Development, relative retention, and productivity of *Tribolium castaneum* on resistant starches

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Introduction

- Red flour beetle (RFB)
  - *Tribolium castaneum* (Herbst)
  - Order: Coleoptera; Family: Tenebrionidae
- Distributed worldwide
- Feeds on stored grain, but prefers milled products
- Damage through feeding, imparts disagreeable taste and odor caused body secretions

http://www.ars.usda.gov/Research/docs.htm?docid=12890

http://www.spike-international-agencies.com/meer_insecten.htm

Basis for the study

- This pest is traditionally controlled using pesticides
- More recently, emphasis has been on exploring alternatives to pesticides
- Limited studies on high-amylose content starches
- Current study explores effects of different amylose content starches on RFB development, preference, and egg laying
- Primarily interested in exploring potential of starches as a pest management tool

Research objectives

- Measure growth of red flour beetle larvae on flour and six starches
- Determine preference (relative retention) of red flour beetle adults to flour and starches
- Determine productivity (eggs laid) on flour and starches

Materials and methods

- RFB: Reared in laboratory at 28C, 65% RH, on flour + 5% yeast (w/w)

- RFB eggs: 50 RFB unsexed adults were placed in plastic cups, after 3 days, eggs were collected by sieving the rearing diet through a No.60 mesh (U.S. Standard Sieve)

- Treatments: white flour (F), 95% white flour with 5% yeast (), waxy corn starch (2% amylose), modified wheat starch (cross linked, 70% total dietary fiber), 70% amylose corn starch, normal corn starch, normal wheat starch (25% amylose), Potato starch (12-20% amylose)

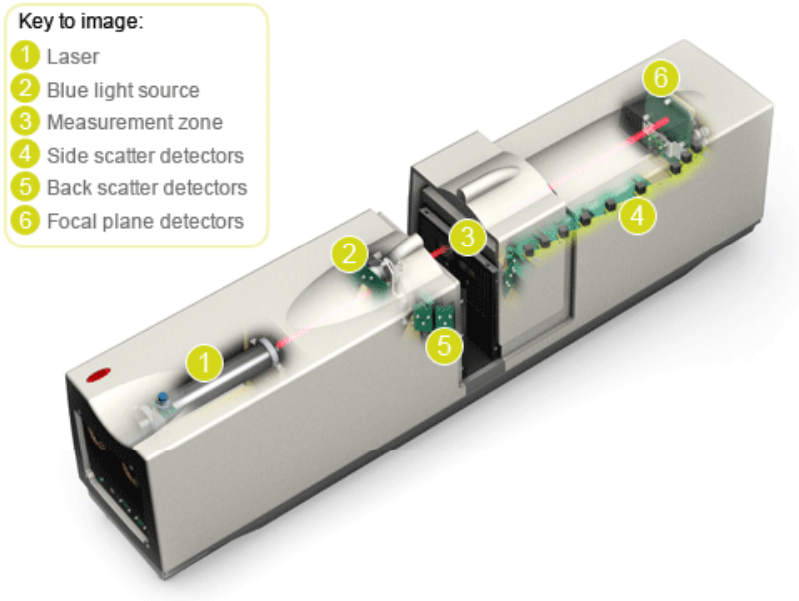
Data collected on:

1. Starch and flour particle size distribution
2. RFB egg hatchability
3. Development of RFB on starches
4. Relative retention of RFB on flour and starches
5. RFB productivity on starches
6. Fecundity with pheromone lure added to flour
7. Oviposition with and without food contact

Methods

1. Particle size analysis

- NanoScale Corporation, Manhattan, KS., U.S.A.
- Malvern Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK)
- 1 g dry powder and a refractive index (RI) of 1.530 was used during each measurement
- All samples were measured in duplicate
- The software-driven Standard Operating Procedures (SOPs) was used to ensure reproducibility



