DEVELOPMENT, RELATIVE RETENTION, AND OVIPOSITION OF THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST), ON DIFFERENT STARCHES

by

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B.E., China Agricultural University, 2008

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2010

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Abstract

The development, relative retention, and oviposition of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), on six different types of starches, wheat flour, and wheat flour plus yeast were investigated in the laboratory. The particle size of starch and flours were different; the mean size of 90% of particles for starches ranged from 15 μm for high amylose corn starch to 58 μm for potato, whereas that of the flour was ≤ 133 μm. Larval length, head capsule width, and weight gain of *T. castaneum* larvae were measured every 3 d on starches, flour, and flour plus 5% (by wt) yeast diet for 30 d at 28°C, 65% r.h., and 14:10 (L:D) photoperiod. Larvae reared on flour and flour plus yeast developed normally and showed better survival compared to those reared on starches. Larvae on the starches failed to develop beyond second, and rarely, third instars. Adults of *T. castaneum* did not show any preference to flour over starches in dual-choice tests in circular arenas. On average, *T. castaneum* laid ≤3 eggs/female over a 15-d period on starches compared to 97 and 109 eggs/female on flour and flour plus yeast diet, respectively. These studies suggest that starches are poor substrates for larval survival and development. Starches were as attractive as flour to adults; however, starches do not appear to be a suitable medium for egg-laying. Both aggregation pheromone and volatiles did not trigger oviposition behavior. Experiments by moving adults between wheat starch and wheat flour and *vice versa* showed that feeding on wheat flour was necessary for egg-laying, indicating the absence of essential nutrients in wheat starch.

On wheat flour, feeding for 0.5 d was necessary to lay eggs. Females that were starved failed to lay eggs, reinforcing that the nutritional status of females and not males was essential for egg-laying. A minimum of 4% of wheat gluten (wheat protein) elicited egg-laying on

starches, although 4-5 times fewer eggs were laid in starch gluten compared with wheat flour alone. Supplementing wheat starch with 1% cholesterol, in addition, to gluten, did not result in an increase in egg-laying by *T. castaneum* females. These findings suggest that starches may have potential in managing development and reproduction of *T. castaneum*—a pest that is common and severe in food-processing facilities. Furthermore, starches can be used as a suitable substrate for studying the nutritional ecology of *T. castaneum*.

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Acknowledgments

I could not complete the thesis without the help of many people. I would like thank all the people who have helped and inspired me during my master's study. First, I would like to show my deepest gratitude to my advisor, Dr. Bhadriraju Subramanyam, whose encouragement, guidance, and support from the initiation of my graduate studies to the completion of my program enabled me to grow professionally and become who I am today. Dr. Subramanyam was always there to listen, to give advice, and encourage me to try new ideas. He showed me different ways to approach a research problem and the need to be persistent to accomplish any goal. Under his direction, the past two years have been some of the important and formative years of my life. Dr. Yong-Cheng Shi of Grain Science and Industry, Kansas State University, and Dr. James Campbell, USDA's Center for Grain and Animal Health Research, Manhattan, Kansas, deserve special mention for being my committee members. They provided on various portions of my thesis. I am deeply indebted to them for their valuable comments and support. They have generously given their time and expertise to improve my thesis.

I convey my sincere gratitude to Dr. Subbaratnam Muthukrishnan, University Distinguished Professor of Chemistry, Kansas State University, for his help on giving advice on the red flour beetle nutrition and reproduction. He gave valuable advice on dissecting insects to study the reproductive organs.

I would like to thank Ms. Mary Roth, Division of Biology, Kansas State University, who provided help in analyzing the lipid content of the wheat starch, and allowed me work in the Lipidomics Center at Kansas State University where I gained experience in lipid analysis.

All my colleagues in the Stored Product Educational and Research Laboratory in Dr. Subramanyam's group made it a fun place to work. My special gratitude goes to Ms. Michelle Hartzer, Research Assistant, who always gave me good suggestions on how to improve my experiments. I would like to thank Dr. Lakshmikantha Channaiah, Khamis Moses, and Monika Brijwani who were a constant source of inspiration to work harder and excel in my studies. I thank my friends Hyma Gajula, Yanjie Bai, and Yxing Zhang for great times in Manhattan. Finally

I thank my family, especially my father and mother, whose unconditional love was a constant source of encourage, and I therefore, dedicate this thesis to these great individuals for who I am today.

Chapter 1 - Development, relative retention, and oviposition of *Tribolium castaneum* on flour, flour plus yeast, and six different starches

Abstract

The development, relative retention and oviposition of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), on wheat flour, wheat flour plus yeast, and six types of starches were investigated in the laboratory. The particle size of starch and flours varied; the mean size of 90% of particles for starches ranged from 15 μm for high amylose corn starch to 58 μm for potato starch, while that of the flour was ≤133 μm. Larval length, head capsule width, and weight of *T. castaneum* were measured every 3 d by rearing larvae on starches, flour, and flour plus 5% (by wt) yeast diet until larvae pupated (30 d) at 28^{o}C, 65% r.h. and 14:10 (L:D) photoperiod. Larvae reared on flour and flour plus yeast developed normally and showed better survival compared to those reared on starches. Larvae reared on starches failed to develop beyond second, and rarely third instars. Adults of *T. castaneum* did not show any preference to flour over starches in dual-choice tests. On average, *T. castaneum* laid less than 3 eggs/female over a 15-d period on starches compared to 97 and 109 eggs/female on flour and flour plus yeast diet, respectively. These studies suggest that starches, despite being as attractive as flour to adults, are poor substrates for larval survival, development, and oviposition. The aggregation pheromone lure had no effect on oviposition in flour and starches. Contact and/or feeding in flour was essential for egg-laying by *T. castaneum* in wheat starch, indicating the absence of essential nutrients essential for egg development in wheat starch. These findings suggest that starches may have potential in managing development and reproduction of *T. castaneum*; a pest that is commonly associated with food-processing facilities.

Keywords: Starch, Red flour beetle, Nutritional ecology, Oviposition behavior triggers

1. Introduction

Starch is second only to cellulose as a major carbohydrate and exists in advanced plants, and it is the main carbohydrate source in human diet (Guilbot and Mercier, 1985). Starches are usually digested in small intestine into glucose, where it is then absorbed and transported by blood to cells for metabolism (Voet et al., 2008). The starch utilization efficiency depends on the activity of amylase and on the type of starch. Englyst et al. (1992) classified starches into rapidly digestible starch, slowly digestible starch, and resistant starch based on the digestibility in the human intestine. Resistant starch (RS) is defined as starch and starch products that are not absorbed in the small intestine of healthy individuals. There are four types of resistant starches (RS) (Baghurst, 1996; Englyst et al., 1992; Woo and Seib, 2002). Starch that is physically encapsulated within a plant cell or tissue such as in whole grains is of the RS1 type. The RS2 type includes granular starch with crystallinity of native B-type starch, which is found in green banana and raw potato. The RS3 refers to non-granular, retrograded amylose, which is usually produced when starch-containing foods are cooked and cooled. The RS4 is chemically modified starch and can be manufactured from various sources, including wheat, potato, tapioca, and high-amylose content maize. Low digestibility of resistant starches in the human small intestine lowers blood glucose levels than other carbohydrates. It has been used in human diet for the management of insulin resistance, diabetes, and coronary heart diseases as well as for weight control (Annison and Topping, 1994).

The red flour beetle, *T. castaneum* (Herbst), is an economically important insect pest in food-processing facilities such as flour mills (Good, 1937; Sinha and Watters, 1985). Starch is the main carbohydrate source for this species. Carbohydrates greatly influence larval growth of *T. castaneum* (Sokoloff, 1974; Rentería-Gutiérrez et al., 2000). Starch is usually hydrolyzed by

amylases in *T. castaneum* (Krishna and Saxena, 1962). Wool and Noiman (1980) and Bremner et al., (1982) detected two amylase isozymes using vertical acrylamide slab gel electrophoresis, and the amylase activity was mainly in the midgut (Wool and Noiman, 1980).

Vohra et al. (1981) studied growth of *T. castaneum* larvae on diets containing 9.5% dried brewer's yeast, 7.5% soybean protein isolate, and 83% rice flour from 17 brown rices and 21 milled rices that differed in starch properties and protein content. Larvae were reared on the diets at 33^{o}C and 70% r.h., and the larval weight was measured at 14 d. They reported faster larval growth on milled than brown rice. Larval growth on rice flours was negatively correlated with the amylose content (Shariff et al., 1981; Vohra et al., 1981). The differences observed in larval growth were attributed to relative digestibility of the starch granules of the rice flours. Vohra et al. (1978) reported larval weigh gain in 14 d to be significantly higher on rice starch and wheat starch than on potato starch, waxy maize starch, and tapioca starch, and in this study these carbohydrates made up 40% of the insect diet. Applebaum (1966) reported 100% larval mortality in 14 d at 32^{o}C on a diet in which raw potato starch (80% by wt) was the only carbohydrate source, whereas mortality on cooked potato starch was 27%. The poor development and survival of larvae on raw potato starch was attributed to starch granule resistance to digestion. However, the average larval weight (1.87 mg/larva) and survival rate (96-98%) of *T. castaneum* at 14 d were essentially similar between potato starch that was extensively digested with salivary amylase and rice starch which was used as the control treatment. Applebaum and Konijn (1965) found that larval weight and percentage of larval survival were similar on diet containing rice starch, corn starch, and wheat starch as the carbohydrate source. Baker et al. (1992) observed *in vitro* and *in vivo* digestion of purified wheat starch granules by *T.*

castaneum larvae and found a similar digestion pattern–scattered attack initially on the surface and then penetration of the granules by enzyme.

Many of the evaluations examining effects of starches on *T. castaneum* involved carbohydrate sources making up a certain percentage of the diet. The effects of pure starches on *T. castaneum* development and reproduction were not explored fully. The present investigation was designed to determine development, preference, and oviposition of *T .castaneum* on six starches varying in amylose content and type of crystalline structure. The goal is to explore the value of starches as a nutritional control method. This method was proposed by Pratt et al. (1972) and involves adding non-nutrients or inert substances to food to render it unsuitable as a source of nutrients.

2. Materials and methods

2.1 Insects

Cultures of *T. castaneum* were reared on wheat flour fortified with 5% by wt of brewer's yeast at 28^{o}C, 65% r.h., and 14:10 (L:D) h photoperiod in a growth chamber (Model I-36 VL, Percival Scientific, Perry, IA) in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS, USA. The *T. castaneum* population used in the present tests has been reared continuously in the laboratory since 1999. To collect *T. castaneum* eggs and pupae, 50 unsexed adults of mixed ages separated from culture jars were placed on 20 g flour in 150-ml plastic containers with lids. The lid had a perforation that was covered with a 250 μm plastic mesh to allow airflow and for preventing adults from escaping. The cups were kept at rearing conditions for 3 d after which the adults were removed by sifting the flour on an 841 μm opening sieve to remove adults. The flour with eggs that passed through was then sifted over a 250 μm opening sieve to retain the eggs. To obtain pupae for oviposition tests, 50 adults were placed on

20 g of flour in plastic containers for 3 d. Eggs collected following procedures described above were placed on 20 g of flour in 150-ml plastic containers with lids and reared at 28°C and 65% r.h. After 30 d, the flour was sifted through an 841 µm sieve to separate pupae from flour. Sexing was done by separating pupae from the medium and by examining their external genitalia under a stereomicroscope (Nikon SMZ 645, Nikon Instruments Inc., Melville, NY, USA) according to a standard method (http://bru.gmprc.ksu.edu/proj/tribolium/wrangle.asp). Male and female pupae were placed in separate 9 cm glass Petri dishes and held at 28°C and 65% r.h. until adult emergence.

