th day, adults may have eventually emerged. Wheat kernels contain naturally-occurring α-amylase enzyme inhibitors (Buonocore et al, 1977; Gatehouse, et al., 1986; Baker et al., 1991; Franco et al., 2002). Conversely, cereal-eating insects like *R. dominica* has α-amylase inside their guts that plays an important role in plant starch digestion for their development and reproduction (Franco et al., 2002; Cinco-Moroyoqui et al., 2006). But, this specific enzyme inhibitor acts to obstruct in insect's starch metabolism and adversely hindering their growth and development (Buenocore et al., 1977; Franco et al., 2002; Cinco-Moroyoqui et al., 2006). Baker et al. (1991) have studied 30 cultivars of eastern soft wheat with varying levels of α-amylase inhibitory activity against *Sitophilus oryzae* (L.) or rice weevil. There were 3 population growth parameters measured such as total progeny production, rate of development, and average days of adult emergence. Their findings showed that there was a positive association between α-amylase inhibitory content and average number of day of adult emergence but not on total progeny production and rate of development. It is finally concluded that it significantly delayed rice weevil's development time with high content of amylase enzyme. The preceding literatures cited might be one of the probable explanation for the SW wheat result of this experiment. An additional and probably in depth chemical analysis and characterization of SW wheat is needed to elucidate more on this observed phenomenon on adult non-emergence.

The duration of percent weight loss monitoring due to first instar to adult feeding started from the 5th to 7th week (27- 50 days). The successful growth and development of first instar to adult and its subsequent feeding showed significant influence on mean % weight loss across all the different sound and AD-kernel treatments infested. HW wheat class experiment clearly showed AD-brush end and sound kernels registered the highest % kernel weight loss of 24.77% and 23.61%, respectively, compared with AD-endosperm and AD-germ treatments. The significantly higher weight loss from sound kernels can be possibly attributed to an early entry into the wheat kernel and gaining quick position into the nutrient-rich germ portion. This could have promoted rapid growth of larvae to adult stage and voracious consumption of kernels that contributed significant kernel weight loss. This result is somewhat similar to the observation made by Khare and Mills ((1968) that when larvae of Angoumois grain moth, *Sitotroga cerealella* (Olivier) entered early into the germ portion of the wheat grain, adult emergence is faster compared to endosperm entry.

In conclusion, these experiments on HW and SW wheat classes involving sound and AD-individual kernel showed that physically-damaged grain facilitated higher chances of first instar infestation versus undamaged grain. The harder the textural property of the grain endosperm, the lower the larval infestation rates whereas the opposite was observed for soft-textured grains. Germ portion was the preferred site of entry over other portions of endosperm and brush end damaged kernel including sound grains. As a result of this anatomical preference for germ portion, our findings showed that developmental rates via larval head capsule width measurement had higher growth ratio. There were heavier and plump larvae observed and percent kernel weight loss was significantly higher. First instar's subsequent development and duration of days to adult emergence was shortened compared with brush end and endosperm portions.

5.5.3. Durum and SRW wheat classes at 21-day *R. dominica* first instar infestation

The durum wheat that were used on this experiment had an 86 HI score. Considering that durum is the hardest of all wheat, our result had an unexpected 42% probability of infestation by

R. dominica first instars on sound kernel treatment at 21 days. This can be due to the fungal infection observed on each infested kernel. The data showed that fungal infection were found on more than 50% or 11 out of 21 infested kernels. Further, 9 of the 11 fungal-infested kernels had a dense mycelial growth found on germ portion while 2 other kernels had infections at the ventral-crease section of the endosperm. This situation seemed to have contributed in increasing the chances of infestation by the first instars and probably served as an additional food for them. On the other hand, the sound kernels of SRW wheat experimented (HI = 18), registered an infestation rate of 26% by first instars. This indicates that the soft texture of the kernels as evidenced by a low hardness index score contributed to its higher chances of infestation.

Regardless of the type of kernel treatments tested, germ was the preferred site of entry for durum wheat and endosperm for SRW wheat class. Based on the visual inspection made by Özkaya et al. (2009) on their experiment differentiating soft and hard wheat infestation by stored-product insects, *R.dominica* is found to have fed mostly on the endosperm portion than the germ of the wheat kernels. However, our statistical analysis results on both wheat classes showed that the 3 sites of entry on wheat kernel anatomy are of equal importance in the infestation success of LGB first instars.

Head capsule width measurement is one technique in determining the number of instars undergone by a particular larva. It is also known as Dyar's rule where the sclerotized part of a larval head does not change in area during a certain stadium and it only increases in size during ecdysis where the changes in head capsule width usually follow a regular geometric pattern in its successive stages (Mayer and Babers, 1944). In durum wheat class experiment, germ entry had a clear advantage over other sites as it showed an advanced growth stage due to increased width of head capsule, either at the 4th or last larval state. Comparatively, brush end and endosperm entries still had early 2nd and 3rd larval stages noted. In fact, even for sound kernel treatment, 1 out of the 19 that entered through the germ portion was already at the pupal stage. Also, it cannot be discounted that the possibility that the "earliness" of first instar entry can be another factor in the speed of development inside the kernel of wheat. Robert Mills (1965), in an experiment conducted on Angoumois grain moth (*Sitotroga cereallella*, Olivier), found that when larvae entered early on a wheat kernel and fed on germ portion, rapid development is observed. Probably it is also true when first instar enter early on through the brush end and endosperm portions as shown in this experiment. But the germ entry advantage would be the rich nutritional composition it offers to the larvae versus the other anatomical sections of the kernel. Similar observations were noted for the SRW wheat class experiment where first instar's head capsule width were influenced positively when germ was the site of entry leading to an advanced growth stage inside the kernel. The single pupa found in the sound kernel treatment via germ entry as well as the 5 pupae at the AD-germ treatment confirmed our initial observation on the positive merit of entering through this site by increasing the head capsule width of larvae.

Both durum (1.13 ± 0.04 mg) and SRW (1.34 ± 0.05 mg) wheat classes experiments showed heavier weight at 21 days when first instars entered through the AD-germ kernel treatment. This was also observed to be true for the sound kernel treatment where first instars chose to enter via the germ section with 1.03 mg weight for durum and 1.07 mg for SRW wheat class. According to Serna-Saldivar (2010), durum usually contains 10.5% to 14% protein and the germ part together with the aleurone layer had the highest concentration. Nutritionally, germ contains the best amino acids (albumins and globulins) that is of good quality because they are satisfactorily digested by the insects and the presence of high amounts of lysine and other essential amino acids give an additional advantage. On the other hand, distributed in endosperm cells of the kernel, is protein fraction of prolamin embedded in protein bodies. Nutrition wise, prolamins are the poorest when it comes to essential amino acid balance. Distribution wise in durum and soft wheat, simple prolamin fractions are 49% and 45%, respectively (Serna-Saldivar, 2010).

Durum wheat's average % kernel weight loss due to larval feeding for 21 days was noted to be highest at 12.46% on sound kernel treatment when it entered via germ site. This was followed by AD-germ treatment where % kernel weight loss registered at 11.50% whereas both AD-brush end and AD-endosperm treatments were noted to have registered the lowest both within the range of 7%. These results seemed to indicate that the chosen site of entry in the kernel played an important role in the successful reduction of kernel weight. It is probable that germ feeding of larvae effectively accelerated their growth through voracious feeding of kernels. This feeding behavior in turn resulted in the reduced weight of kernels. In fact, based on visual observations during kernel dissection, first instars that entered via germ produced high amounts of floury materials or debris compared with other anatomical sections of wheat kernels. For the SRW wheat class results, AD-germ and AD-brush end treatments both got the highest % weight loss at 20.94% and 20.57%, respectively. The probable explanation for AD-brush end high % weight loss was the time factor or an "early entry" into the kernel. It is possible that this gained a headway for first instars and they reached the germ portion earlier through voracious feeding contributing to further kernel weight loss.