Particle size distribution in µm

Starch	d (0.1)	d (0.5)	d (0.9)
Flour	13.5	60.0	132.5
Potato starch	24.4	37.6	57.7
Wheat starch	11.8	17.9	26.7
Cross linked wheat starch	10.7	17.0	26.4
Corn starch	9.1	13.3	19.2
Waxy corn starch	8.1	13.0	20.4
70% amylose corn starch	5.5	9.1	14.8

Based on n=2

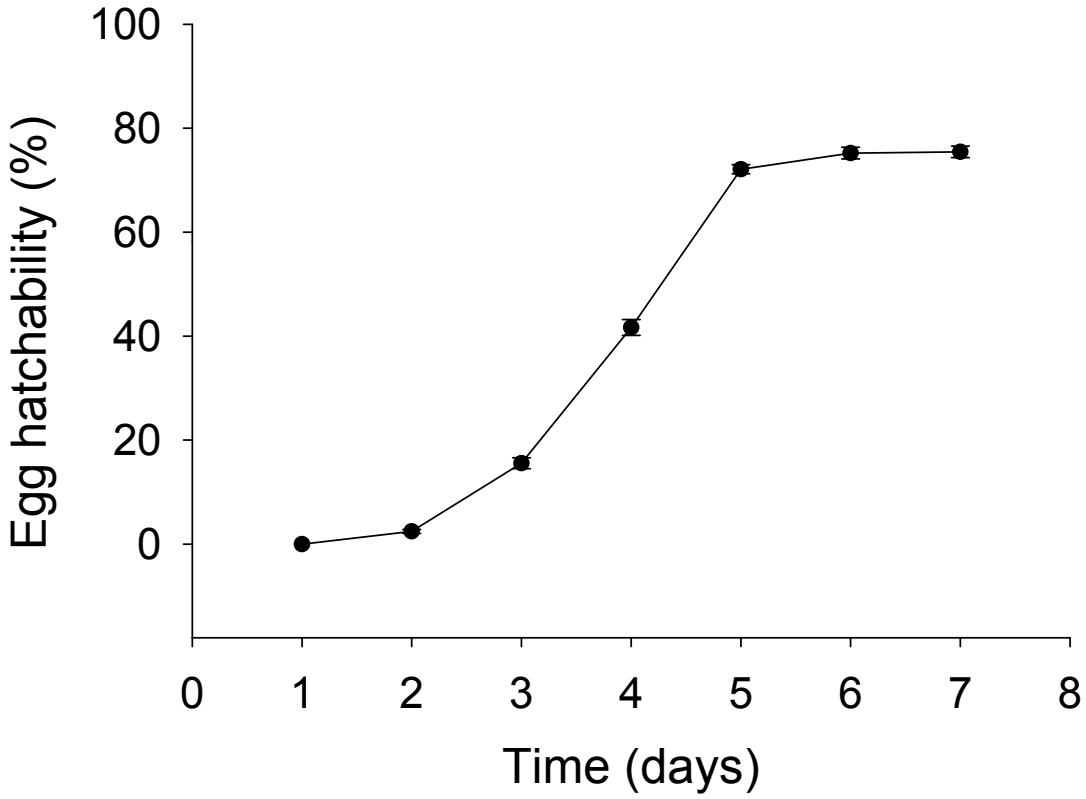
	DF	F	P>F
d (0.1)	6	10565.8	<0.0001
d (0.5)	6	66107.9	<0.0001
d (0.9)	6	33740.2	<0.0001

Data was analyzed by orthoµgonal contrast (α =0.05) (SAS Institute, 2004). Starch particle sizes were compared to flour particle size.

