

Effects of Flour Conditioning on Cannibalism of *T. castaneum* Eggs and Pupae

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ABSTRACT Cannibalism is a very important factor regulating population dynamics of the red flour beetle. After several days of feeding, the flour becomes conditioned by the beetles, which can affect rates of cannibalism. Flour conditioning is caused by an accumulation of feces, pheromones, and ethylquinone, which is a repellent produced by the beetles. We determined the effect of five different levels of flour conditioning on cannibalism of red flour beetle eggs and pupae by adult and larval stages. Larvae had the highest rates of egg cannibalism (12 eggs eaten over the 4-d period) followed by female adults (seven eggs consumed). Adult males had the lowest rates of cannibalism with only two eggs consumed. Cannibalism of eggs by females was correlated negatively with the level of flour conditioning. There was no effect of flour conditioning on egg or pupal cannibalism by larvae or adult males. Cannibalism by adult females may decrease as the level of flour conditioning increases because females may spend less time tunneling in highly conditioned flour and more time trying to disperse to other areas that are better for oviposition.

KEY WORDS cannibalism, flour conditioning, *Tribolium castaneum*, population dynamics

The red flour beetle [*Tribolium castaneum* (Herbst)] is a common insect pest infesting flour mills and stored grain (Fraenkel and Blewett 1943, Campbell et al. 2010). In addition to feeding on flour, cannibalism of egg and pupal stages by adult or larval *T. castaneum* is well documented (Park et al. 1965). Eggs or pupae can provide a rich source of nutrients and moisture for *Tribolium* adult beetles and large larvae. Cannibalism in *T. castaneum* can have a strong affect on population growth. As population density increases, the proportion of the eggs eaten also increases, to the extent that at high densities as much as 98% of the eggs can be destroyed or consumed (Rich 1956). *Tribolium* populations change the composition of the flour as they develop in it, a process called flour conditioning. Flour conditioning is a result of depletion of nutrients and the build up of waste materials, pheromones, and ethylquinone, which is a repellent produced by the beetles (Sonleitner 1961). Conditioning of flour increases over time and the rate is dependent on beetle density. Flour conditioning can influence beetle behavior. For example, flour can be more attractive to *T. castaneum* beetles when first colonized (Hughes 1982), but highly conditioned flour can become repellent (Duehl et al. 2011). Sonleitner (1961) showed that egg can-

nibalism in adult *T. castaneum* increased in conditioned flour compared with fresh flour. However, Park (1935) found that conditioned flour did not result in an increase in egg cannibalism in the closely related species *Tribolium confusum* DuVal. To accurately predict the effects of flour conditioning on rates of cannibalism it is important to characterize the relationship between the level of flour conditioning and rates of cannibalism. While Sonleitner (1961) qualitatively showed that conditioned flour affected the rate of cannibalism, he did not show how rates of cannibalism varied over a range of flour conditioning. We develop a method to quantify flour conditioning (beetle-days), and develop equations to predict the rate of cannibalism for *T. castaneum* females, males, and large larvae, feeding on eggs or pupae over different levels of flour conditioning.

Materials and Methods

Five different levels of conditioned flour were prepared by leaving 500 adults on 100 g of flour for different amounts of time. The flour was sieved weekly with a U.S. Standard no. 50 sieve (0.30-mm opening) to remove eggs from the conditioned flour. One beetle-day of conditioned flour was assumed to be one adult beetle per day feeding on 1 g of flour. The conditioned flour was stored at -18°C in glass containers until it was used in the experiment.

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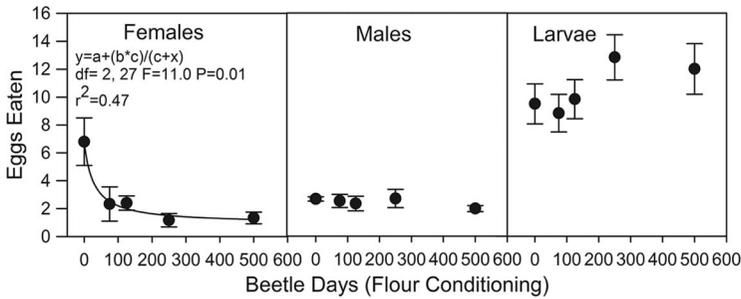


Fig. 1. Cannibalism of eggs by *T. castaneum* larvae, male adults and female adults as a function of flour conditioning.

The arena consisted of a block of medium density particle board with a 5- by 5-cm depression that was 2 mm deep filled with 2 g of organic golden buffalo flour (Heartland Mills, KS). A 5- by 5- by 1-cm square metal band was placed on top of the particle board to keep the insects from leaving the flour. In addition, a 9-cm-diameter petri dish lid was placed on top of the arena to prevent insects from leaving. A 0.5-cm hole was drilled in the center of the petri dish lid for air flow. We selected a flour depth of 2 mm because this was the minimum depth that allowed the adults and larvae to tunnel into the flour. Five different levels of conditioned flour were used (0, 75, 125, 250, or 500 beetle-days). Twenty eggs (1 d old) or pupae (1 d old) were mixed into the 2 g of flour that was placed in the arena. Marked eggs (Rich 1956) were obtained by allowing females to oviposit into flour that contained 1% by weight neutral red dye (Sigma-Aldrich, St. Louis, MO). This allowed us to differentiate them from eggs laid by adult females during the experiment. Particles of the dyed flour remained attached to the eggs and preliminary tests indicated that this did not impact feeding by adult *T. castaneum*.