5.5.4. Durum and SRW wheat classes at 50-day monitoring of *R. dominica* adult emergence

The data on durum and SRW wheat classes showed the significant influence of physical damage on kernels on days of *R. dominica* adult emergence. Both wheat classes on AD-germ treatment revealed shortened growth and development inside the kernel by emerging at 34 days.

Compared with 4 days of delayed emergence for AD-brush end and AD-endosperm treatments under durum wheat and only true for AD-brush end and not for AD-endosperm under SRW experiment. Also, it is worth noting that the control treatments for both classes registered 33 – 34 days of adult emergence similar to the AD-germ treatment. This is due to the data obtained that there were 18 kernels for durum and 5 kernels for SRW that infested via germ entry site. This result further confirmed our initial findings that through germ entry, it effectively shortened developmental time of first instar to adult *R. dominica*.

According to Rao and Wilbur (1972) study, a week after adult emergence from pupal stage inside wheat kernel, lesser grain borer adults inflict the biggest loss in kernel weight. But as time progresses and it grows older before emerging out of the kernel, weigh loss diminishes. In their study, the first week of adult emergence brought about 19.4% loss until it decreased to 6.5% on 4th week. Let it be clear that results of their adult feeding study was based on adult emergence from pupal stage while it is still inside wheat kernel. A group of 10 kernels are weighed every other day starting on the 28th day until the 60th day. Also, a hole at germ portion is drilled to facilitate infestation by first instar. On the other hand, this study's methodology of ascertaining % kernel weight loss started from introduction of first instar until the size of the kernel hole was enough for an adult to emerge out on each kernel and weighed. It was monitored daily from the 27th day and arbitrarily stopped on 50th day due to time constraints. In a way, the semblance of similarity in our methodology was the drilling of holes at the germ portion of the sound wheat kernel and the day of monitoring at 52 days which is close to our 50th day end of observation. Hence, a comparison is not needed between our studies due to obvious reasons cited above. For purposes of discussion of our data, the AD-brush end and sound kernel treatments for durum registered the highest weight losses after adult emergence. It suggests two probable reasons; first the sound kernel treatment had germ as entry point by first instars. Since it is the most nutritious part of the kernel, growth and development is faster and more feeding activity is required to support it. Secondly, perhaps, for AD-brush end treatment, it was more dictated by the longer time spent by the adult inside it consuming its contents. It should be noted that for this treatment, it took adults an average of 38 days to emerge. Based on statements made by Rao and Wilbur (1972), adults are undisturbed inside the wheat kernel and continue to remain so for 3-5 days (Schwardt, 1933) and feed until nothing is left with only the pericarp remained. On the other hand, SRW wheat data showed close numerical values of % kernel weight losses ranging from 23% to 26% on all treatments and found to be not statistically different from each other.

Based on these findings, sound kernels of both classes provided more protection against first instar infestation over damaged kernels. Germ as the feeding site encouraged faster developmental rates for larvae, produced heavier larval weight, shorter days of adult emergence, and higher % kernel weight losses.

5.5.5. HRS and HWS wheat classes at 21-day *R. dominica* first instar infestation

The probability of infestation by *R. dominica* first instars on sound kernel treatments was significantly low on both HRS and HWS wheat classes. This indicated that to a certain extent, seeds or grains that are in good physical condition are less susceptible to first instar infestation. Similarly, Sinha et al (1988) observed that the whole wheat seed with an intact covering versus crushed or cracked seed was found to have lesser risk of damage from lesser grain borer.

Preference for site of entry in a given grain is an insect's response that impacts its successful growth and development for progeny survival. There are 3 possible aspects that might have influenced preference of entry by first instars, that is, physical, chemical, and nutritional factors of the grain. Physical factors could involve the morphological parts of the grain and its condition whether good or damaged. Nutritional factors include biochemical molecules such as protein, carbohydrates, and lipid contents. Chemical factors are those volatile substances released by cereals as a result of natural and artificial damage to the grain. Overall, data showed that the preferred site of entry by first instars for HRS and HWS wheat classes varied differently. The brush end portion was the preference for the former while germ was for the latter wheat class. But, statistical analyses results showed that these site preferences on brush end, endosperm, and germ portions has an equal chance on both wheat classes. These results suggest that this may be due to the similarity of the physical condition of the kernel, that is, all have damage on specific anatomical parts of the grain and hence, the likelihood of infestation was equally similar. Khare and Mills' (1968) experiments have shown that Angoumois grain moth larvae generally entered drilled holes when they were exposed to artificially-drilled wheat at the junction of germ and endosperm and hole at the latter distal from the germ portion. They also added that early germ feeding by larvae resulted in as much as 90% chances of completing development. This could be due to the vitamin-rich component of the wheat germ (Campbell and Sinha, 1976). In addition to the data obtained on these experiments, it was observed that a different scenario for entry site preference was observed on two classes of undamaged wheat kernels. Sound HRS kernel were infested by 3 first instars via the germ site and only 1 first instar entered via the brush end. Meanwhile, on HWS sound kernel, a 2:2:1 ratio on site preference was observed for germ, endosperm, and brush end portions, respectively.

Head capsule width measurement is a laboratory technique in determining the specific larval stages of insects. This procedure was pioneered by H. G. Dyar (1890) when he determined the number of molts of a Lepidepterous larvae. He explained that larval head width in successive phases follow a regular geometric pattern. Measuring its width is the most suitable thing to obtain and this method has been known as Dyar's rule (Mayer and Babers, 1944). This method was applied in this experiment and data on the influence of site of entry on head capsule of larvae infesting HRS and HWS wheat classes showed positive results of germ as feeding site. It clearly showed that on both classes, germ as site of entry produced a bigger head width. This brought about advanced larval stages at 4th instar and pupae. Similarly, Khare and Mills (1968) observed that an early germ feeding on wheat kernels resulted in faster development from larvae to pupae of Angoumois grain moth. Further, germ entry had 4 or 5 instars while via endosperm had 4 to 7 instars. They also observed that the life stage of immature varied more if it entered through the endosperm portion of the wheat kernel which was similarly observed from data generated for these 2 experiements. Wheat germ contains large amounts of protein, high in vitamins B and E, as well as enzymes (Hoseney, 1994) compared with endosperm that contains about 80% carbohydrates (Serna-Saldivar, 2010).

Larval weight is another insect response that is an important parameter in measuring successful insect establishment of this experiment. The data indicated a more positive effect of germ as site of entry over other sites. It conferred a heavier larval weight breaching the mark of more than 1 mg weight from both wheat classes tested. Larval weight response can be associated with the nutritional content of the wheat grain being infested by *R. dominica* first instar. Serna-Saldivar (2010) stated that hard wheat generally contain 10.5% to 14% protein. Anatomically, germ and aleurone layers contain the highest protein concentration in wheat. Specifically, these proteins are globulins and albumins and are found in the germ portion. Nutrition-wise, these proteins are of good quality and considered to have the best balance of amino acid content. This means both contain high amounts of lysine and other essential amino acids and digested well upon consumption. Probably, due to these nutritional advantage on both wheat classes' germ portion, they might have contributed to the heavier weight of larvae monitored.