2.2 Starches and flour

The six starches types used included waxy corn starch (essentially 0% amylose), corn starch (26% amylose), and high amylose corn starch (70% amylose), which were obtained from National Starch and Chemical Co., Bridgewater, NJ, USA. Wheat starch (27% amylose) and cross-linked wheat starch (70% total dietary fiber content) were obtained from MGP Ingredients Inc., Atchison, KS, USA. The potato starch (20% amylose) was obtained from Sigma-Aldrich Inc., St. Louis, MO, USA. Organic wheat flour was purchased from Heartland Mills, Marienthal, KS, USA. Brewer's yeast (Lesaffre Yeast Corporation, Milwaukee, WI, USA) used in this project was ground using a coffee grinder. The yeast after grinding was passed through a sieve with 250 µm openings (Seedburo Equipment Company, Chicago, IL, USA). The particle sizes of all starches and wheat flour (without yeast), in duplicate, were analyzed using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK, USA) by NanoScale Corporation in Manhattan, KS, USA.

2.3 Larval development and survival on starches and flour

About 5 g of each starch, flour, and flour plus 5% by wt yeast diet were transferred separately into 30-ml plastic condiment cups. Fifty *T. castaneum* eggs were added into each cup. The cups were covered with plastic lids. Holes were made in the lids with a pin for ventilation. All cups were placed in a growth chamber at 28°C and 65% r.h. Three cups were removed from the chamber every 3 d, and independent cups were sampled over time for a maximum period of 30 d from the time of egg introduction. Larvae were separated from flour using a 250 µm sieve. The lengths and head capsule widths of 10 larvae collected from each cup were measured, where possible. The lengths and head capsule widths of larvae from a cup were averaged to obtain a single value for that replication. In addition, the number of larvae surviving out of the total (50) was also recorded. To obtain larval weights, all larvae in all three cups were pooled and weighed on a Mettler® balance (Mettler-Toledo, Inc., Columbus, OH, USA).

To determine egg viability, 100 eggs were placed in 10 separate 9 cm glass Petri dishes and held at 28^{o}C and 65% r.h. These dishes were checked daily for 7 d to count number of eggs that hatched.

2.4 Adult retention in starches and flour

To determine the retention of adults of *T. castaneum* in starches and flour, dual-choice tests were conducted in circular arenas as described by Subramanyam (1992). Each arena measured 30 cm in diameter and 8 cm in height. Arena floors were cut from white foam display board into 30 cm diameter circles. Arena walls were cut from white mat board and the interior wall of the arena were covered with plastic tape to prevent adult escape from floor-wall junctions. In each arena, the north and south end were marked by a highlighter. These two locations were used for placing food. Each starch (2.5 g) and flour (2.5 g) was offered to adults

in these dual-choice tests. The location of starch and flour on the north or south end of the arena was selected randomly with a coin toss. After placing starch and flour, 50 unsexed *T. castaneum* of mixed ages were released through a glass funnel at the center of the arena. The arena was covered with a lid made from white foam display board. Each starch type and flour was tested in separate arenas. Arenas were observed at 36, 48, 60 and 72 h after adult introduction to determine number of adults retained in starch and flour. Starch and flour were collected in 9 cm glass Petri dishes and adults in the starch and flour were counted. Each starch and flour comparison and the four observation periods were replicated three times. All tests were conducted at room conditions because of the large space needed to accommodate the arenas. Room temperature and humidity during tests were measured every 2 min using HOBO® data loggers (Onset Computer Corporation, Bourne, MA, U.S.A.), and these environmental variables ranged from 21-27^{o}C and 30-55% r.h. during the test period.

2.5 Oviposition in starches and flour

Male and female pupae in Petri dishes were examined daily for adult emergence. A pair of adults emerging on the same day were paired and placed on 2.5 g of one of the six starches in 30 ml plastic condiment cups. The control treatments consisted of flour alone and flour plus yeast diet, and each was infested with a pair of adults. This experiment was replicated 10 times. The flour was sifted using 841 and 250 µm sieves every 3 d to separate the adults and count the number of eggs laid. The adults were transferred to 2.5 g of fresh starch, flour, or flour plus yeast diet and allowed to lay eggs for an additional 3 d. Beetles were allowed to lay eggs for a total of 15 d in these tests.

2.6 Influence of aggregation pheromone on oviposition

In order to determine if aggregation pheromone produced by male *T. castaneum* stimulated mating and egg-laying on starches, three levels of pheromone were tested. Only cross-linked wheat starch, potato starch, flour, and flour plus yeast were used in this test. To obtain different levels of pheromone, the aggregation pheromone lure for *T. castaneum* (Trécé, Adair, OK, USA) was cut into pieces having a mean ± SE wt (n = 3) of 0.095 ± 0.002 and 0.270 ± 0.002 g. In 30 ml plastic condiment cups, 2.5 g of cross-linked wheat starch, potato starch, flour, or flour plus yeast were taken. A piece of pheromone lure (0.095 or 0.270 g) was added to each of the cups. Cups without any pheromone served as the control treatment (0 g). Each pheromone level-diet combination was replicated five times. All cups were placed at 28^{o}C and 65% r.h. for 6 d, after which the starch and flour were sifted to count the number of eggs laid.

2.7 Effect for food contact on oviposition

A circular screen of 6 cm diameter with 425 µm openings was placed between two mason jar lids of 6 cm diameter. To hold the screen in place the lids were taped using masking tape. A pair of newly-emerged *T. castaneum* adults from pupae was placed in a 125-ml glass jar. The jar was fitted to one of the lids that were taped together. The jar was then inverted over 5 g of wheat flour in 9 cm diameter glass Petri dish. In this test, the adults in jars were physically above the flour and not in contact with it. The purpose of this test was to determine if volatiles from flour stimulated egg laying in *T. castaneum*. In a separate test, a pair of adults was introduced into 125 ml glass jar with 5 g of flour. Each treatment (contact and no contact with flour) was replicated five times. All jars were kept at 28°C and 65% r.h. and after for 6 d the number of eggs laid in flour were counted.

2.8 Diet exchange tests on oviposition

The influence of intermittently changing the diet on oviposition was tested by transferring a pair of *T. castaneum* adults from wheat flour to wheat starch, and *vice versa*. A pair of newly emerged adults was added into each 30 ml plastic condiment cup with 2.5 g starch or flour. Four separate tests were performed at 28^{o}C and 65% r.h. In the first test, a pair of adults was initially placed on flour for 3 d after which it was transferred to fresh starch for an additional 3 d. This pair was then transferred 3 d back again to fresh starch. Such reciprocal transfers were done for a maximum of 21 d. The second test was similar to the first test, except that a pair of adults was initially allowed to lay eggs on starch for 3 d, and then followed by flour-starch transfers for 21 d. The third test involved transferring a pair of adults every 3 d on starch alone, whereas in the fourth test, a pair was transferred every 3 d on flour alone. Each of the tests was replicated five times.

2.9 Ingestion of wheat starch by T. castaneum larvae and adults

Ten young larvae, 10 large larvae, and 10 male and female adults were kept in separate 9 cm diameter glass Petri dishes. The insects were starved for 24 h. Wheat starch was mixed with 0.1% by wt of Lissamine^{TM} Green B dye (Sigma-Aldrich, Inc. St. Louis, MO, USA). Larvae were fed for 5 and 60 min, and adults were allowed to feed for 8 h after which their mid gut was extracted by dissecting the insects. Five larvae and five extricated adult mid guts were examined directly under a stereomicroscope (Nikon SMZ 1000) and photographed using a Nikon COOLPIX 990 camera (Nikon Inc., Melville, NY, USA). The color of the dye in the mid gut indicated feeding by the insects.

2.10 Data analyses

The data on mean percentage egg viability, larval length, head capsule width, larval weight, proportion surviving, and number of eggs laid by *T. castaneum* females in various treatments in the 15- and 21-d tests were plotted over time using the SigmaPlot® 11 software (Systat Software Inc., San Jose, CA, USA). The percentage of particles (10, 50, or 90%) below a certain size among starches and flour were compared using one-way analysis of variance (ANOVA), and means were separated using Ryan-Einot-Gabriel-Welsch (REGWQ) multiple comparison test (SAS Institute, 2002). Differences in the mean number of *T. castaneum* adults retained in flour and a particular starch at each observation time was determined by paired *t*-test after transformation of data to $\log_{10}$ (x) scale (SAS Institute, 2002). The oviposition data were transformed to $\log_{10}$ (x + 1) scale and subjected to analysis of variance (one-way and/or two-way ANOVA), followed by mean separation using the REGWQ test. All statistical comparisons were considered significant at the $\alpha = 0.05$ level.

3. Results

3.1 Particle size distribution of starches and flour

Starch and flour particle size distributions are shown as 10, 50, and 90% percentages of particles below a certain size (Table 1.1). There were differences (df = 6, 7) in particles sizes among starches and flour ([10%]: $F = 6965.71$, $P < 0.0001$; [50%]: $F = 8794.37$, $P < 0.0001$; and [90%]: $F = 4185.59$, $P < 0.0001$). Generally, the particle size of flour was larger than those of the starches with the exception of 10% of the potato starch particles being larger than those of the flour. About 90% of the particles of starches ranged from 15 µm (70% amylases corn starch) to 58 µm (potato starch) while that of flour was 133 µm. Particle sizes of wheat starch and cross-

linked wheat starch were close despite being statistically different; the same relationship was found between corn starch and waxy corn starch.

3.2 Larval development and survival on starches and flour

The eggs of *T. castaneum* collected after infesting wheat flour with adults for 3 d started hatching the second day after collection and all eggs hatched by the sixth day. The mean ± SE egg hatchability in 7 d was 75.4 ± 1.1%.

The development of *T. castaneum* larvae, as determined by larval length, head capsule width, and larval weight, showed that larvae developed normally on flour and flour plus yeast, with the development being slightly better on the latter substrate (Figure 1.1). On starches, development of larvae was adversely affected, and larvae failed to develop beyond second instars. A few larvae became third instars but failed to molt beyond this stage. Additionally, survival on flour and flour plus yeast was far superior to survival on starches. The worst larval survival was on potato starch, followed by cross-linked wheat starch, and 70% amylose corn starch.

3.3 Adult retention in starch and flour

In arena tests, between 48 and 80% of the released adults were retained in the starch and flour substrates. There was no significant difference between the numbers of adults retained on each of the starches and flour ($P > 0.05$) at each observation time (Table 1.2).

3.4 Oviposition on starches and flour

The number of eggs laid by a female on flour and flour plus yeast was greater than those laid on starches (Figure 1.2) over a 15-d period. The peak egg-laying was on day 9. Two-way ANOVA showed that the mean number of eggs laid by females varied among the food substrates ($F = 382.40$; df = 7, 358; $P < 0.0001$) and observation times ($F = 90.52$; df = 4, 358; $P < 0.0001$).

The interaction of substrate and observation time was also significant (F = 26.31; df = 28, 358); P < 0.0001). The pooled data over 15 d showed that a mean of 0.1 to 2.6 eggs/female was laid on starches compared to 97.3-108.7 eggs/female on flour and flour plus yeast, and these differences were significant (F= 126.23; df= 3, 16; P< 0.0001; one-way ANOVA using Type III SS) (Table 1.3).

3.5 Effect of aggregation pheromone and food contact

No eggs were laid on starches in the presence or absence of the added pheromone lure (Table 1.4). In flour and flour plus yeast, a mean of 16 to 41 eggs/female was laid. Two-way ANOVA showed that there were significant differences among food substrates (F = 44.45; df = 3, 24; P < 0.0001), but not the pheromone level (F = 0.19; df = 2, 24; P = 0.8313). The number of eggs/female across the pheromone levels was consistent and therefore the treatment and pheromone level interaction was not significant (F = 0.45; df = 6, 24; P = 0.8385). One-way ANOVA by pheromone level showed that the number of egg/female laid in flour and flour plus yeast were not different from one another, but were different from those on starches (0 eggs/female) (F, range = 9.75 – 32.51; df = 3, 8; P < 0.005).

Female *T. castaneum* failed to lay eggs when the mating pair was above flour and not in contact with it. When beetles were in contact with food, a mean ± SE of 9.0 ± 1.9 eggs/female were laid in 6 d.