2. Egg hatchability

- 100 eggs were placed in 9 cm-diameter glass Petri dishes
- Petri dishes were kept at 28^{o}C and 65% RH
- Number of eggs hatched were counted daily
- 10 replications

Egg hatchability

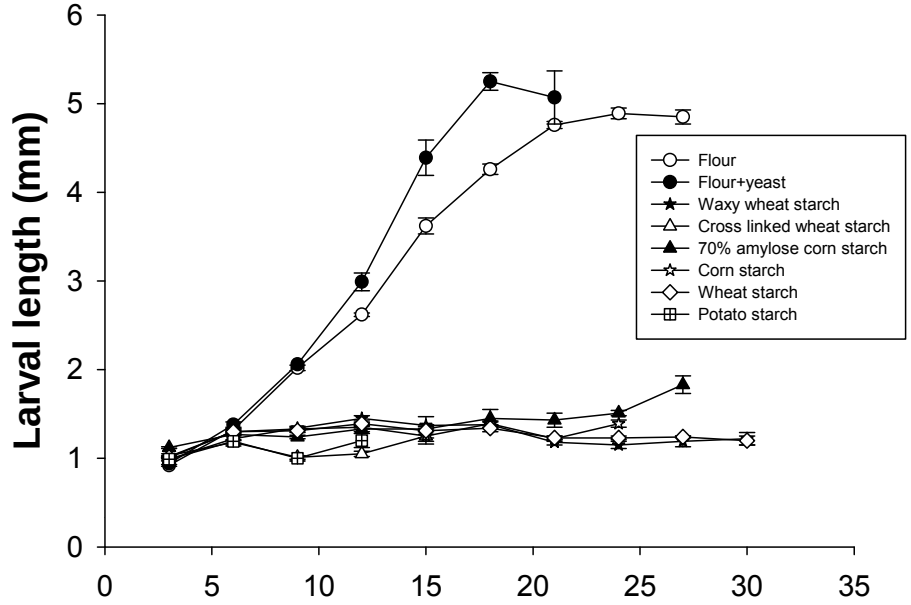


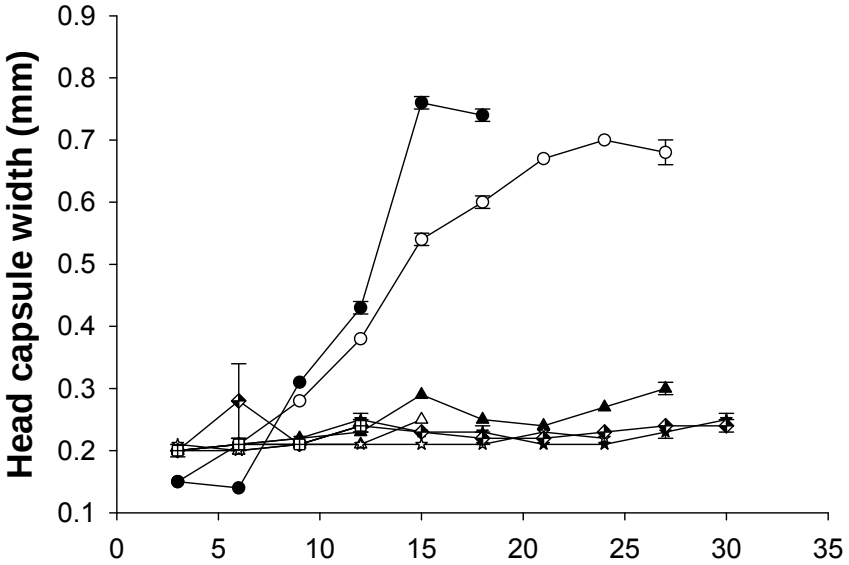
3. RFB development

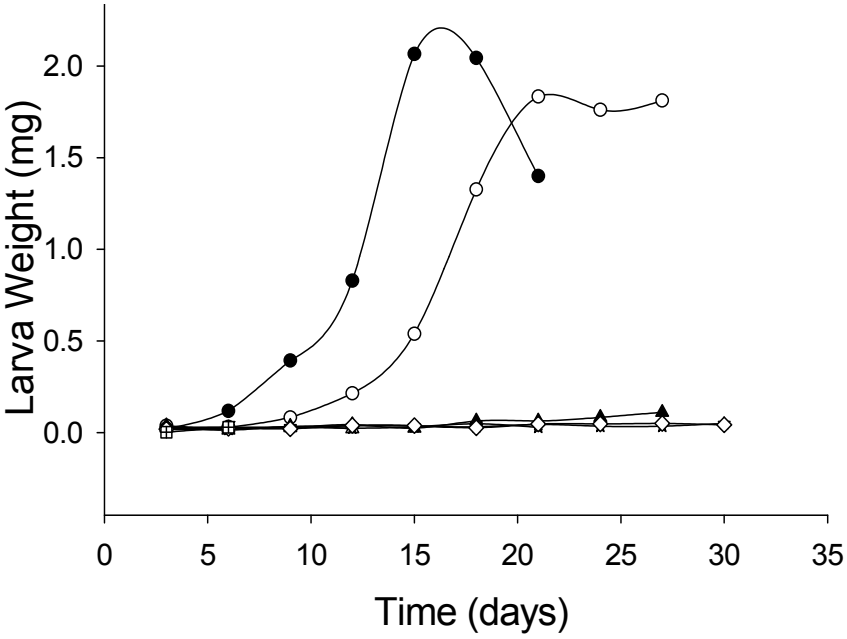
