Development, relative retention, and fecundity of *Tribolium castaneum* (Herbst) on different starches

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Abstract

The development, relative retention, and fecundity of the red flour beetle, *Tribolium castaneum* (Herbst), on six different types of starches, flour, and flour plus yeast were investigated in the laboratory. The size of 90% of particles among the starches was below 15 to 58 µm, while that of the flour was below 133 µm. Larval length, head capsule width, and weight gain of *T. castaneum* were measured by rearing larvae on starches, flour, and flour plus 5% (by wt) Brewer's yeast diet for 30 d at 28oC and 65% r.h. Larvae reared on flour or flour plus yeast developed normally and showed better survival compared to those reared on starches. Larvae 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. *Tribolium castaneum* laid less than 3 eggs/female over a 15 d period on all starches, but laid 97 and 109 eggs/female on flour and flour plus yeast diet, respectively. These initial studies suggest that starches are poor substrates for development, and currently experiments are in progress to improve larval survival and development by incorporating specific nutrients in starches. Starches were as attractive as flour to adults; however, starches do not appear to be a suitable medium for egg-laying. Preliminary experiments by moving adults between starches and flour and vice versa showed that feeding on suitable diets is essential for eliciting oviposition. Although preliminary, these interesting 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.

Keywords: Nutritional control, *Tribolium castaneum*, Resistant starches, Development, Oviposition, Pest management

1. Introduction

Natural starch is an abundant nutrient carbohydrate, (C6H10O5)n, found mainly in the seeds, fruits, tubers, roots, and stem pith of plants, notably in corn, potatoes, wheat, and rice, and varying widely in appearance according to source but commonly prepared as a white amorphous tasteless powder. Natural starch usually consists of 25% amylose (linear starch polymers) and 75% amylopectin (branched starch polymers). Resistant starches (RS) include starches that are resistant to enzymatic hydrolysis in the small intestines of humans. RS are classified as RS1, RS2, RS3 and RS4. RS1 refers to resistant starch that is physically encased by whole grains. RS2 is a granular resistant starch. RS3 refers to non-granular, retrograded or crystalline resistant starch, and RS4 is a manufactured resistant starch. Whole grains can deliver RS1, green bananas deliver RS2, and RS3 is found in ready-to-eat breakfast cereals, bread crusts, cooked and cooled potatoes and cooked and cooled pasta. RS4 is manufactured from various sources, including wheat, potato and tapioca, and is available from a variety of ingredient suppliers.

Applebaum (1966) used raw and cooked potato starch as the carbohydrate source and evaluated larval development of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleptera: Tenebrionidae), an economically important pest associated with stored grain and food-processing facilities (Hagstrum and Subramanyam, 2009). Applebaum (1966) found that larval mortality reached 100% when larvae fed raw potato starch, while it was 72% on cooked potato starch. Larval weight was 0.44 mg on cooked potato starch compared to 1.84 mg on rice starch (control). Pupation rate was 79% for T. castaneum larvae fed potato starch and it was 90% on corn starch (Pant and Kamlesh, 1965). Applebaum and Konijn (1965) found that larval weight and percentage of larval survival was 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.

The present investigation was designed to determine development, preference, and oviposition of *T. castaneum* on six different starches with varying amylose content. 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. Flour and starches

The six types of starches used included waxy corn starch (2% amylose), corn starch (25% amylose), wheat starch (25% amylose), cross-linked wheat starch (70% total dietary fiber), high amylose corn starch (70% amylose), and potato starch (12-20% amylose). In addition to these starches, wheat flour purchased from Heartland Mills, Marienthal, KS, U.S.A., was also used as the control treatment. The particle sizes of all starches and flour were analyzed using a Malvern Mastersizer 2000 by The NanoScale Corporation, located in Manhattan, KS, U.S.A.

2.2. Insects

Cultures of *T. castaneum* have been maintained in the Department of Grain Science and Industry's Stored-Product Entomology Research and Education Laboratory since 1999 on wheat flour plus 5% (by wt) of Brewer's yeast diet at 28oC and 65% r.h. in growth chambers. To collect *T. castaneum* eggs, 50 adults of mixed ages and sexes from cultures were placed on 20 g of flour in separate 150-mL round plastic containers with perforated lids. The flour used was previously sifted through a 250 µm opening sieve. The containers were closed with lids that had perforations which were covered with plastic mesh for ventilation and to prevent adult escape. The containers were held at the rearing conditions for 3 d after which the adults were removed and sifted over two sets of sieves. The top 840 µm sieve retained the adults, while allowing the flour and eggs to pass through. The eggs were retained on the 250 µm sieve.