3.6 Diet exchange tests on oviposition

No or few eggs were laid when a *T. castaneum* pair was initially on starch, but when transferred to flour, and had 3 d started to lay eggs. When transferred back to starch, they continued to lay eggs in the starches although the numbers were smaller than those laid on flour (Figure 1.3). The total number of eggs laid per female over 21 d was significantly different

among the four tests (F = 126.23; df = 3, 16; $P < 0.0001$). Although the mean numbers of eggs laid by females varied from 52 to 91 on flour, flour-starch exchanges, and starch-flour exchanges (Table 1.5), there were no significant differences among these three treatments. However, these three treatments had significantly more eggs ($P < 0.05$) than those found in starch alone (0.4 eggs/female).

3.7 Feeding

Young larvae, old larvae, and male and female adults of *T. castaneum* were capable of feeding on wheat starch, as evidenced by the presence of the dyed-starch within their digestive tract (Figure 1.4, A-F).

4. Discussion

The observed egg hatchability of 75% was 16% less than that observed by Sokoloff (1974). This difference could be related to strain differences. The development of *T. castaneum* is less effective on intact grain while the development rate and reproduction rate increases on damaged kernels, and a maximum population increase and growth were found on insects reared on 149 µm size flour particles (Sokoloff, 1974; White, 1982; Li and Arbogast, 1991). Starch and flour particle size analysis proved that starch particles are smaller than the flour particles and can be easily ingested by *T. castaneum* larvae. The dyed-wheat starch was visible through the digestive tract of larvae and adults supporting this view.

After ingestion, the utilization of starch starts with digestion by enzymes in the insect's digestive system. Wool and Noiman (1980) reported the amylase activity of a wild type strain of the confused flour beetle, *Tribolium confusum* Jacquelin du Val, which were reared on flour fortified with brewer's yeast (95: 5) at 30^{o}C and 60% r.h., varied with larval age. The amylase activity increased with larval age up to 16 d after hatching, and was stable between 16 and 22 d

(0.4 mg maltose equivalents). Amylase activity was low in pupae (0.1 mg maltose equivalents), while amylase activity began to increase at 64-72 h after adult emergence to more than twice the highest larval level. Although larvae are able to digest starch, their development on all six pure starches were adversely affected. This may be due to the lack of essential nutrients such as protein, lipid, steroids, vitamins, and/or minerals in starches (Sokoloff, 1974). Notable differences in larval development and survival were observed on different starches. Larval mortality dramatically increased on potato starch, cross-linked wheat starch, and 70% amylose corn starch. This observation coincides with the low digestibility by α-amylase of these starches. Potato starch and high amylose corn starch, both have B-type X-ray diffraction patterns. They are more resistant to α-amylase digestion than waxy and normal cereal starches, which have an A-type X-ray diffraction pattern (Gallant et al., 1997; Gerard et al., 2001; McCleary and Monaghan, 2002; Evans and Thompson, 2004). In cross-linked wheat starch, the phosphate cross-linking can decrease starch digestion by α-amylase(Woo and Seib, 2002; Shin et al., 2004).

Although larval development and oviposition were adversely affected by feeding on starch, adult relative retention on starches was equal to that on wheat flour. The retention of insects in food patches depends on two the presence of aggregate stimuli which attract insects to come in contact with food from both long range and short range, and on the presence of arrestant stimuli that retain the beetles in food once they contact it (Subramanyam, 1992).

The lack of significant difference in the number of beetles retained in starches and flour suggest that these substrates provided a physical harborage for the adults or the adults were arrested once they contacted the substrates because of presence of volatiles. Sayaslan et al. (2000) reported existence of hexanal in commercial corn and potato starches, 2-ethyl-1-hexanol and benzaldehyde in wheat starch, pentyl, 2-methyl-1-butyl, benzyl, isobornyl acetates,

citronella, and 1, 8-cineole in waxy corn starch. Majority of the specific compounds detected appear to be products from lipid peroxidation. Many volatiles detected in these starches also exist in the wheat kernels (Maga, 1978) and wheat flour (Wang, 1981). Although wheat volatiles enhance egg-laying by beetles in flour (Wang, 1981), such an effect was not observed in starches. This suggests that in addition to volatile compounds, enhanced egg-laying may also require essential nutrients that were present in flour.

The pheromone mediated mating in *Tribolium* spp. is complex, but appears to be due to the production of male-produced aggregation pheromone, 4,8-dimethydecanal (Olsson et al., 2006). In addition to the aggregation pheromone, in *Tribolium* spp. volatiles including a sex pheromone, from glands on other locations of the body also help mediate mating. The amount of 4,8-dimethyldecanal produced increases with the nutritionally quality of diets (Obeng-Ofori and Coaker, 1990; Ming and Lewis, 2010). In our test, artificially increasing the aggregation pheromone level had no impact on egg-laying in cross-linked wheat starch, potato starch, and flour substrates.

The contact and/or feeding on flour was necessary for *T. castaneum* to lay eggs based on two separate experiments—one in contact with flour and the other with wheat starch-flour diet exchanges. Adults fed for 3 d on flour had the ability to lay eggs on starch. Adults that exclusively fed on starches laid little or no eggs. Adults of *T. castaneum* that failed to lay eggs on starches for 3 d when transferred to flour laid eggs in the next 3 d. This observation indicated that essential nutrients, perhaps protein and lipids, in flour were necessary for egg development and egg-laying. The role of nutrients in development of *T. castaneum* is well known (Sokoloff, 1974), and the role of nutrients and triggers for oviposition are poorly understood. In additional studies, wheat starch was used as a substrate and specific nutrients (protein and lipids) were

added to wheat starch to study the oviposition behavior of *T. castaneum*. Although starches did not support *T. castaneum* development, they can be useful as substrates to study effects of levels of specific nutrients or compounds on growth, development, and reproduction.

The ultimate goal of an IPM program should be to reduce the number of insects by limiting their movement and emigration rate, while also reducing their reproductive potential. The adverse effects of starch on development and oviposition of *T. castaneum* suggest that it can be used for management of this species. Furthermore, pure starches can be used as a substrate to study the role of specific nutrients on *T. castaneum* reproduction.

Figure 1.1 Development and survival of *T. castaneum* larvae on six starches, flour, and flour plus yeast as measured by larval length, head capsule width, larval weight, and proportion surviving every 3 d for 30 d.

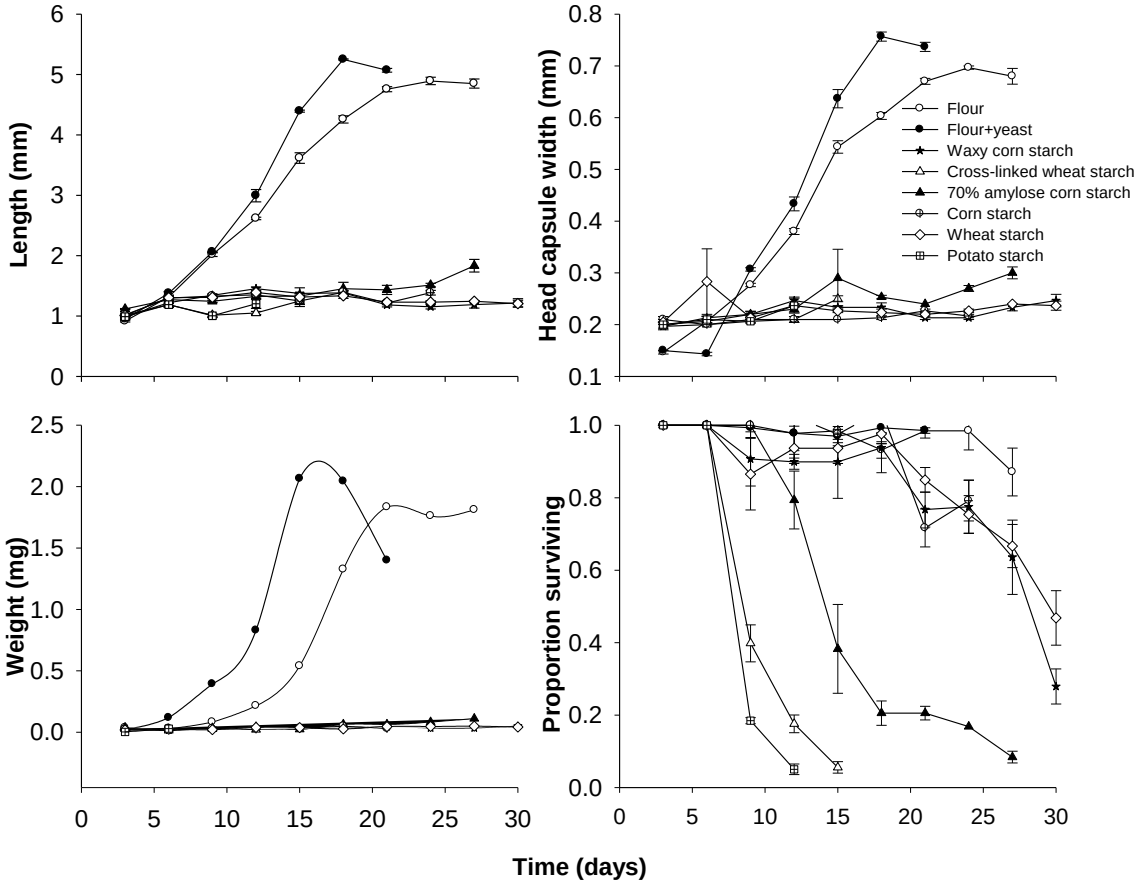

Figure 1.2 Number of eggs (mean ± SE) laid by each *T. castaneum* female on flour, flour plus yeast, and different starches every 3 d for 15 d. Each mean is based on *n* = 10, except for flour on day 12 and 15 where *n* = 9. In flour plus yeast, the number of mating pairs laying eggs on day 3, 6, 9, 12, 15 were 0, 9, 10, 10, and 10, respectively. In flour, number of mating pairs laying eggs on day 3, 6, 9, 12, 15 were 0, 9, 10, 9, and 9, respectively. In waxy corn starch, number of mating pairs laying eggs on day 3, 6, 9, 12, 15 were 0, 0, 0, 1, and 0, respectively. In cross-linked wheat starch, number of mating pairs laying eggs on day 3, 6, 9, 12, 15 were 0, 2, 2, 1, 2, respectively. In 70% amylose corn starch, number of mating pairs laying eggs on day 3, 6, 9, 12, 15 were 0, 1, 2, 1, and 2, respectively. In corn starch, number of mating pairs laying eggs on day 3, 6, 9, 12, 15 were 0, 1, 3, 3, and 2, respectively. In wheat starch, number of mating pairs laying eggs on day 3, 6, 9, 12, 15 were 0, 0, 4, 1, and 2, respectively. In potato starch, number of mating pairs laying eggs on day 3, 6, 9, 12, 15 were 0, 3, 6, 2, and 2, respectively.

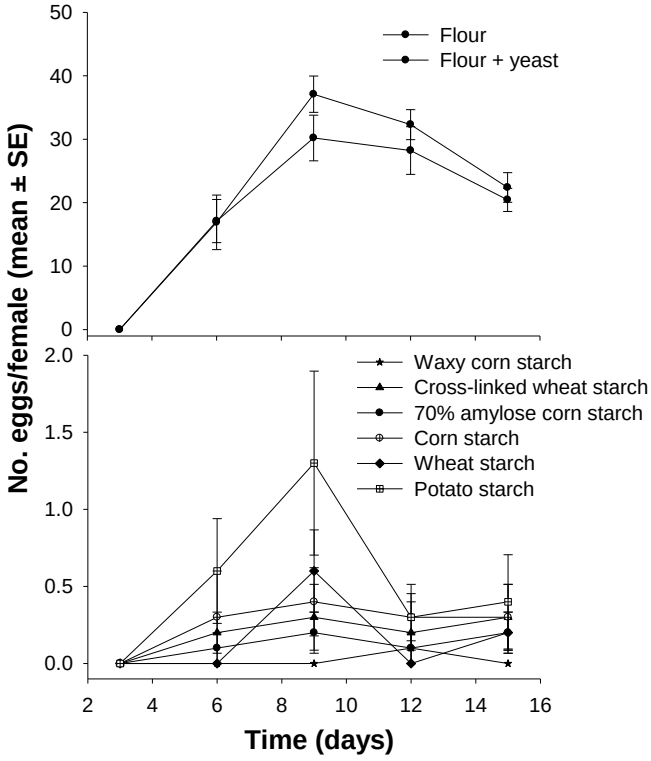

Figure 1.3 Number of eggs (mean ± SE) laid by each *T. castaneum* female every 3 d for 21 d on flour alone, starch alone, flour-starch exchange, and starch-flour exchange.