On grain weight loss assessment due to insect feeding, Campbell and Sinha (1976) suggested the inclusion of site of entry and the quantity of frass produced. In these experiments, we have only considered the influence of site of entry into the grain tested. *R. dominica* larvae at 21st day and entered via the germ portion consumed the highest average of 10.5% of HRS kernel while about 7% mean weight loss for HWS kernel for the same number of days of feeding. This value for HRS wheat class was higher than the 9.5% wheat weight loss recorded at 20-day monitoring period by Rao and Wilbur (1972). But, our value for HWS wheat kernel % weight loss was lower by 2.5% than cited by same authors. It is also worth mentioning that these authors also drilled holes on the germ portion of the wheat kernel tested.

5.5.6. HRS and HWS wheat classes at 50-day monitoring of *R. dominica* adult emergence

Monitoring of duration to adult emergence for this study was not done on a 24-hour basis. It was done on a daily inspection from 28th to 50th day and emergence was based on the procedure earlier mentioned on this chapter. Earliest adult emergence at almost 34 days was recorded for both wheat classes of HRS and HWS when larvae initially entered via germ portion. In contrast, there was a 6-day delay (40 days) if it entered through the brush end and endosperm parts of the kernel. These early days of adult emergence results have parallelism by Osuji (1982) study on *R. dominica* development on corn kernels as influenced by site of larval entry. He demonstrated that it only took 38 days for adults to emerge when early germ entry occurred while 58 days for endosperm entry at the crown end of the corn kernel.

Percent kernel weight loss due to first instar to adult feeding at the germ entry site for HRS wheat class recorded the highest average at 17.5% (33 days mean emergence).

Comparatively, HWS wheat kernels had the highest recorded mean weight loss at 13% (39 days average emergence) at endosperm feeding site. The obtained value for HRS was almost equal to the 17.8% (32 days) recorded mean weight loss after adult emergence by Rao and Wilbur (1972) but lower for HWS kernel weight loss of 12.2% for 33 days at germ feeding site. In addition, Campbell and Sinha (1976) *R. dominica* adult feeding for 2 days recorded a 6.17% weight loss which was 2x the recorded 1.6% mean weight loss per day by Rao and Wilbur (1972).

In conclusion, regardless of wheat class, sound kernels had a lesser probability of infestation from *R. dominica* first instars. Feeding at germ entry site highly influenced larval developmental rates that led to earlier emergence of adults and higher kernel consumption.

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Table 5.1. Probability of infestation by *R. dominica* first instars on HW wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Number of un-infested kernels	Infestation (%)^{a,*}
Sound kernels	5	45	10
AD-brush end	36	14	72
AD-endosperm	33	17	66
AD-germ	41	9	82

[a] *n* = 50 kernels/treatment.

*There were significant differences among treatments (*F* = 14.19; df = 3, 196; *P* < 0.0001; PROC GLIMMIX, fixed effects; Type III SS).

Table 5.2. Results of least squares means test with Bonferroni adjustment comparing pair-wise probability of infestation as shown in Table 5.1.

Treatments compared	*t*-value (df = 196)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	0.65	1.0000
AD-brush end vs. AD-germ	-1.18	1.0000
AD-endosperm vs. AD-germ	-1.80	1.0000
AD-brush end vs. Sound kernel	5.54	< 0.0001*
AD-endosperm vs. Sound kernel	5.13	< 0.0001*
AD-germ vs. Sound kernel	6.21	< 0.0001*

[*] Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.3. Preferred site of entry by *R. dominica* first instars on HW wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels*				% Infestation
	Brush end	Endosperm	Germ	Total	(Multinomial 95% CI)[a]
AD-brush end	35	1	0	36	31.3 (21.7 – 41.2)
AD-endosperm	0	32	1	33	28.7 (19.1 – 38.6)
AD-germ	0	0	37/4 pupae	41	35.7 (26.0 – 45.5)
Sound kernels	0	1	4	5	4.4 (0.0 – 14.2)
Total				115	

[a] Percentages infestation are based on a total of 115 kernels.

*Significant at α level = 0.05 ($\chi^2 = 27.2957$; df = 3; $P = < 0.0001$; PROC FREQ Chi-square test).

Table 5.4. Influence of site of entry on head capsule widths by *R. dominica* first instars on HW wheat at 21 days post infestation.

Site of entry	Instar					Number of infested kernels	Mean ± SE head capsule width (mm)*
	1	2	3	4	Pupae		
Sound kernels	0	0	0	5	-	5	0.43 ± 0.01
AD-brush end	1	0	24	11	-	36	0.33 ± 0.01
AD-endosperm	6	1	18	8	-	33	0.30 ± 0.02
AD-germ	0	0	2	35	4	41	0.43 ± 0.00

*There were significant differences among treatments ($F = 28.28$; df = 3, 107; $P < 0.0001$; PROC GLIMMIX, Type III SS).

Table 5.5. Results of least squares means test with Bonferroni adjustment comparing pair-wise head capsule width among treatments of first instars as shown in Table 5.4.

Treatments compared	*t*-value (df = 107)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	1.58	0.7045
AD-brush end vs. AD-germ	-6.79	< 0.0001*
AD-brush end vs. Sound kernel	-3.43	0.0052*
AD-endosperm vs. AD-germ	-8.22	< 0.0001*
AD-endosperm vs. Sound kernel	-4.20	0.0003*
AD-germ vs. Sound kernel	-0.10	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.6. Mean larval weight of *R. dominica* on HW wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Mean ± SE larval weight (mg)*
Sound kernels	5	1.14 ± 0.06
AD-brush end	28	0.39 ± 0.04
AD-endosperm	13	0.63 ± 0.1
AD-germ	37	1.11 ± 0.04

[*] There were significant differences among treatments ($F = 43.54$; df = 3, 79; $P < 0.0001$; PROC GLIMMIX, Type III SS).

Table 5.7. Results of least squares means test with Bonferroni adjustment comparing pair-wise larval weights among treatments as shown in Table 5.6.

Treatments compared	*t*-value (df = 79)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-2.74	0.0456*
AD-brush end vs. AD-germ	10.85	< 0.0001*
AD-brush end vs. Sound kernel	-5.76	< 0.0001*
AD-endosperm vs. AD-germ	-5.58	< 0.0001*
AD-endosperm vs. Sound kernel	-3.57	0.0037*
AD-germ vs. Sound kernel	-0.17	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.8. Mean kernel weight loss of HW wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Mean ± SE kernel weight loss (%)*
Sound kernels	5	17.80 ± 2.2
AD-brush end	36	7.21 ± 0.6
AD-endosperm	33	3.61 ± 0.8
AD-germ	41	12.71 ± 0.7

*There were significant differences among treatments ($F = 35.80$; df = 3, 111; $P < 0.0001$; PROC GLIMMIX, Type III SS).

Table 5.9. Results of least squares means test with Bonferroni adjustment comparing pair-wise kernel weight loss among treatments as shown in Table 5.8.

Treatments compared	*t*-value (df = 111)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	3.45	0.0048*
AD-brush end vs. AD-germ	-5.57	< 0.0001*
AD-brush end vs. Sound kernel	-5.14	< 0.0001*
AD-endosperm vs. AD-germ	-9.00	< 0.0001*
AD-endosperm vs. Sound kernel	-6.84	< 0.0001*
AD-germ vs. Sound kernel	-2.49	0.0862

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.10. Successful adult emergence of *R.domnica* first instars on HW wheat sound and AD-kernels.