One hundred eggs were placed in 9-cm glass Petri dishes and held at 28oC and 65% r.h. and examined daily to measure hatchability. There were 10 such dishes (n = 10 replications).

To obtain pupae for fecundity tests, after 3 d the adults were removed using the 850 µm sieve and the flour and eggs were placed back in the 150-mL plastic containers and held at 28oC and 65% r.h. After 30 d the contents were sifted using an 850 µm sieve and pupae retained on the surface of the sieve were collected. After sexing, male and female pupae were placed separately in 9-cm glass Petri dishes and held at 28oC and 65% r.h.

2.3. Procedures for measuring development

About 5 g of each starch, flour, or flour plus 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 (n = 3 replications) were removed from the chamber every 3 d, and independent cups were sampled over time. Larvae were sifted using the 250 µm sieve to separate developing larvae. The lengths and head capsule widths of 10 larvae collected from each cup were measured. 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, U.S.A.).

2.4. Preference tests

Dual-choice tests were conducted in circular arenas following techniques modified from Subramanyam (1992). Each arena measured 30 cm in diameter and 8 cm in height. Arena floors were cutout from white foam display board into 30 cm diameter circles. Arena walls were made from white mat board and the interior wall of the arena was covered with plastic tapes to close any gaps between the floor-wall junctions. In each arena, 2.5 g of one of the six starches and 2.5 g of flour were placed in the north and south ends of the arena. These locations were randomly selected by a coin toss. Fifty unsexed adults of

T. castaneum were released at the center of the arena. All arenas were covered with round lids made from the same material used for floors.

Arenas were observed at 36, 48, 60 and 72 h after adult introduction to determine number of adults retained in starch or flour. Starch and flour were collected in 9-cm glass Petri dishes and adults in the starch or flour were counted. Each starch and flour comparison and the four observation periods were replicated three times. All tests were conducted at room conditions. Room temperature and humidity during tests were measured using HOBO® data loggers (Onset Computer Corporation, Bourne, MA, U.S.A.), and these environmental variables ranged from 21-27oC and 30-55% r.h.

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. The flour was sifted using 840 and 250 µm sieves every 3 d to separate the adults and eggs as explained above. The pair of adults retained on 840 µm sieve was transferred to 2.5 g of fresh diet. The total number of eggs laid in diet were counted every 3 d for 15 d. This experiment was replicated 10 times.

2.6. Data analysis

Particle size data on 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 Ryan-Einot-Gabriel-Welsch (REGWQ) multiple comparison test (SAS Institute, 2002). Data (x) on adult retention in a starches or flour at each observation time were transformed to log (x + 1) scale and subjected to a paired t-test. Oviposition data were transformed to logarithmic scale and subjected to one-way ANOVA; treatment means were separated using REGWQ test. All statistical differences were considered significant at the $\alpha = 0.05$ level.

3. Results and discussion

Starch and flour particle size distributions are shown with 10, 50, and 90% percentages of particles below a certain size (Table 1). In general, the flour particles were bigger than that of the starches, and differences among flour and starches were significant ($P < 0.05$). Differences were also noted among the starches. Therefore, particle size may not be a limiting factor for consumption of starches by *T. castaneum* larvae.

Table 1 Particle size analysis of flour and starches.

Treatment	Percentage of particles below a certain size (in µm)a,b		
	10%	50%	90%
Flour	13.52 ± 0.12a	60.04 ± 0.02a	132.50 ± 0.39a
Potato starch	24.36 ± 0.04b	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 ± 0.06e	20.38 ± 0.03e
70% Amylose corn starch	5.52 ± 0.01g	9.15 ± 0.16f	14.76 ± 0.46f

a = Each mean is based on n = 2 replications; b = Means followed by different letters are significantly different ($P < 0.05$; REGWQ test).

The mean egg hatchability of *T. castaneum* eggs was 75.4 ± 1.1%. The observed hatchability was 16% less than that observed by Sokoloff (1974).