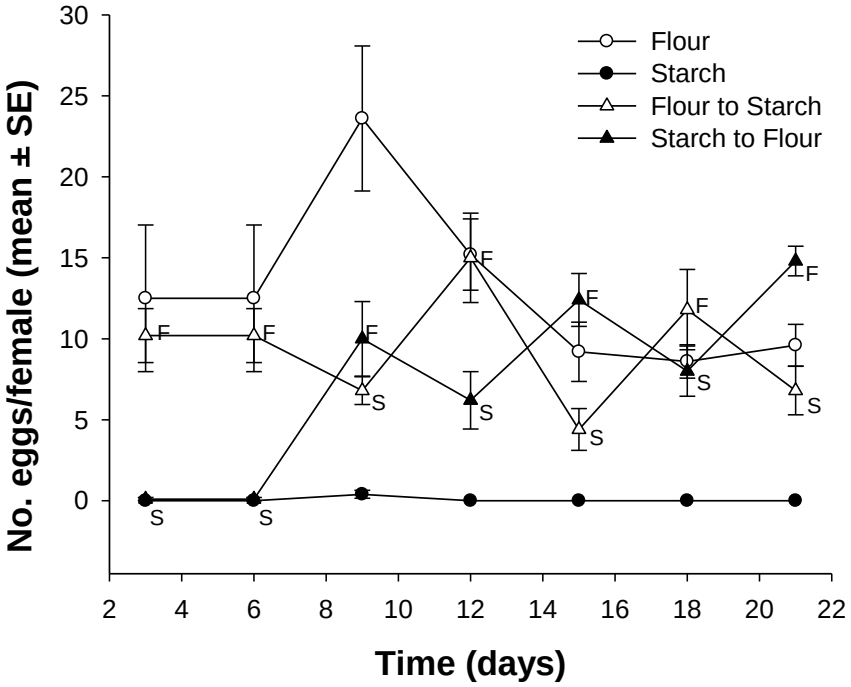

Figure 1.4 Larvae and adults of *T. castaneum* fed dyed wheat starch showing dyed-starch within the digestive tract. Young larva after 5 (A) and 60 min (B) after feeding on dyed starch; old larva after 5 (C) and 60 min (D) after feeding on dyed starch; and male (E) and female (B) adult showing dyed starch in extricated digestive tract.

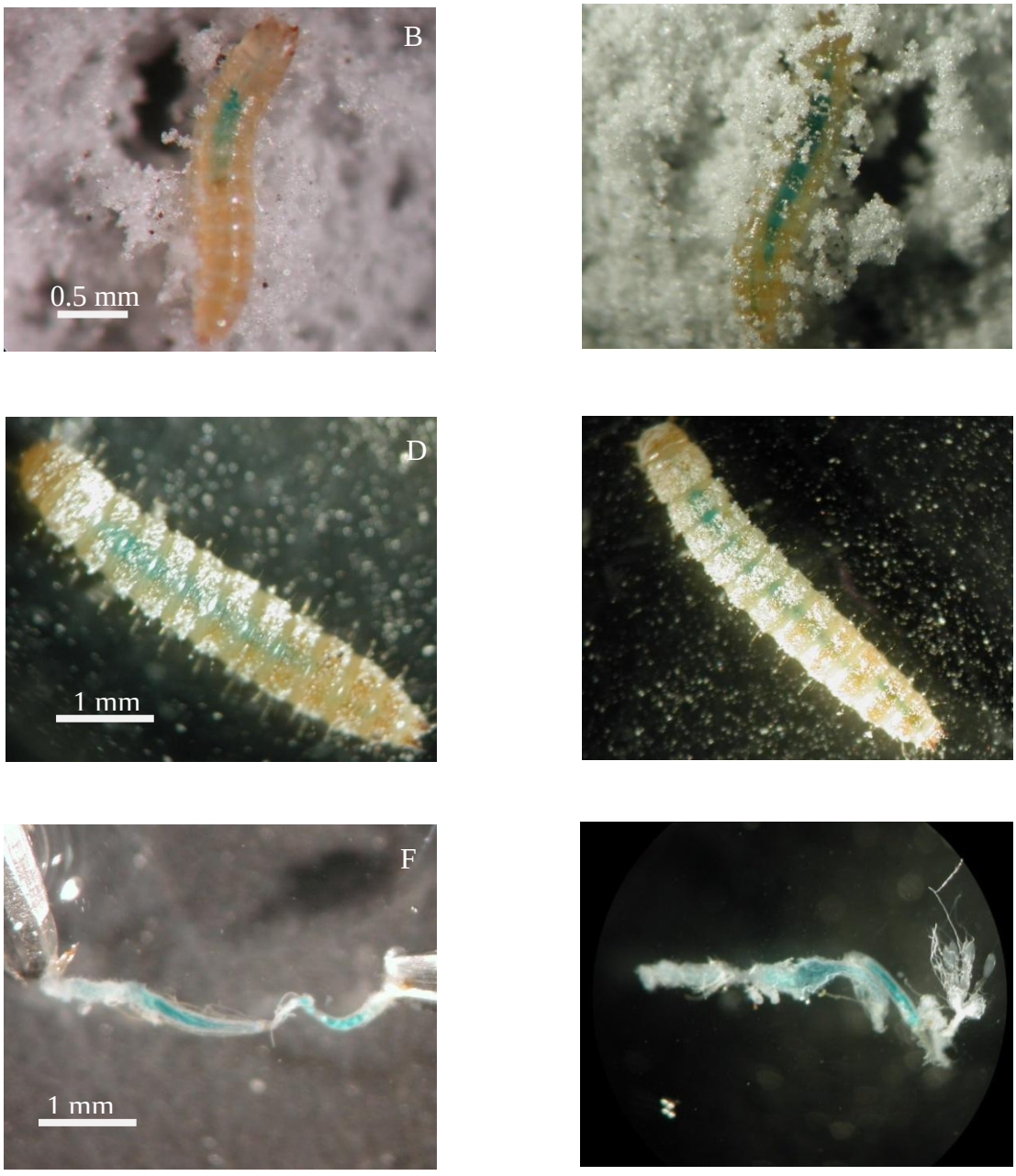

Table 1.1 Particle size distribution of wheat flour and six starches.

Treatment	Mean ± SE[a] particle size (µm) distribution:		
	10%[b]	50%[b]	90%[b]
Flour	13.52 ± 0.12b	60.04 ± 0.02a	132.50 ± 0.39a
Potato starch	24.36 ± 0.04a	37.65 ± 0.04b	57.65 ± 0.04b
Wheat starch	11.81 ± 0.00c	17.88 ± 0.01c	26.74 ± 0.03c
Cross-linked wheat starch	10.69 ± 0.01d	16.98 ± 0.01d	26.44 ± 0.00c
Corn starch	9.14 ± 0.06e	13.32 ± 0.07e	19.21 ± 0.05d
Waxy corn starch	8.13 ± 0.07f	12.97 ± 0.06f	20.38 ± 0.03e
70% amylose corn starch	5.52 ± 0.01g	9.15 ± 0.16g	14.76 ± 0.46f

[a]Mean size (n = 2) of 10, 50, and 90% of the particles.

[b]Means followed by different letters are significantly different ($P < 0.05$; REGWQ test).

Table 1.2 Relative retention of *T. castaneum* adults in starch and flour in dual-choice tests in circular arenas.

Time (h)	Flour vs. Starch/ starch name	Mean ± SE[a] adults retained in: Starch	Flour	*t* (df=2)[b]	*P*-value[c]
36	Potato starch	11.0 ± 5.7	14.0 ± 3.0	-1.50	0.27
	70% amylose corn starch	14.3 ± 5.2	25.7 ± 7.8	-1.70	0.23
	Corn starch	5.7 ± 2.6	24.7 ± 7.4	1.79	0.22
	Cross-linked wheat starch	20.0 ± 4.7	12.7 ± 2.9	-0.83	0.49
	Wheat starch	23.7 ± 6.4	12.0 ± 0.0	1.92	0.20
	Waxy corn starch	13.0 ± 5.5	19.0 ± 1.0	-0.79	0.51
48	Potato starch	11.7 ± 1.8	12.3 ± 2.3	-1.22	0.35
	70% amylose corn starch	14.0 ± 5.6	14.6 ± 3.8	-0.84	0.49
	Corn starch	9.0 ± 4.6	16.3 ± 8.3	-0.44	0.70
	Cross-linked wheat starch	15.7 ± 2.3	17.7 ± 2.4	-0.31	0.87
	Wheat starch	12.0 ± 3.5	17.7 ± 2.6	-2.26	0.15
	Waxy corn starch	10.7 ± 3.5	16.3 ± 4.5	-0.18	0.87

Time (h)	Flour vs. Starch/ starch name	Mean ± SE[a] adults retained in: Starch	Flour	*t* (df=2)[b]	*P*-value[c]
60	Potato starch	9.7 ± 5.1	13.3 ± 2.9	-1.57	0.25
	70% amylose corn starch	13.3 ± 1.9	20.7 ± 3.4	-3.67	0.07
	Corn starch	5.0 ± 1.3	25.7 ± 2.8	-0.77	0.52
	Cross-linked wheat starch	12.7 ± 1.9	19.0 ± 4.6	-0.41	0.72
	Wheat starch	21.0 ± 7.2	18.3 ± 3.7	0.04	0.26
	Waxy corn starch	7.3 ± 1.4	21.3 ± 5.8	-1.16	0.36
72	Potato starch	8.3 ± 1.2	16.7 ± 6.3	-1.86	0.20
	70% amylose corn starch	12.0 ± 4.9	21.7 ± 9.4	-0.25	0.82
	Corn starch	14.0 ± 5.7	13.0 ± 1.0	-0.05	0.97
	Cross-linked wheat starch	15.3 ± 1.8	17.0 ± 5.6	-0.43	0.71
	Wheat starch	13.0 ± 4.7	15.3 ± 1.4	-0.90	0.46
	Waxy corn starch	10.0 ± 4.4	17.0 ± 4.5	-1.61	0.20

[a]$n = 3$.

[b]Paired *t*-test; data were transformed to $\log_{10}$ (x) scale for analysis.

[c]Not significant ($P > 0.05$).

Table 1.3 Number of eggs laid by each *T. castaneum* female over 15 d in flour plus yeast, flour, and six starches.

Treatment	Mean ± SE[a] number of eggs/female
Flour + yeast	108.7 ± 5.7a
Flour[a]	97.3 ± 6.9a
Potato starch	2.6 ± 0.5b
Cross linked wheat starch	1.0 ± 0.2c
Corn starch	1.3 ± 0.5c
Wheat starch	0.8 ± 0.2c
70% amylose corn starch	0.6 ± 0.3c
Waxy corn starch[a]	0.1 ± 0.1c

[a]$n = 9$, all other treatments were based on $n = 10$.

[b]Means followed by different letters are significantly different ($P < 0.05$; REGWQ test). Data were transformed to $\log_{10}(x + 1)$ scale for analysis.

Table 1.4 Number of eggs laid by each *T. castaneum* female over 6 d in flour plus yeast, flour, cross-linked wheat starch, and potato starch in the absence and presence of the aggregation pheromone lure.