Treatment	Number of infested kernels[a]	Infestation (%)[b]	Number of adults emerged	Emergence (%)[b]	Mean ± SE duration to adult emergence (days)*
Sound kernels	4	8	3	6	33.33 ± 0.9
AD-brush end	40	80	38	76	40.84 ± 0.6
AD-endosperm	33	66	28	56	42.42 ± 0.9
AD-germ	42	84	38	76	34.24 ± 0.4

[a] Based on observing kernels for boring hole/dust on day 27 under a steromicroscope.

[b] Percentages based on 50 kernels.

*There were significant differences among treatments ($F = 36.83$; df = 3, 103; $P < 0.0001$; PROC GLIMMIX; Type III SS).

Table 5.11. Results of least squares means test with Bonferroni adjustment comparing pair-wise adult emergence among treatments as shown in Table 5.10.

Treatments compared	*t*-value (df = 103)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-1.79	0.4631
AD-brush end vs. AD-germ	8.07	< 0.0001*
AD-brush end vs. Sound kernel	3.51	0.0040*
AD-endosperm vs. AD-germ	9.22	< 0.0001*
AD-endosperm vs. Sound kernel	4.20	0.0003*
AD-germ vs. Sound kernel	0.42	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons test).

Table 5.12. Mean kernel weight loss at 50 days after adult emergence on HW wheat sound and AD-kernels.

Treatment	Number of infested kernels	Number of adults emerged	Mean ± SE kernel weight loss (%)*
Sound kernels	4	3	23.61 ± 0.7
AD-brush end	40	38	24.77 ± 1.1
AD-endosperm	33	28	18.79 ± 0.9
AD-germ	42	38	19.51 ± 1.0

[1] There were significant differences among treatments ($F = 7.03$; df = 3, 103; $P = 0.0002$; PROC GLIMMIX; Type III SS).

Table 5.13. Results of least squares means test with Bonferroni adjustment comparing pair-wise kernel weight loss among treatments as shown in Table 5.12.

Treatments compared	*t*-value (df = 103)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	3.97	0.0008*
AD-brush end vs. AD-germ	3.79	0.0015*
AD-brush end vs. Sound kernel	0.32	1.0000
AD-endosperm vs. AD-germ	-0.48	1.0000
AD-endosperm vs. Sound kernel	-1.31	1.0000
AD-germ vs. Sound kernel	-1.13	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons test).

Table 5.14. Probability of infestation by *R. dominica* first instars on SW wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Number of un-infested kernels	Infestation (%)[a,*]
Sound kernels	28	22	56
AD-brush end	48	2	96
AD-endosperm	48	2	96
AD-germ	38	12	76

[a] Percentages are based on 50 kernels for each treatment.

*There were significant differences among treatments ($F = 8.51$; df = 3, 196; $P < 0.0001$; PROC GLIMMIX, fixed effects; Type III SS).

Table 5.15. Results of least squares means test with Bonferroni adjustment comparing pair-wise probability of infestation as shown in Table 5.14.

Treatments compared	*t*-value (df = 196)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	0.00	1.0000
AD-brush end vs. AD-germ	2.55	0.0691
AD-brush end vs. Sound kernel	3.79	0.0012*
AD-endosperm vs. AD-germ	2.55	0.0691
AD-endosperm vs. Sound kernel	3.79	0.0012*
AD-germ vs. Sound kernel	2.09	0.2293

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.16. Preferred site of entry by *R. dominica* first instars on SW wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels*				% Infestation
	Brush end	Endosperm	Germ	Total	(Multinomial 95% CI)[a]
AD-brush end	41	2	5	48	29.6 (21.6 – 37.8)
AD-endosperm	1	43	4	48	29.6 (21.6 – 37.8)
AD-germ	0	1	37	38	23.5 (15.4 – 31.6)
Sound kernels	2	16	10	28	17.3 (9.2 – 25.4)
Total				162	

[a] Percentages infestation are based on a total of 162 kernels.

*There were no significant differences among sites of entry ($\chi^2 = 6.7901$; df = 3; $P = 0.0789$; PROC FREQ Chi-square test).

Table 5.17. Influence of site of entry on head capsule widths of *R. dominica* first instars on SW wheat sound and AD-kernels at 21 days post infestation.

Site of entry	Instar					Number of infested kernels	Mean ± SE head capsule width (mm)*
	1	2	3	4	Pupa		
Sound kernels	0	1	2	25	-	28	0.41 ± 0.01
AD-brush end	1	1	9	37	-	48	0.39 ± 0.01
AD-endosperm	1	1	17	29	-	48	0.38 ± 0.01
AD-germ	0	0	3	35	-	38	0.42 ± 0.01

*There were significant differences among sites of entry ($F = 4.21$; df = 3, 158; $P = 0.0068$; PROC GLIMMIX, Type III SS).

Table 5.18. Results of least squares means test with Bonferroni adjustment comparing pair-wise head capsule width among treatments of first instars as shown in Table 5.17.

Treatments compared	*t*-value (df = 158)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	1.73	0.5146
AD-brush end vs. AD-germ	-1.71	0.5355
AD-brush end vs. Sound kernel	-0.97	1.0000
AD-endosperm vs. AD-germ	-3.34	0.0064*
AD-endosperm vs. Sound kernel	-2.45	0.0921
AD-germ vs. Sound kernel	0.57	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.19. Mean larval weight of *R. dominica* on SW wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Mean ± SE larval weight (mg)*
Sound kernels	28	0.70 ± 0.05
AD-brush end	47	0.62 ± 0.04
AD-endosperm	46	0.61 ± 0.05
AD-germ	38	0.84 ± 0.06

*There were significant differences among treatments ($F = 4.82$; df = 3, 155; $P = 0.0031$; PROC GLIMMIX, Type III SS).

Table 5.20. Results of least squares means test with Bonferroni adjustment comparing pair-wise larval weights among treatments as shown in Table 5.19.

Treatments compared	*t*-value (df = 155)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	0.16	1.0000
AD-brush end vs. AD-germ	-3.26	0.0082*
AD-brush end vs. Sound kernel	-1.13	1.0000
AD-endosperm vs. AD-germ	-3.40	0.0052*
AD-endosperm vs. Sound kernel	-1.26	1.0000
AD-germ vs. Sound kernel	1.78	0.4666

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.21. Mean kernel weight loss of SW wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Mean ± SE kernel weight loss (%)*
Sound kernels	28	12.59 ± 0.9
AD-brush end	47	7.29 ± 0.6
AD-endosperm	46	5.94 ± 0.7
AD-germ	38	10.45 ± 0.9

*There were significant differences among treatments (df = 3, 155; $F = 13.67$; $P < 0.0001$; PROC GLIMMIX, Type III SS).

Table 5.22. Results of least squares means test with Bonferroni adjustment comparing pair-wise kernel weight loss among treatments as shown in Table 5.21.

Treatments compared	*t*-value (df = 155)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	1.33	1.0000
AD-brush end vs. AD-germ	-2.96	0.0212*
AD-brush end vs. Sound kernel	-4.54	< 0.0001*
AD-endosperm vs. AD-germ	-4.21	0.0003*
AD-endosperm vs. Sound kernel	-5.67	< 0.0001*
AD-germ vs. Sound kernel	-1.75	0.4883

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.23. Unsuccessful adult emergence of *R.domnica* first instars on SW wheat sound and AD- kernels.