The development of *T. castaneum* larvae, as determined by larval length, head capsule width, and weight gain, showed that larvae developed normally on flour and flour plus yeast, with the development being better on the latter substrate. 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 (Fig. 1). The

worst larval survival was on potato starch, followed by cross-linked wheat starch, and 70% amylose corn starch. Potato starch which belongs to RS2 type is resistant to enzyme digestion due to the enriched phosphorus content in large starch granules resulting in a compact structure with crystallinity (Englyst and Cummings, 1987). The cross-linked wheat starch and 70% amylose corn starch are resistant to enzyme digestion, because their tightly packed structure reduces enzyme access to the starch side chains (Woo and Seib, 2002). The larval development results are consistent with this finding. The adverse effects of starches on growth of *T. castaneum* larvae compared to flour or flour plus yeast diet suggest that the starches lack certain essential nutrients for normal development. What these constituents are at this point is unclear, but we speculate that it could be protein components which these starches lack.

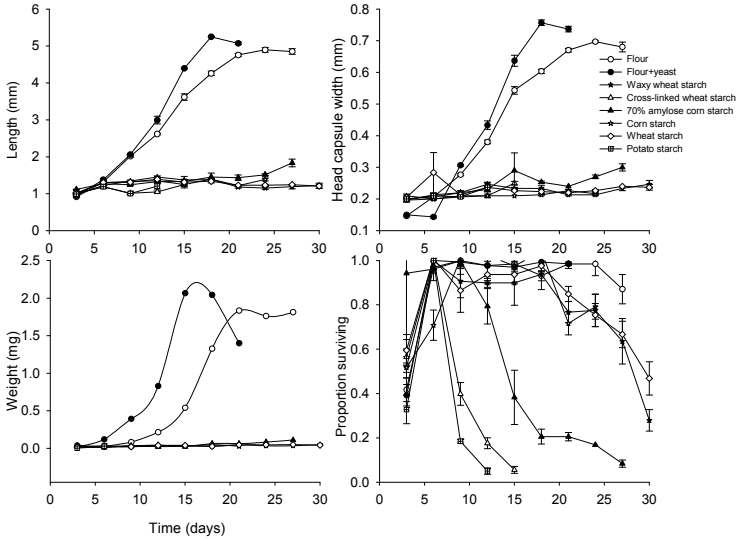

Figure 1 Development and survival of *T. castaneum* larvae on the six starches, flour, and flour plus yeast.

In arena tests, about 48-80% of the released adults were retained in the starch and/or flour substrates. There was no significant difference between the number of adults retained on each of the starches and flour ($P > 0.05$) at each observation time (t, among observation times = -3.67 – 1.79; df = 2; $P > 0.05$). Therefore, only data for 72 h is shown in Table 2. Adults of T. castaneum evaluate suitable media by visual, tactile, and chemical cues (Campbell and Runnion, 2003). Commercial starches are usually odor-free materials. However, lack of significant differences between starches and flour suggest that they are all equally attractive to *T. castaneum*.

Table 2 Relative retention of T. castaneum adults on a specific starch and flour in dual-choice testsa.

Time	Flour vs. starch/ starch type	No. adults retained in:		t (df=2)	P-value*
		Starch	Flour		
72 h	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

n = 3 replications; data (x) transformed to log10 (x) scale to normalize variances; a = Data for the other observation times were similar to data presented for 72 h; *All P-values are not significant ($P > 0.05$; paired t-test).

Very few eggs were laid on all starches (<3 eggs/female) during the 15 d test period compared with 97 to 109 on flour and flour plus yeast diet (Table 3). The low number of eggs laid suggests that triggers for egg laying are absent in starches. A simple test in the laboratory showed that *T. castaneum* adults placed

on a sieve above flour do not lay eggs and need to be in contact with the flour. This suggested that feeding may stimulate egg-laying. In additional tests not reported here, we observed that *T. castaneum* placed on flour and then transferred to starches after 3 d laid eggs. Similarly, when beetles that failed to lay eggs on starches after 3 d when placed on flour laid eggs during the next 3 d. Currently, tests are underway with different percentages of wheat gluten admixed with wheat starch to determine whether protein plays an important role in development, survival, and oviposition of *T. castaneum*.

Table 3 Oviposition of *T. castaneum* on flour plus yeast, flour, and starches over a 15-d period.

Treatment	No. eggs/female (mean ± SE)a
Flour + yeast	108.7 ± 5.7a
Flour	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	0.1 ± 0.1c

n = 10 replications, except for flour and waxy corn starch where there were 9 replications;
a = Means followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

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Mention of trade or proprietary names in this publication does not imply an endorsement by Kansas State University or the USDA.

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