Treatment	Mean ± SE[a] number of eggs/female at pheromone level (g)		
	0[b]	0.095[b]	0.270[b]
Flour + yeast	37.0 ± 12.6a	26.3 ± 14.5a	41.0 ± 11.9a
Flour	22.7 ± 11.7a	19.7 ± 8.4a	16.3 ± 9.8ab
Cross-linked wheat starch	0b	0b	0b
Potato starch	0b	0b	0b

[a]*n* = 5.

[b]Means within a column followed by different letters is significantly different ($P < 0.05$; REGWQ test). Data were transformed to $\log_{10}$ (x+1) scale for analysis.

Table 1.5 Number of eggs laid by each *T. castaneum* female over 21 d in flour alone, starch alone, and in flour to starch, and starch to flour exchange tests.

Treatment	Mean ± SE[a,b] number of eggs/female
Flour	91.2 ± 12.3a
Flour to starch	65.2 ± 10.7a
Starch to flour	51.6 ± 7.2a
Starch	0.4 ± 0.2b

[a]$n = 5$.

[b]Means followed by different letters are significantly different ($P < 0.05$; REGWQ test). Data were transformed to $\log_{10}(x+1)$ scale for analysis.

References

Annison, G., Topping, D.L., 1994. Nutritional role of resistant starch: chemical structure vs. physiological function. Annual Review of Nutrition 14, 297-320.

Applebaum, S.W., 1966. Digestion of potato starch by larvae of flour beetle *Tribolium castaneum*. The Journal of Nutrition 90, 235-239.

Applebaum, S.W., Konijn, A., 1965. Utilization of starch by larvae of flour beetle *Tribolium castaneum*. The Journal of Nutrition 85, 275-282.

Baghurst, K.L., 1996. Dietary fiber, non-starch polysaccharides and resistant starch - A review. Food Australia 48, S3-S35.

Baker, J., Woo S., Silvers, S., 1992. Observations on in vitro and in vivo digestion of wheat-starch granules by larvae of *Tribolium castaneum* (Coleoptera: Tenebrionidae). Annals of the Entomological Society of America 85, 612-615.

Bremner, T.A., Anderson, M.D., Pope, G.J., Anderson, R.M., 1982. Genetic polymorphism of amylase in three species of *Tribolium* (Coleoptera: Tenebrionidae). Comparative Biochemistry and Physiology 74, 755-758.

Englyst, H.N., Kingman, S.M., Cummings, J.H., 1992. Classification and measurement of nutritionally important starch fractions. European Journal of Clinical Nutrition 46, S33-S50.

Evans, A., Thompson, D. A., 2004. Resistance to α-amylase digestion in four native high-amylose maize starches. Cereal Chemistry 81, 31-37.

Gallant, D. J., Bouchet, B., Baldwin, P. M., 1997. Microscopy of starch: Evidence of a new level of granule organization. Carbohydrate Polymers 32, 177-191.

Gerard, C., Colonna, P., Buleon, A., 2001. Amylolysis of maize mutant starches. Journal of the Science of Food and Agriculture 81, 1281-1287.

Good, N.E., 1937. Insects found in the milling streams of flour mills in the southwestern milling area. Journal of Kansas Entomological Society 10, 135-148.

Guilbot A., Mercier C., 1985. Starch. In: Aspinall, G. (Ed), The Polysaccharides. Academic Press, New York, pp. 209-282.

Krishna, S.S., Saxena, K.N., 1962. Digestion and absorption of food in *Tribolium castaneum* (Herbst). Physiological Zoology 35, 66-78.

Li, L., Arbogast, R., 1991. The effect of grain breakage on fecundity, development, survival, and population increase in maize of *Tribolium-castaneum* (Herbst) (Coleoptera: Tenebrionidae). Journal of Stored Products Research 27, 87-94.

Maga, J.A., 1978. Cereal volatiles, a review. Journal of Agricultural Food Chemistry 26, 175-179.

McCleary, B.V., Monaghan, D. A., 2002. Measurement of resistant starch. Journal of Association of Official Analytical Chemists International 85, 665-675.

Ming, Q.L., Lewis, S.M., 2010. Pheromone production of male *Tribolium castaneum* (Coleoptera: Tenebrionidae) is influenced by diet quality. Journal of Economic Entomology 103, 1915-1919.

Obeng-Ofori, D., Coaker, T.H., 1990. Some factors affecting responses of four stored product beetles (Coleoptera: Tenebrionidae & Bostrichidae) to pheromones. Bulletin of Entomological Research 80, 433–441.

Olsson, C.P.O., Ryne, C., Walle, R., Olle, A., Lofstedt, C., 2006. Male-produced sex pheromone in *Tribolium confusum*:Behaviour and investigation of pheromone production locations. Journal of Stored Product Research 42, 173-182.

Pratt, J.J. Jr., House, H.L., and Mansingh, A., 1972. Insect control strategies based on nutritional principles: a prospectus. In: Rodriguez, J.G. (Ed), Insect and Mite Nutrition. North-Holland Publishing Company, Amsterdam, London, pp. 651-668.

Rentería-Gutiérrez, T.R., Moreno-Martinez, E., Barron-Hoyos, J.M., 2000. Population growth of *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (H.) in different wheat varieties and groups commercially produced in Sonora, Mexico. Southwestern Entomologist 25, 213-220.

SAS Institute., 2002. SAS/STAT User's Guide, Version 9.1. Sas Institute, Cary, North Carolina.

Sayaslan, A., Okkyung, K.C., Seib, P.A., Seitz, L.M., 2000. Volatile compounds in five starches. Cereal Chemistry 77, 248-253.

Shariff, G., Vohra, P., Qualset, C.O., 1981. Further studies on the nutritional evaluation of wheat, triticale, and rice grains using the red flour beetle. Cereal Chemistry 58, 86-89.

Shin, M., Song, J., Seib, P.A., 2004. In vitro digestibility of cross-linked starches – RS4. Starch – Stärke 56, 478–483.

Sinha, R.N., Watters, F.L., 1985. Insect pests of flour mills, grain elevators, and feed mills and their control: Agriculture Canada Publication 1776. Canadian Government Publishing Centre, Ottawa, Canada.

Sokoloff, A., 1974. The Biology of *Tribolium*, With Special Emphasis on Genetic Aspects, Volume II. Oxford University Press, Ely House, London.

Subramanyam, Bh., 1992. Relative retention of adult sawtoothed grain beetles in potential food baits. Postharvest Biology and Technology 2, 73-82.

Voet, D.J., Voet, J.G., Pratt, C.W. 2008. Saccharide chemistry. In: Principles of Biochemistry. Wiley, Chichester, UK, pp. 219-244.

Vohra, P., Shariff, G., Robinson, D., Qualset, C., Gall, G., 1978. Nutritional-evaluation of triticale, wheat and rye grain using red flour beetle (*Tribolium-castaneum*) larvae and chickens. Nutrition Reports International 18, 289-300.

Vohra, P., Shariff, G., and Juliano, B.O., 1981. Relative nutritional value of some rices for growth of *Tribolium castaneum* larvae. Plant Foods for Human Nutrition (formerly Qualitas Plantarum) 30, 187-193.

Wang V.S., 1981. Studies of volatile chemicals from wheat flour for the correlation of active vibrational frequencies to the oviposition and olfaction response of the red flour beetle (*Tribolium castaneum*, Herbst). M.Sc. Thesis, Kansas State University.

White, G., 1982. The effect of grain damage on development in wheat of *Tribolium-castaneum* (Herbst) (Coleoptera: Tenebrionidae). Journal of Stored Products Research 18, 115-9.

Woo, K.S., Seib, P.A., 2002. Cross-linked resistant starch: preparation and properties. Cereal Chemistry 79, 819-825.

Wool, D., Noiman, S., 1980. Amylase activity in adults and immature of *Tribolium confusum* (Coleoptera: Tenebrionidae). Zeitschrift fur Angewandte Entomologie 90, 382-390.

Chapter 2 - The effects of feeding time, nutrient deprivation, and specific nutrients on *Tribolium castaneum* oviposition

Abstract

The study examined the effect of feeding time on wheat flour, feeding status of male and female on wheat flour, and addition of specific nutrients to an unsuitable food substrate (wheat starch) on the number of eggs laid and percentage egg hatchability of newly-emerged red flour beetle, *Tribolium castaneum* (Herbst), adults at 28^{o}C and 65% r.h. in the laboratory. A minimum feeding time of 1 d on flour was necessary to lay eggs. Irrespective of the feeding time, egg hatchability was not significantly different in adults fed for 1 to 3 d on flour. Female feeding on flour was more important than male feeding to lay eggs. Very few or no eggs were laid by *T. castaneum* in wheat starch. Male starvation had no effect on egg laying or egg hatchability. More eggs (3-5 times) were consistently produced on flour compared to wheat starch, or wheat starch supplemented with gluten, cholesterol, or both. Gluten at 4-16% gluten by wt in wheat starch elicited egg laying. Cholesterol at 1% by wt in wheat starch did not improve egg laying of *T. castaneum*. These studies suggest wheat starch to be a suitable substrate to study the effects of individual or combination of nutrients on *T. castaneum* egg laying. The increased egg laying by *T. castaneum* in flour compared to wheat starch supplemented with gluten suggests the existence of certain essential nutrients that promote egg development.

Keywords: Red Flour Beetle, Nutritional ecology, Reproduction

1. Introduction

Oviposition behavior in flour beetle, *Tribolium* spp. (Coleoptera: Tenebrionidae), is affected by environmental conditions, such as temperature and relative humidity (Howe, 1962; King and Dawson, 1972; Hagstrum and Flinn, 1990). Flour conditioning by quinines, which are secreted by beetles from a pair of odoriferous glands, also reduces fecundity and delayed onset of oviposition of *Tribolium* spp. (Ghent 1963; Matsumura and Yoshida, 1988; Sonleitner and Guthrie, 1991). The red flour beetle, *Tribolium castaneum* (Herbst) oviposition can be affected by yeast, vitamin B-complex mixture, sucrose, and fructose (Sokoloff, 1974; Singh and Krishna, 1983; Krishna, 1987). Rentería-Gutiérrez et al. (2000) reported that correlation coefficients between chemical characteristics of four wheat flour sample and growth stages for *T. castaneum* showed that starch content had the greatest influence on the number of progeny, whereas the effect of protein content was not significant. Also, *T. castaneum* oviposition ability decreased with increasing starvation period followed by 7 d recovery on flour (Daglish, 2006). Another area of insect oviposition studies focused on bimolecular aspects, which point out that the successful reproduction requires ovarian maturation, synthesis of yolk protein, vitellogenin, and its deposition into the ovary, and finally the egg maturation (Bhat and Gill, 1980; Izumi et al., 1994). In order to achieve successful reproduction, a threshold level of stored nutrients (Frisch, 2002) or ingested nutrients (Juliano et al., 2004), and organ size (Davidowitz et al., 2005) must be met to ensure critical transitions, such as the maturation of ovary and initiation of oviposition. The feeding threshold for oviposition has been studied for the lubber grasshopper, *Romalea microptera* Beauvois (Juliano et al., 2004) and yellow fever mosquitoe, *Aedes aegypti* (L.) (Feinsod and Spielman, 1980), but not in stored-product insects.

With the decreasing number of chemicals that can be used for stored product insect, further understanding of the nutritional factors on insect oviposition, which largely decide the population growth rate, is becoming more important (Schneider et al., 2003; Baker and Loschiavo, 1987; Ferry et al., 2006; Kos et al., 2009). In a previous study (Xue, 2010), female *T. castaneum* laid very few eggs or failed to lay eggs on six different starches, but laid eggs when fed for a minimum of 3 d on wheat flour suggesting the absence of specific nutrients required for egg-laying in starches. The present investigation was undertaken to determine the minimum feeding time required for *T. castaneum* to lay eggs, determine effect of male and female starvation status on *T. castaneum* ability to lay eggs, and determine addition of specific nutrients, specifically protein and lipids, on ability of *T. castaneum* to oviposit in wheat starch. In addition to number of eggs laid, the percentage of the eggs laid was also determined.