Treatment	Number of infested kernels[a]	Infestation (%)[b]	Number of adults emerged	Emergence (%)	Mean ± SE duration to adult emergence (days)
Sound kernels	23	46	0	0	0.00 ± 0.000
AD-brush end	50	100	0	0	0.00 ± 0.000
AD-endosperm	48	96	0	0	0.00 ± 0.000
AD-germ	46	92	0	0	0.00 ± 0.000

[a] Based on observing kernels for boring hole/dust on day 27 under a steromicroscope.

[b] Percentages based on 50 kernels.

Table 5.24. Probability of infestation by *R. dominica* first instars on Durum wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Number of un-infested kernels	Infestation (%)[a,*]
Sound kernels	21	29	42
AD-brush end	47	3	94
AD-endosperm	42	8	84
AD-germ	43	7	86

A Percentage infestation is based on 50 kernels/treatment.

*There were significant differences among treatments ($F = 12.25$; df = 3, 196; $P < 0.0001$; PROC GLIMMIX, fixed effects; Type III SS).

Table 5.25. Results of least squares means test with Bonferroni adjustment comparing pair-wise probability of infestation as shown in Table 5.24.

Treatments compared	*t*-value (df = 196)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	1.54	0.7497
AD-brush end vs. AD-germ	1.30	1.0000
AD-brush end vs. Sound kernel	4.65	< 0.0001*
AD-endosperm vs. AD-germ	-0.28	1.0000
AD-endosperm vs. Sound kernel	4.12	0.0003*
AD-germ vs. Sound kernel	4.29	0.0002*

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.26. Preferred site of entry by *R. dominica* first instars on Durum wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels*				% Infestation
	Brush end	Endosperm	Germ	Total	(Multinomial 95% CI)[a]
AD-brush end	42	1	4	47	30.7 (22.9 – 39.6)
AD-endosperm	0	39	3	42	27.5 (19.6 – 36.3)
AD-germ	1	4	37/1 pupa	43	28.1 (20.3 – 36.9)
Sound kernels	0	2	18/1 pupa	21	13.7 (5.9 – 22.6)
Total				153	

[a] Percentages infestation are based on a total of 153 kernels.

*There were significant differences among sites of entry ($\chi^2 = 10.7386$; df = 3; $P = 0.0132$; PROC FREQ Chi-Square Test).

Table 5.27. Influence of site of entry on head capsule widths by *R. dominica* first instars on Durum wheat at 21 days post infestation.

Site of entry	Instar					Number of infested kernels	Mean ± SE Head capsule width (mm)*
	1	2	3	4	Pupa		
Sound kernels	0	0	0	20	1	21	0.42 ± 0.01
AD-brush end	0	0	14	33	-	47	0.39 ± 0.01
AD-endosperm	0	0	7	35	-	42	0.40 ± 0.01
AD-germ	0	1	2	39	1	43	0.43 ± 0.01

*There were significant differences among treatments ($F = 5.49$; df = 3, 147; $P = 0.0013$; PROC GLIMMIX, Type III SS).

Table 5.28. Results of least squares means test with Bonferroni adjustment comparing pair-wise head capsule width among treatments of first instars as shown in Table 5.27.

Treatments compared	*t*-value (df = 147)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-1.55	0.7354
AD-brush end vs. AD-germ	-3.97	0.0007*
AD-brush end vs. Sound kernel	-2.10	0.2262
AD-endosperm vs. AD-germ	-2.35	0.1209
AD-endosperm vs. Sound kernel	-0.85	1.0000
AD-germ vs. Sound kernel	1.04	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.29. Mean larval weight of *R. dominica* on Durum wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Mean ± SE larval weight (mg)*
Sound kernels	20	1.03 ± 0.1
AD-brush end	47	0.56 ± 0.03
AD-endosperm	42	0.59 ± 0.1
AD-germ	42	1.13 ± 0.04

*There were significant differences among treatments ($F = 33.72$; df = 3, 147; $P < 0.0001$; PROC GLIMMIX, Type III SS).

Table 5.30. Results of least squares means test with Bonferroni adjustment comparing pair-wise larval weights among treatments as shown in Table 5.29.

Treatments compared	*t*-value (df = 147)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-0.52	1.0000
AD-brush end vs. AD-germ	-8.48	< 0.0001*
AD-brush end vs. Sound kernel	-5.60	< 0.0001*
AD-endosperm vs. AD-germ	-7.75	< 0.0001*
AD-endosperm vs. Sound kernel	-5.10	< 0.0001*
AD-germ vs. Sound kernel	1.13	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.31. Mean kernel weight loss of Durum wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Mean ± SE kernel weight loss (%)*
Sound kernels	21	12.46 ± 0.9
AD-brush end	47	7.44 ± 0.6
AD-endosperm	41	7.82 ± 0.6
AD-germ	43	11.50 ± 0.9

*There were significant differences among treatments ($F = 10.60$; df = 3, 148; $P < 0.0001$; PROC GLIMMIX, Type III SS).

Table 5.32. Results of least squares means test with Bonferroni adjustment comparing pair-wise kernel weight loss among treatments as shown in Table 5.31.

Treatments compared	*t*-value (df = 148)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-0.38	1.0000
AD-brush end vs. AD-germ	-4.19	0.0003*
AD-brush end vs. Sound kernel	-4.17	0.0003*
AD-endosperm vs. AD-germ	-3.68	0.0020*
AD-endosperm vs. Sound kernel	-3.77	0.0014*
AD-germ vs. Sound kernel	-0.79	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.33. Successful adult emergence of *R. domnica* first instars on Durum wheat sound and AD-kernels.

Treatment	Number of infested kernels[a]	Infestation (%)[b]	Number of adults emerged	Emergence (%)[b]	Mean ± SE duration to adult emergence (days)*
Sound kernels	18	36	18	36	34.17 ± 0.6
AD-brush end	48	96	41	82	38.59 ± 0.6
AD-endosperm	42	84	41	82	38.73 ± 0.6
AD-germ	44	88	44	88	34.52 ± 0.6

[a] Based on observing kernels for boring hole/dust on day 27 under a steromicroscope.

[b] Percentages based on 50 kernels.

*There were significant differences among treatments ($F = 15.05$; df = 3, 140; $P < 0.0001$; PROC GLIMMIX, Type III SS).

Table 5.34. Results of least squares means test with Bonferroni adjustment comparing pair-wise adult emergence among treatments as shown in Table 5.33.

Treatments compared	*t*-value (df = 140)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-0.18	1.0000
AD-brush end vs. AD-germ	4.98	< 0.0001*
AD-brush end vs. Sound kernel	4.16	0.0003*
AD-endosperm vs. AD-germ	5.16	< 0.0001*
AD-endosperm vs. Sound kernel	4.30	0.0002*
AD-germ vs. Sound kernel	0.34	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.35. Mean kernel weight loss at 50 days after adult emergence on Durum wheat sound and AD-kernels.

Treatment	Number of infested kernels	Number of adults emerged	Mean ± SE kernel weight loss (%)*
Sound kernels	18	18	20.21 ± 0.9
AD-brush end	48	41	20.51 ± 0.7
AD-endosperm	42	41	17.64 ± 0.6
AD-germ	44	44	18.85 ± 0.8

*There were significant differences among treatments (*F* = 3.24; df = 3, 140; *P* = 0.0242; PROC GLIMMIX; Type III SS).