- 50 eggs were placed in each 30 ml plastic condiment cup containing 5 g of flour or starch sample. All condiment cups were kept in growth chambers at 28^{o}C and 65% RH

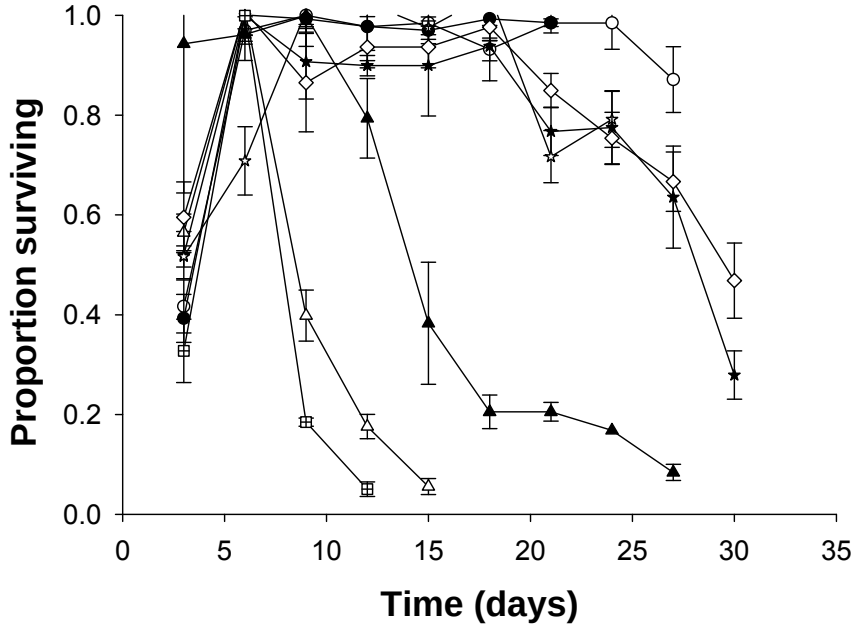
- Cups checked every 3 d

- Measured larval length and weight, head capsule width, and survivorship


Larval length (mm)
Flour
Flour+yeast
Waxy wheat starch
Cross linked wheat starch
70% amylose corn starch
Corn starch
Wheat starch
Potato starch