2. Materials and methods

2.1 Food substrates

Wheat starch (27% amylose) was obtained from MGP Ingredients Inc., Atchison, KS, USA. Wheat flour was purchased from Heartland Mills, Marienthal, KS, USA. Brewer's yeast (Lesaffre Yeast Corporation, Milwaukee, WI, USA) that was used in this project was ground using a coffee grinder, and particles that passed through a US Standard Sieve Number 60 with 250 μm openings (Seedburo Equipment Company, Chicago, IL) were add to the standard *T. castaneum* rearing diet. Cholesterol and wheat gluten were purchased from Sigma-Aldrich Inc., St. Louis, MO, USA.

2.2 Insects

Cultures of *T. castaneum* were reared on wheat flour fortified with 5% by wt brewer's yeast at 28°C, 65% r.h., and 14:10 (L:D) h photoperiod in a growth chamber (Model I-36 VL, Percival Scientific, Perry, IA) in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS, USA. These insects have been in culture in the Department of Grain Science and Industry since 1999. Unsexed adults (50) of mixed ages separated from culture jars were placed on 20 g flour in 150 ml plastic containers with lids. The lid had a perforation that was covered with a 250 µm plastic mesh to allow airflow and for preventing adults from escaping. The cups were kept at rearing conditions for 3 d after which the adults were removed by sifting the flour on an 841 µm opening sieve to remove adults. The flour with eggs that passed through was then sifted over a 250 µm opening sieve to retain the eggs. These eggs were reared at 28°C and 65% r.h. in 150 ml plastic containers containing 20 g of flour. After 30 d, the flour was sifted through an 841 µm sieve to separate pupae from flour. Sexing was done by separating pupae from the medium and by examining their external genitalia under a stereomicroscope (Nikon SMZ 645, Nikon Instruments Inc., Melville, NY, USA) according to a standard method (http://bru.gmprc.ksu.edu/proj/tribolium/wrangle.asp). Male and female pupae were placed in separate 9 cm glass Petri dishes and held at 28°C and 65% r.h. These dishes were examined daily for adult emergence and a pair of newly-emerged, virgin, male and female were paired in various oviposition tests.

2.3 Effect of feeding time on oviposition and egg hatchability

A pair of newly-emerged adults was introduced to each of several 30 ml plastic condiment cups containing 2.5 of flour. The cups were closed with lids. Holes were made on the lids with a pin to facilitate air diffusion. All cups were held at 28°C and 65% r.h. Each pair

was allowed to feed in flour for 0.5, 1, 1.5, 3, 2.5, or 3 d. After this minimum feeding time, a pair was separated from the flour and transferred to 2.5 g of fresh wheat starch in 30 ml plastic condiment cups with lids and held at 28^{o}C and 65% r.h. for specific time periods ranging from 1 to 6 d. Starch was used only as an egg-laying substrate because feeding on starch does not result in egg-laying in *T. castaneum* adults (Xue, 2010). Therefore, any additional feeding on starch does not influence egg laying in *T. castaneum*, and thus any eggs laid on starch are due to feeding on flour alone. A pair of adults transferred to separate cups of starch after 0.5, 1.5, and 3 d on flour was examined on day 1, 2, 3, 4, 5, and 6 to count the number of eggs laid, and separate cups with starch were used for these different days. Similarly, cups with starch receiving a pair of adults that feed on flour for 1, 2, and 2.5 d were examined on day 3 and 6 for number of eggs laid, and separate cups with starch were used for these two observation days. The eggs after counting were placed in 9 cm glass Petri dishes and held at 28oC and 65% r.h. for 7 d to determine percentage egg hatchability. Each feeding time on flour and each observation time on flour were replicated six times.

2.4 Effect of feeding status on oviposition and egg hatchability

Newly-emerged, virgin, male and female adults were fed on 2.5 g of flour or starved for 6 d in 30 ml plastic condiment cups with lids at 28^{o}C and 65% r.h. After 6 d, the following reciprocal crosses were made: fed female was paired with a starved male, fed female was mated with a fed male, starved female was mated with a fed male, and starved female was mated with a starved male. Each of these pairs was placed in 30 ml plastic condiment cups with 2.5 g of wheat starch. The number of eggs laid by each pair after 6 d in wheat starch and egg hatchability was enumerated.

2.5 Oviposition and egg hatchability on wheat starch supplemented with gluten and cholesterol

Two separate tests were conducted. In the first test, gluten at 4, 8, 12, and 16% by wt was added to wheat starch in separate 30 ml plastic condiment cups to make up a total amount of 2.5 g. The positive control consisted of 2.5 g of flour in plastic condiment cups, and the negative control consisted of 2.5 g of starch alone in cups. A pair of newly-emerged, virgin, adults was added to each cup. After 3 d each cup contents were sifted using a 250 µm sieve to separate eggs from starch or flour. The number of eggs laid was counted. The pair of adults from each cup was transferred to fresh starch, starch plus a specific level of gluten, and flour. After 3 d, the contents of each cup were sifted to count the number of eggs laid and the pair transferred for an additional 3 d to cups with fresh food. Such 3 d transfers occurred during a 21 d egg-laying period. Each treatment and observation time was replicated five times.

In the second test, 2.5 g of flour, 2.5 g of wheat starch containing 8% by wt of gluten and 1% by wt of cholesterol, 2.5 g of wheat starch containing 8% by wt of gluten, and 2.5 g of wheat starch plus 1% by wt of cholesterol were taken in individual 30 ml plastic condiment cups with lids. A pair of newly emerged, virgin, *T. castaneum* adults was introduced into each cup. Cups were closed with lids and held at 28oC and 65% r.h. Like the first test, contents of the cups were sifted every 3 d and the pair transferred to fresh food of the same type over a 21-d period. In addition to counting number of eggs laid every 3 d, the hatchability of the eggs was also determined by collecting the eggs in 9 cm diameter glass dishes and examining the after 7d. Each treatment and observation time was replicated five times.

2.6 Lipid analysis of gluten sample

Gluten in wheat flour contains 1.6% by wt of lipid (range, 1.5 – 2.5%) (Belitz et al., 2009). In order to determine free and bound lipid, gluten was extracted using methods described by Greenblatt et al. (1995) with modifications. Gluten sample (1 g), run in duplicates, was mixed with 10 ml hexane in a 15 ml tube using a vortex mixture, and incubated for 30 minutes at ambient temperature (21^{o}C). The solution was then decanted into another 15 ml tube and dried under nitrogen. Free lipids were saved at -20^{o}C for lipid analyses. Bound lipids were extracted from the pellet using 6 ml isopropanol/water (90/10; v/v/). This mixture was incubated for 15 minutes at ambient temperature, and centrifuged at 10,000 g for 15 minutes. The precipitate was discarded and the supernatant was dried under nitrogen and saved at -20^{o}C for lipid analyses. Just before analysis, the dried free and bound lipids were redissolved in 1 ml chloroform and transferred into 2 ml glass vial with Teflon-lined screw cap. Direct infusion electrospray ionization tandem mass spectrometry was used to identify and quantitatively describe lipid species (Finnie et al., 2009). Targeted lipid species were polar lipids, triacylglycerols (TAG) and diacylglycerol (DAG). The instrument acquisition and method were developed at the Kansas Lipidomics Research Center in Kansas State University, Manhattan, KS.

2.7 Data analysis

The mean number of eggs laid by each *T. castaneum* female every 3 d over 21 d in flour-feeding time tests and in tests with gluten and cholesterol were plotted using SigmaPlot® 11 software (Systat Software Inc., San Jose, CA, USA). At feeding times ranging from 0.5 to 3 d, only observations (number of eggs laid and percentage egg hatchability) on starch made on days 3 and 6 were common to the six feeding times. Data on the number of eggs laid was transformed to log10 (x + 1) for analysis, while the percentage egg hatchability data was transformed to angular

values (Zar, 1984). Significant differences in the mean number of eggs laid and percentage egg hatchability on days 3 and 6 on starch across the six feeding times were determined by one-way analysis of variance (ANOVA) and Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test (SAS Institute, 2002). In the feeding status test, only pairs in which the female was fed laid eggs. Therefore, the number of eggs laid and percentage egg hatchability in treatments where the fed female was mated with fed male and fed female was mated with starved female were compared using two-sample *t*-tests for equal variance (SAS Institute, 2002). Two-way ANOVA was used to compare differences in number of eggs laid among flour, wheat starch, and what starch with various levels of gluten, after transformation of data to $\log_{10}(x + 1)$ scale. The mean number of eggs laid over 21 d was compared by one-way ANOVA and REGWQ test was used for mean separation. The numbers of eggs laid and egg hatchability data every 3 d for 21 d in flour, wheat starch plus gluten, wheat starch plus cholesterol, and wheat starch plus gluten and cholesterol, after transformation to log10 (x +1) scale and angular values, respectively, were subjected to two-way ANOVA. These data were averaged over the 21 d and differences among treatments were determined using one-way ANOVA and REGWQ test. All treatments were considered significant at the $\alpha = 0.05$ level. Although data were transformed for analysis, in tables and graphs, the means and associated standard errors on untransformed scale are presented.

3. Results

3.1 Effect of feeding time on oviposition and egg hatchability

A paid allowed for only 0.5 d on flour failed to lay eggs on wheat starch during the 1 to 6 d observation time. Generally, a minimum of 1 d of feeding on flour is necessary for egg laying in *T. castaneum* (Table 2.1). The number of pairs out of 6 laying eggs increased when pairs were fed for 2.5 and 3 d compared to pairs fed for 1 to 2 d on flour. A pair fed on flour for 1.5 d did

not lay eggs on starch on the first two days but laid eggs on the third day. However, a pair fed on flour for 3 days laid eggs on starch on the first day. The mean number of eggs laid on days 3 and 6 on starches among the six feeding times was different (F = 7.84; df = 9, 50; P < 0.0001). Pairs that fed on flour for 2 or more days laid significantly (P < 0.05) more eggs than those fed for 1 and 1.5 d. Similarly, the percentage egg hatchability was also different (F = 3.68; df = 9, 50; P = 0.0014) among feeding times, but there was no consistent pattern.

3.2 Effect of feeding status on oviposition and egg hatchability

Eggs were not laid in pairs where the starved female was mated with flour-fed male and where both sexes were starved. Both the number of eggs/female and egg hatchability observed in cases where the flour-fed females were mated with fed males and flour-fed females were mated with starved males were not statistically different from one another (P > 0.05) (Table 2.2).

3.3 Oviposition and egg hatchability on wheat starch supplemented with gluten and cholesterol

When starch was supplemented with gluten, *T. castaneum* oviposition was elicited. However, the number of eggs laid was lower than those laid on flour, but higher than those laid on starch alone (Figure 2.1). The number of eggs laid peaked on day 12 on flour, and on day 9 in all starch plus gluten treatments. The number of eggs laid were different among the treatments (F = 127.05; df = 5, 168; P < 0.001) and among observation times (F = 107.23; df = 6, 168; P < 0.0001). The treatment and observation time interaction was also significant (F = 8.64; df = 30, 168; P < 0.0001), suggesting that the number of eggs laid were not consistent over time among the treatments. One-way ANOVA showed that the mean number of eggs laid by a female in 21 d was different among the five treatments (F = 168.21; df = 5, 24; P < 0.0001). The greatest number of eggs (111 egg/female) was laid on flour and the least number in starch (0.4

eggs/female); in the four gluten treatments the number of eggs laid were similar ($P > 0.05$) and ranged from 21-24 eggs/female (Table 2.3).