Table 5.36. Results of least squares means test with Bonferroni adjustment comparing pair-wise kernel weight loss among treatments as shown in Table 5.35.

Treatments compared	*t*-value (df = 140)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	2.91	0.0252*
AD-brush end vs. AD-germ	1.72	0.5260
AD-brush end vs. Sound kernel	0.24	1.0000
AD-endosperm vs. AD-germ	-1.24	1.0000
AD-endosperm vs. Sound kernel	-2.03	0.2627
AD-germ vs. Sound kernel	1.09	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.37. Probability of infestation by *R. dominica* first instars on SRW wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Number of un-infested kernels	Infestation (%)[a,*]
Sound kernels	13	37	26
AD-brush end	41	9	82
AD-endosperm	41	9	82
AD-germ	42	8	84

[a] *n* = 50 kernels/treatment.

*There were significant differences among treatments (*F* = 15.08; df = 3, 196; *P* < 0.0001; PROC GLIMMIX, fixed effects; Type III SS).

Table 5.38. Results of least squares means test with Bonferroni adjustment comparing pair-wise probability of infestation as shown in Table 5.37.

Treatments compared	*t*-value (df = 196)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-0.00	1.0000
AD-brush end vs. AD-germ	-0.27	1.0000
AD-brush end vs. Sound kernel	5.24	< 0.0001*
AD-endosperm vs. AD-germ	-0.27	1.0000
AD-endosperm vs. Sound kernel	5.24	< 0.0001*
AD-germ vs. Sound kernel	5.38	< 0.0001*

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.39. Preferred site of entry by *R. dominica* first instars on SRW wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels*				% Infestation
	Brush end	Endosperm	Germ	Total	(Multinomial 95% CI)[a]
AD-brush end	35	5	1	41	29.9 (21.2 – 38.9)
AD-endosperm	1	40	0	41	29.9 (21.2 – 38.9)
AD-germ	0	0	37/5 pupae	42	30.7 (21.9 – 39.6)
Sound kernels	2	9	1/1 pupa	13	9.49 (0.7 – 18.4)
Total				137	

[a] Percentages infestation are based on a total of 137 kernels.

*There were significant differences among sites of entry ($\chi^2 = 17.5985$; df = 3; $P = 0.0005$; PROC FREQ Chi-Square Test).

Table 5.40. Influence of site of entry on head capsule widths of *R. dominica* first instars on SRW wheat at 21 days post infestation.

Site of entry	Instar					Number of infested kernels	Mean ± SE head capsule width (mm)*
	1	2	3	4	Pupae		
Sound kernels	0	0	2	10	1	13	0.41 ± 0.02
AD-brush end	0	1	9	31	-	41	0.40 ± 0.01
AD-endosperm	0	1	6	33	-	40**	0.41 ± 0.01
AD-germ	0	0	0	37	5	42	0.44 ± 0.00

*There were significant differences among treatments ($F = 4.03$; df = 3, 126; $P = 0.0090$; PROC GLIMMIX, Type III SS).

**One (1) larva lost while being weighed at the analytical balance.

Table 5.41. Results of least squares means test with Bonferroni adjustment comparing pair-wise head capsule width among treatments of first instars as shown in Table 5.40.

Treatments compared	*t*-value (df = 126)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-0.45	1.0000
AD-brush end vs. AD-germ	-3.20	0.0103*
AD-brush end vs. Soundn kernel	-0.37	1.0000
AD-endosperm vs. AD-germ	-2.75	0.0408*
AD-endosperm vs. Sound kernel	-0.07	1.0000
AD-germ vs. Sound kernel	1.82	0.4268

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.42. Mean larval weight of *R. dominica* on SRW wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Mean ± SE larval weight (mg)*
Sound kernels	12	1.07 ± 0.1
AD-brush end	38	0.85 ± 0.06
AD-endosperm	36	0.91 ± 0.06
AD-germ	37	1.34 ± 0.05

*There were significant differences among treatments ($F = 12.96$; df = 3, 119; $P < 0.0001$; PROC GLIMMIX, Type III SS).

Table 5.43. Results of least squares means test with Bonferroni adjustment comparing pair-wise larval weights among treatments as shown in Table 5.42.

Treatments compared	*t*-value (df = 119)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-0.62	1.0000
AD-brush end vs. AD-germ	-5.70	< 0.0001*
AD-brush end vs. Sound kernel	-1.83	0.4162
AD-endosperm vs. AD-germ	-5.01	< 0.0001*
AD-endosperm vs. Sound kernel	-1.39	1.0000
AD-germ vs. Sound kernel	2.14	0.2080

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.44. Mean kernel weight loss of SRW wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Mean ± SE kernel weight loss (%)*
Sound kernels	12	16.26 ± 1.6
AD-brush end	41	20.57 ± 1.1
AD-endosperm	41	15.84 ± 1.1
AD-germ	42	20.94 ± 0.7

*There were significant differences among treatments (*F* = 6.74; df = 3, 132; *P* = 0.0003; PROC GLIMMIX, Type III SS).

Table 5.45. Results of least squares means test with Bonferroni adjustment comparing pair-wise kernel weight loss among treatments as shown in Table 5.44.

Treatments compared	*t*-value (df = 132)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	3.50	0.0038*
AD-brush end vs. AD-germ	-0.28	1.0000
AD-brush end vs. Sound kernel	2.14	0.2031
AD-endosperm vs. AD-germ	-3.80	0.0013*
AD-endosperm vs. Sound kernel	-0.21	1.0000
AD-germ vs. Sound kernel	2.34	0.1256

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.46. Successful adult emergence of *R.domnica* first instars on SRW wheat sound and AD-kernels.

Treatment	Number of infested kernels[a]	Infestation (%)[b]	Number of adults emerged	Emergence (%)[b]	Mean ± SE duration to adult emergence (days)*
Sound kernels	8	16	8	16	33.88 ± 0.6
AD-brush end	45	90	45	90	38.47 ± 0.5
AD-endosperm	44	88	44	88	35.75 ± 0.5
AD-germ	38	76	38	76	34.37 ± 0.4

[a] Based on observing kernels for boring hole/dust on day 27 under a stereomicroscope.

[b] Percentages based on 50 kernels.

*There were significant differences among treatments (*F* = 13.74; df = 3, 131; *P* < 0.0001; PROC GLIMMIX; Type III SS).

Table 5.47. Results of least squares means test with Bonferroni adjustment comparing pair-wise adult emergence among treatments as shown in Table 5.46.

Treatments compared	*t*-value (df = 131)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	4.06	0.0005*
AD-brush end vs. AD-germ	5.89	< 0.0001*
AD-brush end vs. Sound kernel	3.79	0.0014*
AD-endosperm vs. AD-germ	1.98	0.3012
AD-endosperm vs. Sound kernel	1.55	0.7478
AD-germ vs. Sound kernel	0.40	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.48. Mean kernel weight loss at 50 days after adult emergence on SRW wheat sound and AD-kernels.

Treatment	Number of infested kernels	Number of adults emerged	Mean ± SE kernel weight loss (%)*
Sound kernels	8	8	24.79 ± 1.5
AD-brush end	45	45	24.09 ± 0.9
AD-endosperm	44	44	23.41 ± 0.8
AD-germ	38	35	26.38 ± 0.9

*There were no significant differences among treatments (*F* = 1.92; df = 3, 128; *P* = 0.1295; PROC GLIMMIX; Type III SS).