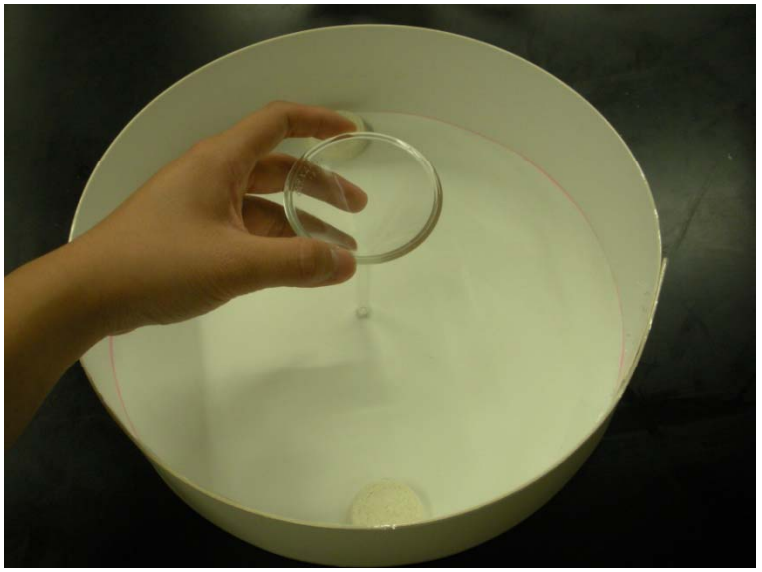

Head capsule width (mm)

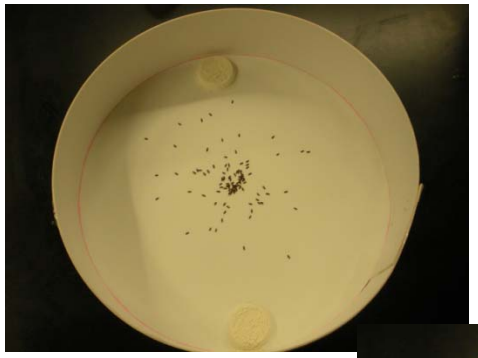

Larva Weight (mg)
Time (days)

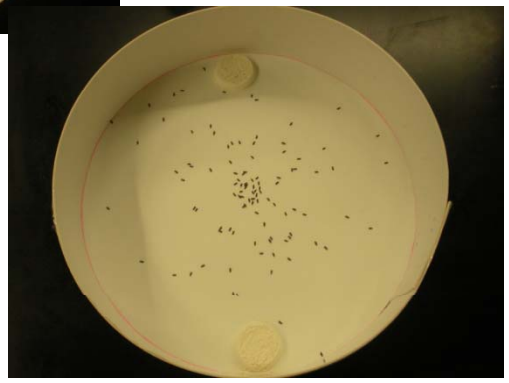

Proportion surviving
Time (days)

4. Relative retention

- 2.5 g flour and 2.5 g starch sample were placed in one arena (diameter=30 cm, height=8 cm)-dual choice tests
- 50 newly emerged RFB adults were released in the center of the arena which were immediately covered with lids and kept at room temperature
- Experiments were replicated 3 times. Flour arenas in each replicate were checked at 36 h, 48 h, 60 h, and 72 h respectively. The number of RFB adults in flour and starch were counted
- Temperatures were recorded during the experiment by HOBO data loggers







Number of adults retained-36h

Starch VS Flour	Adults in starch	Adults in flour	*t* (df=2)	*P*>*t*
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	1.92	0.20
Waxy corn starch	13.0±5.5	19.0±1.0	-0.79	0.51

n = 3; data transformed to *n*=log10 (*n)* to normalize variances

Number of adults retained-48h

Starch VS Flour	Adults in starch	Adults in flour	*t* (df=2)	*P*>*t*
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

Number of adults retained-60h

Starch VS Flour	Adults in starch	Adults in flour	*t* (df=2)	*P*>*t*
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

Number of adults retained-72h

Starch VS Flour	Adults in starch	Adults in flour	*t* (df=2)	*P*>*t*
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

5. RFB productivity on starches

- Male and female RFB were sexed in the pupal stage
- After adult emergence, one pair of RFB was placed on 2.5 g of a starch sample or flour in 30-ml plastic condiment cups
- RFB adults were changed to new diet every 3 d; the number of eggs in old diet were collected and counted
- Data summed over 14 days