Addition of 1% cholesterol to wheat starch did not improve egg-laying of *T. castaneum* (Figure 2). The number of eggs laid by females in starch plus 8% gluten and starch plus 8% gluten and 1% cholesterol was comparable. More eggs were laid by *T. castaneum* in flour compared to other treatments. In flour the peak egg laying time spanned days 6 through 9, whereas in the two starches plus gluten treatments peak egg laying occurred on day 9. Two-way ANOVA showed significant differences among treatments ($F = 463.03$; df = 3, 112; $P < 0.0001$) and observation times ($F = 7.69$; df = 6, 112; $P < 0.0001$). The treatment and observation interaction was also significant ($F = 3.45$; df = 18, 112; $P < 0.0001$) indicating that the number of eggs laid varied over time among the four treatments.

The egg hatchability was lowest in the starch plus 1% cholesterol treatment over 21 d compared to other treatments (Figure 2.2). Egg hatchability over time in flour, starch plus 8% gluten, and starch plus 8% gluten and 1% cholesterol followed a similar trend over time. Two-way ANOVA showed significant differences in egg hatchability among the four treatments ($F = 109.22$; df = 3, 112; $P < 0.0001$) and observation times ($F = 3.42$; df = 6, 112; $P = 0.0039$). The treatment and observation time interaction was not significant ($F = 1.45$; df = 18, 112; $P = 0.1224$), because in three out of the four treatments there were consistent trends over time on egg hatchability.

One-way ANOVA showed that the mean number of eggs laid by a female in 21 d and egg hatchability among the four treatments were significantly different (eggs laid: $F = 338.33$; df = 3, 16; $P < 0.001$; egg hatchability: $F = 72.46$; df = 3, 16; $P < 0.0001$). The mean number of egg laid in the flour treatment was significantly greater ($P < 0.05$) than those laid in the two

starch plus gluten and starch plus cholesterol treatments (Table 2.4). The number of eggs laid in the two starch plus gluten treatments were similar and were higher than those laid in starch plsu cholesterol treatment. The egg hatchability was similar among the flour and the two starch plus gluten treatments, and higher than those observed in starch plus cholesterol treatment (Table 2.4).

3.4 Lipid analysis of gluten sample

In the gluten sample 79 polar lipids and 63 non-polar lipids were identified. The latter are further classified as diacylglycerols (DAG) and TAG (triacylglycerols). The amount of polar lipid in the gluten sample was 2638 nmol/g of gluten sample. In the non-polar lipid categories, DAG made up 1026 nmol/g and the TAG made up 268 nmol/g of the gluten sample. The 79 polar lipids, which are bound lipids, include 13 digalactosyldiglycerides (1456 nmol/g), 10 monogalactosyldiclycerides (764 nmol/g), 7 phosphatidylglycerol (28 nmol/g), 3 lysophosphatidylglycerol (1 nmol/g), 5 lysophosphatidylcholine (75 nmol/g), 4 lysophosphatidylethanoleamine (1 nmol/g), 12 phosphotidylcholine (331 nmol/g), 8 phosphatidyl ethanolamine (41 nmol/g), 6 phosphatidylinositol (28 nmol/g), 3 phospatidylserine (5 nmol/g), and 8 phosphatidicacid (106 nmol/g). No sulfoquinonevosydiaacylglycerol was detected. Non-polar lipids were identified as 23 DAG (including 2 DAG 16:1, 5 DAG 16:0, 5 DAG 18:3, 6 DAG 18:2, and 5 DAG 18:1) and 40 TAG (including 1 TAG 16:1, 11 TAG 16:0, 5 TAG 18:3, 12 TAG 18:2, and 11 TAG 18:1). Usually, one or two abundant molecular species were identified within each class. The predominant species was 36:4 in digalacosyldiglycerides, monogalacosyldiglycerides, and phosphatidylethanolamine, 34:2 in phosphatidylglycerol, 34:2 and 36:4 in phosphatidylcholine, phosphatidylinositol, and phosphatiodicacid, 16:0 and 18:2 in

lysophosphatidylglycerol and lysophosphatidylcholine, 18:2 in phosphatidylethanolamine, 40:2 and 34:2 in phosphatidylserine.

4. Discussion

Nutritional status of an insect affects its reproduction. A cumulative feeding threshold of 4.0 g dry mass of Romaine lettuce is required for the *R. microptera* grasshopper to initiate vitellogenesis and ultimately oviposition (Juliano et al., 2004). In *T. castaneum*, no studies have examined the feeding threshold for oviposition. Because of the difficulty to quantify the amount of the food consumed by beetles, in our study, minimum contact/feeding time was chosen as a criterion for evaluating oviposition. In a previous study, Xue (2010) showed that male and female adults were capable of ingesting dyed-wheat starch within 8 h. Therefore, the minimum feeding time used was 0.5 d (12 h). This observation is consistent with those made by Krishna and Saxena (1962) that 10 h was sufficient for dyed-wheat flour to traverse the whole gut of an adult. A minimum feeding time of 1 day on flour was necessary to elicit oviposition in *T. castaneum*, and longer duration spent in flour (3 d) elicited egg laying within the next day on starch. However, feeding for a short time required close to 3 d to elicit oviposition in starch. Flour, in addition to starch, contains up to 12% of protein, mainly as gluten and 1.6% of lipid (et al., 2009). As Xue (2010) noted, the failure of *T. castaneum* to lay eggs on pure starch, devoid of protein and lipid, suggest that these components in flour are responsible for mobilizing nutrients required for egg development and subsequent egg laying.

The feeding status tests, clearly demonstrated that female feeding was essential for egg laying in *T. castaneum*. The fact that no eggs were produced when starved female was mated with fed male indicates that female feeding and mobilization of nutrients to the developing ovaries is needed for eggs to be produced and laid. The lack of differences in the number of eggs

laid and percentage egg hatchability where fed female was crossed with fed male and fed female was crossed with starved male suggests that in these species maternal investment in egg production and hatchability is more important than paternal investment. In *T. castaneum*, starvation of males leads to decreased size of accessory glands and number of stored sperms (Fedina and Lewis, 2006). Further studies are needed to determine the effect of feeding status on nutrient mobilization to ovaries, ovary development, and egg laying.

Addition of gluten to wheat starch resulted in egg laying, but lack of differences between the four gluten levels suggests that at least 4% by wt of gluten admixed with starch is necessary to elicit oviposition. Gluten provided the necessary amino acid building blocks for ovarian development and egg formation (Izumi et al. 1994; Voet et al., 2008). However, the fact that more eggs were laid in flour compared with starch plus gluten indicates the presence of certain other nutritional supplements in flour. The fact that the number of eggs laid were unaffected when wheat starch with 1% cholesterol by wt and wheat starch with 8% gluten by wt and 1% cholesterol suggests that cholesterol at the level added does not enhance *T. castaneum* oviposition. The lack of differences in egg hatchability between eggs laid in flour and those laid in starch plus gluten treatments suggests that the eggs laid in starch plus gluten treatments were of the same quality as those laid in flour. The decreased number of eggs laid in starch plus gluten treatments could be due to some other nutritional factor present in flour or due to reduced gluten utilization efficiency (Krishna and Saxena, 1962). The gluten sample in this study contained small amounts of both polar and non-polar lipids, thus made it difficult to rule out the role of these lipid components in *T. castaneum* oviposition. Separating the flour into wheat gluten, wheat starch, and water solubles (Hoseney et al., 1969), and adding each of these fractions to wheat starch at various levels may shed light on the importance of these fractions in *T.*

castaneum oviposition. Examining the hemolymph of adults for various nutrients and hormones following periods of starvation and ingestion of flour or starch with specific added nutrients would be a fruitful approach to understand the nutritional ecology and its effect on reproduction of *T. castaneum*. Understanding these aspects may lead to bio-rational approaches to managing this economically important insect pest.

Figure 2.1 Mean ± SE (*n* = 5) number of eggs laid every 3 d for 21 d by each *T. castaneum* female on flour, wheat starch, and wheat starch supplemented with four levels of gluten.

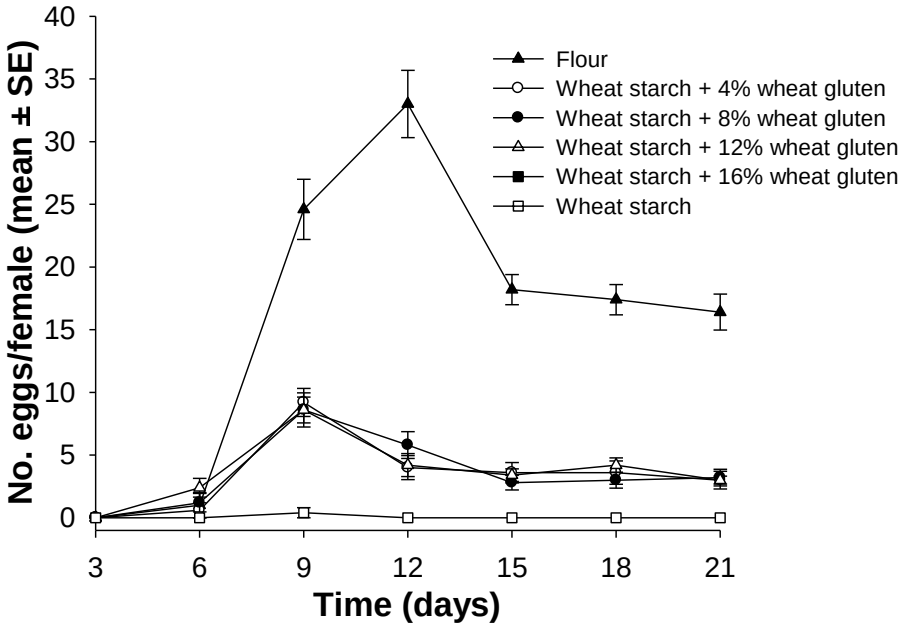

Figure 2.2 Mean ± SE (*n* = 5) number of eggs laid by each *T. castaneum* female and percentage egg hatchability measured every 3 d for 21 d on flour, wheat starch plus 1% by wt of cholesterol, wheat starch plus 8% by wt gluten, and wheat starch plus 8% by wt of gluten and 1% by wt of cholesterol.

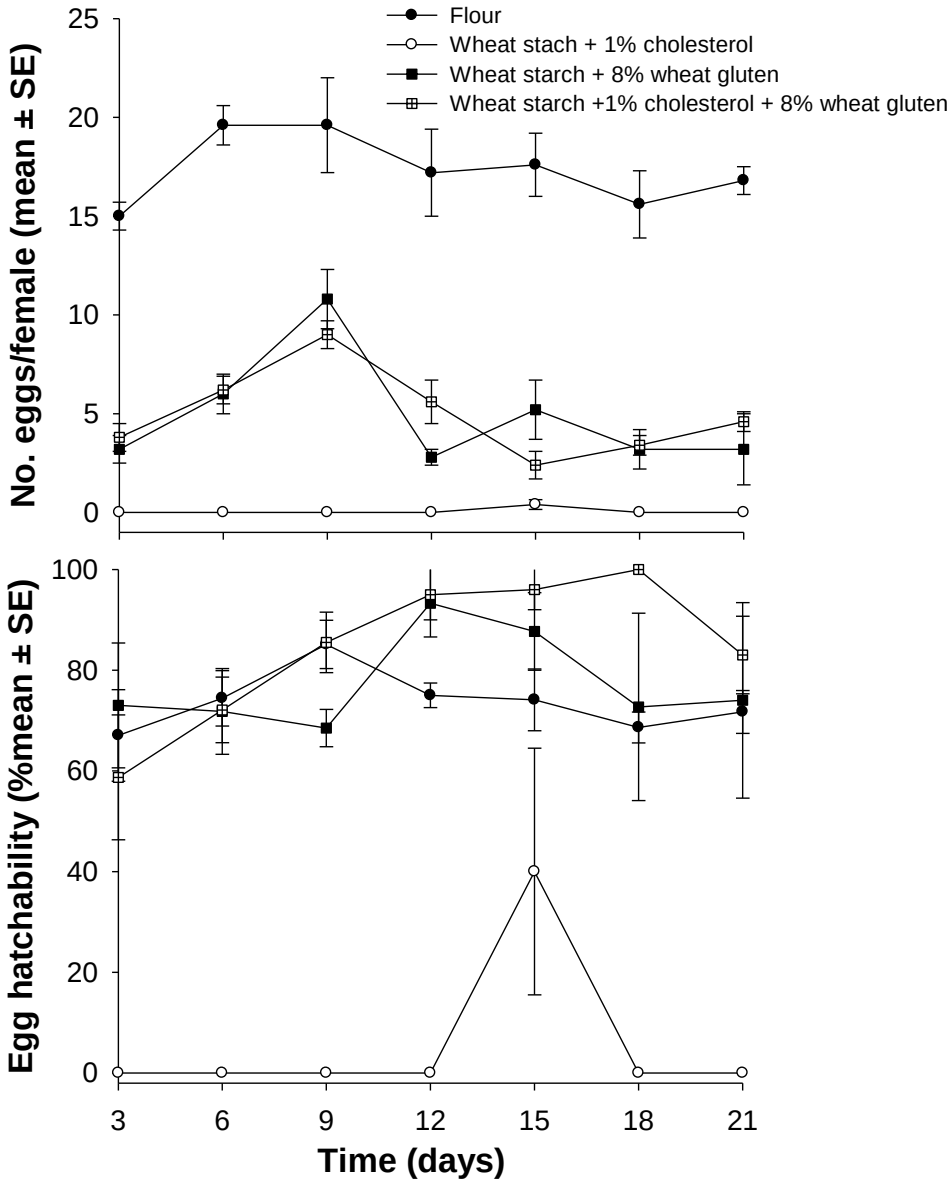

Table 2.1 The effect of feeding time on flour on the number of eggs laid by each *T. castaneum* female on different days in wheat starch.