Table 5.49. Probability of infestation by *R. dominica* first instars on HRS wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Number of un-infested kernels	Infestation (%)[a, *]
Sound kernels	4	46	8
AD-brush end	47	3	94
AD-endosperm	44	6	88
AD-germ	45	5	90

[a] *n* = 50 kernels/treatment.

*There were significant differences among treatments ($F = 21.13$; df = 3, 196; $P < 0.0001$; PROC GLIMMIX, fixed effects; Type III SS).

Table 5.50. Results of least squares means test with Bonferroni adjustment comparing pair-wise probability of infestation as shown in Table 5.49.

Treatments compared	*t*-value (df = 196)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	1.03	1.0000
AD-brush end vs. AD-germ	0.73	1.0000
AD-brush end vs. Sound kernel	6.56	< 0.0001*
AD-endosperm vs. AD-germ	-0.32	1.0000
AD-endosperm vs. Sound kernel	6.53	< 0.0001*
AD-germ vs. Sound kernel	6.60	< 0.0001*

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.51. Preferred site of entry by *R. dominica* first instars on HRS wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels*				% Infestation
	Brush end	Endosperm	Germ	Total	(Multinomial 95% CI)[a]
AD-brush end	44	0	3	47	33.6 (25.0 – 42.8)
AD-endosperm	0	43	1	44	31.4 (22.9 – 40.7)
AD-germ	0	0	45	45	32.1 (23.6 – 41.4)
Sound kernels	1	0	3	4	2.9 (0.0 – 12.1)
Total				140	

[a] Percentages infestation are based on a total of 140 kernels.

* There were significant differences among sites of entry (χ^2 = 36.7429; df = 3; P = < 0.0001; PROC FREQ Chi-Square Test).

Table 5.52. Influence of site of entry on head capsule widths by *R. dominica* first instars on HRS wheat at 21 days post infestation.

Site of entry	Instar					Number of infested kernels	Mean ± SE Head capsule width (mm)*
	1	2	3	4	Pupa		
Sound kernels	0	0	1	3	-	4	0.39 ± 0.04
AD-brush end	7	0	22	17	-	46**	0.33 ± 0.01
AD-endosperm	9	0	25	10	-	44	0.30 ± 0.01
AD-germ	0	0	0	44	1	45	0.43 ± 0.00

*There were significant differences on head capsule width among treatments ($F = 21.94$; df = 3, 134; $P = < 0.0001$; PROC GLIMMIX, Type III SS).

**One (1) larva lost during weighing using an analytical balance.

Table 5.53. Results of least squares means test with Bonferroni adjustment comparing pair-wise head capsule width among treatments of first instars as shown in Table 5.52.

Treatments compared	*t*-value (df = 134)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	1.59	0.6850
AD-brush end vs. AD-germ	-6.10	<0.0001*
AD-brush end vs. Sound kernel	-1.66	0.6001
AD-endosperm vs. AD-germ	-7.60	<0.0001*
AD-endosperm vs. Sound kernel	-2.30	0.1396
AD-germ vs. Sound kernel	0.81	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.54. Mean larval weight of *R. dominica* on HRS wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Mean ± SE larval weight (mg)*
Sound kernels	3	0.90 ± 0.2
AD-brush end	23	0.46 ± 0.09
AD-endosperm	9	0.48 ± 0.1
AD-germ	43	1.22 ± 0.04

*There were significant differences among treatments ($F = 34.63$; df = 3, 74; $P < 0.0001$; PROC GLIMMIX, Type III SS).

Table 5.55. Results of least squares means test with Bonferroni adjustment comparing pair-wise larval weights among treatments as shown in Table 5.54.

Treatments compared	*t*-value (df = 74)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-0.17	1.0000
AD-brush end vs. AD-germ	-9.28	< 0.0001*
AD-brush end vs. Sound kernel	-2.25	0.1663
AD-endosperm vs. AD-germ	-6.36	< 0.0001*
AD-endosperm vs. Sound kernel	-1.97	0.3164
AD-germ vs. Sound kernel	1.71	0.5522

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.56. Mean kernel weight loss of HRS wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Mean ± SE kernel weight loss (%)*
Sound kernels	4	5.52 ± 1.7
AD-brush end	40	2.64 ± 0.5
AD-endosperm	31	1.62 ± 0.3
AD-germ	44	10.49 ± 0.5

*There were significant differences among treatments ($F = 74.39$; df = 3, 115; $P < 0.0001$; PROC GLIMMIX, Type III SS).

Table 5.57. Results of least squares means test with Bonferroni adjustment comparing pair-wise kernel weight loss among treatments as shown in Table 5.56.

Treatments compared	*t*-value (df = 115)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	1.45	0.8912
AD-brush end vs. AD-germ	-12.36	<0.0001*
AD-brush end vs. Sound kernel	-1.89	0.3675
AD-endosperm vs. AD-germ	-13.00	<0.0001*
AD-endosperm vs. Sound kernel	-2.52	0.0785
AD-germ vs. Sound kernel	3.27	0.0084*

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.58. Successful adult emergence of *R. domnica* first instars on HRS wheat sound and AD-kernels.

Treatment	Number of infested kernels[a]	Infestation (%)[b]	Number of adults emerged	Emergence (%)[b]	Mean ± SE duration to adult emergence (days)*
Sound kernels	6	12	5	10	36.40 ± 2.4
AD-brush end	48	96	45	90	40.62 ± 0.4
AD-endosperm	44	88	39	78	40.49 ± 1.2
AD-germ	44	88	44	88	33.82 ± 0.4

[a] Based on observing kernels for boring hole/dust on day 27 under a stereomicroscope.

[b] Percentages based on 50 kernels.

*There were significant differences among treatments ($F = 19.18$; df = 3, 129; $P < 0.0001$; PROC GLIMMIX, Type III SS).

Table 5.59. Results of least squares means test with Bonferroni adjustment comparing pair-wise adult emergence among treatments as shown in Table 5.58.

Treatments compared	*t*-value (df = 129)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	0.13	1.0000
AD-brush end vs. AD-germ	6.68	< 0.0001*
AD-brush end vs. Sound kernel	1.86	0.3860
AD-endosperm vs. AD-germ	6.31	< 0.0001*
AD-endosperm vs. Sound kernel	1.79	0.4546
AD-germ vs. Sound kernel	-1.14	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.60. Mean kernel weight loss at 50 days after adult emergence on HRS wheat sound and AD-kernels.

Treatment	Number of infested kernels	Number of adults emerged	Mean ± SE kernel weight loss (%)*
Sound kernels	6	5	17.10 ± 1.6
AD-brush end	48	45	15.82 ± 0.6
AD-endosperm	44	37	17.43 ± 0.6
AD-germ	44	44	17.53 ± 0.7

[1] There were no significant differences among treatments (*F* = 1.48; df = 3, 127; *P* = 0.2232; PROC GLIMMIX; Type III SS).

Table 5.61. Probability of infestation by *R. dominica* first instars on HWS wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Number of un-infested kernels	Infestation (%)[a, *]
Sound kernels	5	45	10
AD-brush end	43	7	86
AD-endosperm	43	7	86
AD-germ	46	4	92

[a] $n = 50$ kernels/treatment.

*There were significant differences among treatments ($F = 20.54$; df = 3, 196; $P < 0.0001$; PROC GLIMMIX, fixed effects; Type III SS).