Productivity (eggs laid over 14 d)

Treatment	No. reps	Mean± SE
Flour +yeast	10	108.7±5.7 a
Flour	9	97.3±6.9 a
Potato starch	10	2.6±0.5 b
Cross linked wheat starch	10	1.0±0.2 c
Corn starch	10	1.3±0.5 c
Wheat starch	10	0.8±0.2 c
70% amylose corn starch	10	0.6±0.3 c
Waxy corn starch	9	0.1±0.1 c

Values followed by different letters are significantly different ($P < 0.05$; REGWQ test)

6. Effect of pheromone on oviposition

- 2.5 g sample was placed in 30-ml plastic condiment cup

- Pheromone lure was introduced into each cup. For every starch sample, three levels of lure were used based on weight (A: 0 g, B: 0.095 ± 0.002 g, C: 0.270 ± 0.002 g)

- Cups with different amounts of lure were kept separately in three chambers

- After 6 days, eggs in each cup were collected and counted

Oviposition (over 6 days) with pheromone added to substrates

	0 g	0.095 ±0.002 g	0.270 ±0.002 g
DF	11	11	11
F value	32.51	14.4	9.75
P	<.0001	0.0014	0.0048

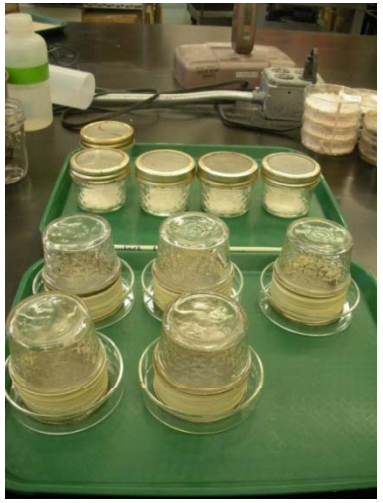
Statistical analysis at three pheromone levels.

Treatment	0 g	0.095 ±0.002 g	0.270 ±0.002 g
Flour	22.7±11.7 a	19.7±8.4 a	16.3±9.8 ab
Flour +yeast	37.0±12.6 a	26.3±14.5 a	41.0±11.9 a
Cross linked wheat starch	0 b	0 b	0 b
Potato starch	0 b	0 b	0 b

n = 3. Data were transformed to log10 (*n*+1) scale to normalize variances.

7. Oviposition with and without food contact

- One pair of newly emerged RFB adults were placed in 125 ml glass jar with 40 mesh lid, the lid is coupled surface-to-surface with another lid and taped together
- The jar with RFB will be then turned over on a Petri dish (9 cm in diameter) which contained 5 g of flour in the center



- In control group, a pair of newly emerged adults were placed in a glass jar with 5 g flour and the jar was closed with a lid with 40 mesh
- All jars were then placed in a growth chamber
- Eggs in the flour in all jars were checked after 6 days

Conclusions

- Hatchability of eggs was 75%
- Starches particle sizes were significantly smaller than flour particle size--starches could be consumed by RFB larvae
- Feeding on starches delayed larval development and also retarded larval growth
- Potato starch and cross linked wheat starch (70% total dietary fiber content) hindered larval development to a greater extent than other starches
- Adults did not show any preference to flour over starches
- However, adults laid significantly little or no eggs on starches compared with flour

Conclusions

- The aggregation pheromone does not trigger RFB oviposition on flour with and without yeast
- Ovipostion was not stimulated by aggregation pheromone when RFB were fed on starches
- Volatiles from flour do not stimulate RFB oviposition, and food contact was necessary for egg laying
- What components of flour elicit egg laying is still a mystery!

Future work

- Reasons for egg laying need to be explored by switching adults between starches and flour and *vice versa*, and by providing access to flour for different time periods
- Determine egg laying by progressively adding various percentages of gluten to starches between 0.1 and 12%
- Characterize RFB midgut amylase and inhibition by starches

Thank you!