Feeding time on flour (d)	Time on starch (d)	No. pairs laying eggs out of 6	Mean ± SE[a]	
			No. eggs/female[b]	Egg hatchability (%)[b]
1.0	3	0	0.0 ± 0.0d	0.0 ± 0.0b
	6	4	2.0 ± 0.8bcd	20.0 ± 10.0ab
1.5	1	0	0.0 ± 0.0	0.0 ± 0.0
	2	0	0.0 ± 0.0	0.0 ± 0.0
	3	1	0.3 ± 0.3cd	16.7 ± 16.7ab
	4	1	0.2 ± 0.2	16.7 ± 16.7
	5	1	0.2 ± 0.2	16.7 ± 16.7
	6	1	0.3 ± 0.3cd	16.7 ± 16.7ab
2	3	4	2.8 ± 1.0bcd	43.9 ± 14.4ab
	6	5	4.2 ± 1.7abc	61.9 ± 14.1a
2.5	3	6	9.7 ± 1.1a	62.1 ± 12.8ab
	6	5	4.2 ± 1.7abc	63.9 ± 15.8a

Feeding time on flour (d)	Time on starch (d)	No. pairs laying eggs out of 6	Mean ± SE	
			No. eggs/female[a]	Egg hatchability (%)[b]
3	1	3	1.8 ± 1.4	46.3 ± 21.0
	2	5	3.7 ± 1.1	54.4 ± 16.4
	3	6	5.3 ± 1.5ab	74.6 ± 9.2a
	4	5	3.8 ± 1.1	75.4 ± 16.0
	5	5	6.3 ± 2.0	66.3 ± 14.2
	6	5	3.7 ± 0.8ab	36.4 ± 8.9ab

[a]$n = 6$.

[b]Data for number of eggs laid and egg hatchability for days 3 and 6 on starch among the six feeding times were compared after transformation of egg data to $\log_{10}$ (x + 1) and hatchability to angular values. Means within a column followed by different letters are significantly different ($P < 0.05$; REGWQ test).

Table 2.2 The effect of male and female on *T. castaneum* feeding status on number of eggs laid and egg hatchability.

Treatment	Mean ± SE[a]	
	No. eggs/female	Egg hatchability (%)
Female fed x male fed	6.5 ± 1.1	59.7 ± 6.3
Female fed x male starved	5.3 ± 1.2	65.3 ± 13.8
t (df = 10)	0.72	-0.37
P-value*	0.4882	0.7174

[a]*n* = 6.

*Not significant (*P* > 0.05; two-sample *t*-test).

Table 2.3 Number of eggs laid by each *T. castaneum* female over 21 d in flour, wheat starch, and wheat starch supplemented with 4-16% by wt of wheat gluten.

Treatment	Mean ± SE no. eggs/female/21 d[a,b]
Flour	110.6 ± 5.0a
Wheat starch + 4% gluten	24.0 ± 1.6b
Wheat starch + 8% gluten	24.6 ± 1.2b
Wheat starch + 12% gluten	25.8 ± 1.4b
Wheat starch + 16% gluten	21.2 ± 2.8b
Wheat starch	0.4 ± 0.4c

[a]$n = 5$.

[b]Means followed by different letters are significantly different ($P < 0.05$, REGWQ test). Data were transformed to $\log_{10}$ (x+1) scale for analysis.

Table 2.4 Number of eggs laid by each *T. castaneum* female over 21 d in flour and wheat starch supplemented with gluten, cholesterol, or both.

Treatment	Mean ± SE[a]	
	No. eggs/female/21 d[b]	Egg hatchability (%)[b]
Wheat flour	121.4 ± 4.1a	73.3 ± 1.2a
Wheat starch + 8% gluten	34.4 ± 3.1a	77.3 ± 3.4b
Wheat starch + 1% cholesterol + 8% gluten	35.0 ± 2.6a	84.3 ± 2.4b
Wheat starch + 1% cholesterol[c]	0.4 ± 0.2b	5.7 ± 3.5c

[a]$n = 5$.

[b]Means within a column followed by different letters are significantly different ($P < 0.05$, REGWQ test). Egg data were transformed to $\log_{10}$ (x+1) scale and egg hatchability data were to angular values for analysis.

[c]Only 1 mating pair out of 5 laid eggs. In the other treatments all 5 mating pairs laid eggs.

References

Baker, J.E., Loschiavo, S.R., 1987. Nutritional ecology of stored-product insects. In: Slansky, F. Jr., and Rodriguez, J.G. (Ed.), Nutritional ecology of insects, mites, spiders and related invertebrates. John Wiley & Sons, New York, pp. 321–344.

Belitz, H.-D., Grosch W., Schieberle, P., 2009. Food chemistry, 4th revised and extended edition. Springer Publishing Company, New York, NY.

Bhat, P.P., Gill, K.S., 1980. Growth and maturation of ovary in *Tribolium castaneum*. Indian Journal of Animal Science 50, 270-273.

Daglish, G.J., 2006. Survival and reproduction of *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) following periods of starvation. Journal of Stored Products Research 42, 328-338.

Davidowitz, G., Roff, D.A., Nijhout, H.F., 2005. A physiological perspective on the response of body size and development time to simultaneous directional selection. Integrative and Comparative Biology 45, 525-531.

Fedina, T.Y., Lewis, S.M., 2006. Proximal traits and mechanisms of biasing paternity in the red flour beetle *Tribolium castaeum* (Coleoptera: Tenebrionidae). Behavioral Ecology Sociobiology 20, 844-853.

Feinsod, F.M., Spielman, A., 1980. Nutrient-mediated juvenile hormone secretion in mosquitoes. Journal of Physiology 26, 113-117.

Ferry, N., Edwards, M., Gatehouse, J., Capell, T., Christou, P., Gatehouse, A., 2006. Transgenic plants for insect pest control: A forward looking scientific perspective. Transgenic Research 15, 13-19.

Finnie, S.M., Jeannotte, R., Faubion, J.M., 2009. Quantitative Characterization of Polar Lipids from Wheat Whole Meal, Flour, and Starch. Cereal Chemistry 86, 637-645.

Fronstin, R.B., Hatle, J.D., 2007. A cumulative feeding threshold required for vitellogenesis can be obviated with juvenile hormone treatment in lubber grasshoppers. The Journal of Experimental Biology 211, 79-85.

Ghent, A., 1963. Studies of behavior of *Tribolium* flour beetles 1. Contrasting responses of *T. castaneum* and *T. confusum* to fresh and conditioned flours. Ecology 44, 269-283.

Greenblatt, G., Bettge, A., Morris, C., 1995. Relationship between endosperm texture and the occurrence of friabilin and bound polar lipids on wheat starch. Cereal Chemistry 72, 172-176.

Hagstrum, D.W., Flinn, P.W., 1990. Simulations comparing insect species differences in response to wheat storage conditions and management practices. Journal of Economic Entomology 83, 2469-2475.

Hoseney, R.C., Finney, K.F., Shogren, M.D., Pomeranz, Y., 1969. Functional (breadmaking) and biochemical properties of wheat flour components II. Role of water solubles. Cereal Chemistry 46, 117-125.

Howe, R.W., 1962. The effects of temperature and humidity on the oviposition rate of *Tribolium castaneum* (Hbst.) (Coleoptera: Tenebrionidae). Bulletin of Entomological Research 53, 301-310.

Izumi, S., Yano, K., Yamamoto, Y., Takahashi, S.Y., 1994. Yolk proteins from insect eggs-structure, biosynthesis and programmed degradation during embryogenesis. Journal of Insect Physiology 40, 735-746.

Juliano S.A., Olson, J.R., Tillman, E.G and Hatle, J.D., 2004. Plasticity and canalization of insect reproduction: testing alternative models of life history transitions. Ecology 85, 2986-2996.

King, C.E., Dawson, P.S., 1972. Habitat selection by flour beetles in complex environments. Physiological Zoology 46, 297-309.

Kos, M., Loon, J., Dicke, M., and L. Vet., 2009. Transgenic plants as vital components of integrated pest management. Trends in Biotechnology 27, 621-627.

Krishna, S.S., 1987. Nutritional modulation of reproduction in two phytophagous insect pest. Proceedings of the Indian Academy of Sciences, Animal Sciences 96, 153-169.

Krishna, S.S., Saxena, K.N., 1962. Digestion and absorption of food in *Tribolium castanem* (Herbst). Physiological Zoology 35, 66-78.

Matsumura, M., Yoshida T., 1988. Reproductive ability and the effect of biological conditioning of medium on surviviorship and oviposition of *Tribolium freeman* Hinton (Coleoptera: Tenebrionidae). Applied Entomology and Zoology 23, 1-7.

Rentería-Gutiérrez, T.R., Moreno-Martínez, E., and Barrón-Hoyos, J.M., 2000. Population growth of *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (H.) in different wheat varieties and groups commercially produced in Sonora, Mexico. Southwestern Entomologist 25, 213-220.

SAS Institute., 2002. SAS/STAT User's Guide, Version 9.1. Sas Institute, Cary, North Carolina.

Schneider, S.M., Rosskopf, E.N., Leesch, J.G., Chellemi, D.O., Bull, C.T., Mazzola, M., 2003. United States Department of Agriculture—Agricultural Research Service research on alternatives to methyl bromide: pre-plant and post-harvest. Pest Management Science 59, 814-826.

Singh, R., Krishna, S.S., 1983. The impact of variation in adult food on the reproductive programming in *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). New Entomologist 32, 23-30.

Sinha, R.N., Watters, F.L., 1985. Insect pests of flour mills, grain elevators, and feed mills and their control: Agriculture Canada Publication 1776. Canadian Government Publishing Centre, Ottawa, Canada.

Sokoloff, A., 1974. The Biology of *Tribolium*, With Special Emphasis on Genetic Aspects, Volume II. Oxford University Press, Ely House, London.

Sonleitner, F.J., Gutherie. J., 1991. Factors affecting oviposition rate in the flour beetle *Tribolium castaneum* and the origin of the population regulation mechanism. Researches on Population Ecology 33, 1-11.

Voet, D.J., Voet, J.G., Pratt, C.W., 2008. Principles of biochemistry. Wiley, Chichester.

Zar, J. H. 1984. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Xue, M., 2010. Development, relative retention, and oviposition of the red flour beetle, *Tribolium castaneum*(Herbst), on different starches. M.S. Thesis, Kansas State University, Manhattan, KS.