Table 5.62. Results of least squares means test with Bonferroni adjustment comparing pair-wise probability of infestation as shown in Table 5.61.

Treatments compared	*t*-value (df = 196)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-0.00	1.0000
AD-brush end vs. AD-germ	-0.95	1.0000
AD-brush end vs. Sound	6.44	< 0.0001*
AD-endosperm vs. AD-germ	-0.95	1.0000
AD-endosperm vs. Sound	6.44	< 0.0001*
AD-germ vs. Sound	6.60	< 0.0001*

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.63. Preferred site of entry establishment across all sound and AD-kernels of HWS wheat by *R. dominica* first instars at 21 days.

Treatment	Number of infested kernels*				% Infestation
	Brush end	Endosperm	Germ	Total	(Multinomial 95% CI)[a]
AD-brush end	36	0	7	43	31.3 (22.6 – 40.5)
AD-endosperm	0	39	4	43	31.3 (22.6 – 40.5)
AD-germ	0	0	36/10 pupae	46	33.6 (24.8 – 42.8)
Sound kernels	1	2	2	5	3.7 (0.0 – 12.9)
Total				137	

[a] Percentages infestation are based on a total of 137 kernels.

* There were significant differences among sites of entry ($\chi^2 = 33.4818$; df = 3; $P = < 0.0001$; PROC FREQ Chi-square test).

Table 5.64. Influence of site of entry on head capsule widths of *R. dominica* first instars on HWS wheat at 21 days post infestation.

Site of entry	Instar					Number of infested kernels	Mean ± SE head capsule width (mm)[1]
	1	2	3	4	Pupae		
Sound kernels	0	0	1	4	-	5	0.40 ± 0.03
AD-brush end	0	1	10	32	-	43	0.38 ± 0.01
AD-endosperm	3	1	26	13	-	43	0.32 ± 0.01
AD-germ	0	0	4	32	10	46	0.42 ± 0.01

[1] There were significant differences among treatments (*F* = 18.44; df = 3, 123; *P* = < 0.0001; PROC GLIMMIX, Type III SS).

Table 5.65. Results of least squares means test with Bonferroni adjustment comparing pair-wise head capsule width among treatments of first instars as shown in Table 5.64.

Treatments compared	*t*-value (df = 123)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	4.80	<0.0001*
AD-brush end vs. AD-germ	-2.61	0.0613
AD-brush end vs. Sound kernel	-0.66	1.0000
AD-endosperm vs. AD-germ	-7.19	<0.0001*
AD-endosperm vs. Sound kernel	-2.85	0.0306*
AD-germ vs. Sound kernel	0.58	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.66. Mean larval weight of *R.dominica* on HWS wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Mean ± SE larval weight (mg)*
AD-brush end	17	0.89 ± 0.05
AD-endosperm	8	0.90 ± 0.2
AD-germ	29	1.13 ± 0.05

*There were significant differences among treatments (*F* = 4.55; df = 2, 51; *P* = 0.0151; PROC GLIMMIX, Type III SS); Sound kernel data excluded from analysis due to ≤ 5 data points obtained.that weakened the test analysis.

Table 5.67. Results of least squares means test with Bonferroni adjustment comparing pair-wise larval weights among treatments as shown in Table 5.66.

Treatments compared	*t*-value (df = 51)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-0.09	1.0000
AD-brush end vs. AD-germ	-2.74	0.0255*
AD-endosperm vs. AD-germ	-1.99	0.1551

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.68. Mean kernel weight loss of HWS wheat sound and AD-kernels at 21 days.

Treatment	Number of infested kernels	Mean ± SE kernel weight loss (%)*
Sound kernels	5	3.97 ± 1.4
AD-brush end	37	3.21 ± 0.5
AD-endosperm	35	2.74 ± 0.5
AD-germ	46	6.93 ± 0.5

[1] There were significant differences among treatments ($F = 16.64$; df = 3, 119; $P < 0.0001$; PROC GLIMMIX, Type III SS).

Table 5.69. Results of least squares means test with Bonferroni adjustment comparing pair-wise kernel weight loss among treatments as shown in Table 5.68.

Treatments compared	*t*-value (df = 119)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	0.66	1.0000
AD-brush end vs. AD-germ	-5.66	<0.0001*
AD-brush end vs. Sound kernel	-0.54	1.0000
AD-endosperm vs. AD-germ	-6.27	<0.0001*
AD-endosperm vs. Sound kernel	-0.86	1.0000
AD-germ vs. Sound kernel	2.11	0.2219

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.70. Successful adult emergence of *R domnica* first instars on HWS wheat sound and AD-kernels.

Treatment	Number of infested kernels[a]	Infestation (%)[b]	Number of adults emerged	Emergence (%)[b]	Mean ± SE duration to adult emergence (days)*
Sound kernels	14	28	14	28	33.50 ± 0.5
AD-brush end	44	88	42	84	37.86 ± 0.6
AD-endosperm	47	94	44	88	39.36 ± 0.7
AD-germ	49	98	49	98	33.86 ± 0.5

[a] Based on observing kernels for boring hole/dust on day 27 under a stereomicroscope.

[b] Percentages based on 50 kernels.

*There were significant differences among treatments (*F* = 22.01; df = 3, 145; *P* < 0.0001; PROC GLIMMIX; Type III SS).

Table 5.71. Results of least squares means test with Bonferroni adjustment comparing pair-wise adult emergence among treatments as shown in Table 5.70.

Treatments compared	*t*-value (df = 145)	Adjusted *P*-value
AD-brush end vs. AD-endosperm	-1.89	0.3681
AD-brush end vs. AD-germ	5.14	< 0.0001*
AD-brush end vs. Sound kernel	3.81	0.0012*
AD-endosperm vs. AD-germ	7.16	< 0.0001*
AD-endosperm vs. Sound kernel	5.16	< 0.0001*
AD-germ vs. Sound kernel	0.32	1.0000

*Significant ($P < 0.05$; by Bonferroni multiple comparisons adjustment test).

Table 5.72. Mean kernel weight loss at 50 days after adult emergence on HWS wheat sound and AD-kernels.

Treatment	Number of infested kernels	Number of adults emerged	Mean ± SE kernel weight loss 1(%)*
Sound kernels	14	14	11.83 ± 0.9
AD-brush end	44	42	12.49 ± 0.4
AD-endosperm	47	44	13.37 ± 0.7
AD-germ	49	49	12.21 ± 0.4

*There were no significant differences among treatments (*F* = 1.19; df = 3, 145; *P* = 0.3141; PROC GLIMMIX; Type III SS).

Chapter 6

Future Research

Although many goals of this research were addressed, there are still additional studies that remain to be tackled and answered. Since the study determined that *R. dominica* first instars preferred germ portion as site of entry that facilitated its growth and development, a behavioral research focusing on how quickly first instar enter a sound versus an artificially-damaged wheat kernel for successful development should be determined. Second, a research investigating the role of chemical factors (volatiles and nutrients) affecting first instar orientation to germ anatomical site either on natural- and mechanically-damaged wheat kernels is needed. Third, a research that characterizes the degree of hardness of the three anatomical portions (germ, endosperm, or brush end) of the wheat kernel in relation to infestation must be determined. Lastly, an assessment of *R. dominica* first instar and adult infestation of wild ancestors of wheat compared to modern varieties is needed to determine when in the evolution wheat did *R. dominica* become a pest of wheat.