Four adult males or females (2–3-wk-old), or four larvae (fifth–sixth instar) were placed in each arena. Arenas then were placed in a growth chamber at 24°C, a photoperiod of 12:12 (L:D) h, and held for 4 d. A 7.5-W incandescent bulb at a distance of 2 m was used so that the beetles would not be disturbed during the experiment (two lux at site of arenas). Cannibalism of eggs or pupae was determined by examining marked eggs. If the number of marked eggs or pupae was less than the original count, or if marked eggs or pupae were partially eaten, then they were assumed to have been cannibalized. Each treatment was replicated six times and blocked over time.

PROC REG and PROC NLIN (SAS version 9.2) (SAS Institute 1988) were used to fit linear and non-linear equations to the data for adult females, adult males, with eggs or pupae eaten as the dependent variable and beetle-days as the independent variable.

Results

Larvae provided with eggs had the highest rates of cannibalism with ≈ 12 eggs eaten over the 4-d period (Fig. 1). Adult females had the second highest rates of cannibalism with ≈ 7 eggs consumed. Adult males had

the lowest rates of cannibalism with only two eggs. The amount of flour conditioning did not affect rates of cannibalism for males ($F_{1,28} = 3.05$, $P > 0.05$) and larvae ($F_{1,28} = 3.05$, $P > 0.05$). However for female adults, the rates of cannibalism decreased in a nonlinear fashion with increasing flour conditioning ($F_{2,27} = 11.03$, $P < 0.01$, $r^2 = 0.47$). The equation $y = a + (b*c)/(c*x)$ fit the data well. Coefficients and SE for a , b , and c were 5.89 ± 1.43 , 27.35 ± 32.29 , and 0.91 ± 1.05 , respectively. The level of flour conditioning affected the number of new eggs laid by females during the experiment. New eggs could be determined because they were not marked with dyed flour. The number of new eggs laid by females \pm SE was 13.8 ± 3.9 , 5.2 ± 2.4 , 5.2 ± 2.3 , 3.2 ± 1.4 , and 4.2 ± 2.6 , for beetle days of 0, 75, 125, 250, and 500, respectively. This indicates that even the lowest level of flour conditioning inhibited oviposition by females.

Female adults provided with pupae had the highest rates of cannibalism with ≈ 7 pupae consumed (Fig. 2). Larvae provided pupae had almost the same rates of cannibalism as female adults. Adult males had the lowest rates of cannibalism feeding on pupae with only 0.5 pupae consumed during the 4-d period. The amount of flour conditioning did not affect adult males or larvae cannibalizing pupae ($F_{1,28} = 3.47$, $P > 0.05$). For female adults, the rate of cannibalism decreased in a nonlinear fashion with increasing flour conditioning ($F_{2,27} = 6.68$, $P < 0.01$, $r^2 = 0.33$). The equation $y = a + (b*c)/(c*x)$ fit the data well. Coefficients and SE for a , b , and c were 4.55 ± 1.47 , 29.61 ± 45.74 , and 1.44 ± 1.13 , respectively.

Discussion

Cannibalism can provide fitness benefits directly in terms of increased survival, development, and fecundity or indirectly through reduced competition. For *Tribolium* spp. the benefits of cannibalism are predicted to change depending on population density, which can be signaled by chemical cues associated with level of conditioning of flour. Adult males exhibited low levels of cannibalism, whereas larvae had relatively high levels of cannibalism, regardless of flour condition. Only adult females responded to level of flour conditioning, with greater cannibalism in uninfested flour. This increased cannibalism in unconditioned flour could result from females perceiving

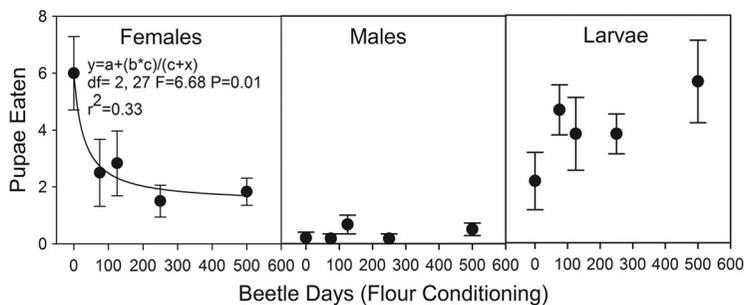


Fig. 2. Cannibalism of pupae by *T. castaneum* larvae, male adults and female adults as a function of flour conditioning.

patch quality differently. As discussed in more detail below, they could be reducing cannibalism rates when avoiding conditioned flour because it is of poor quality or increasing cannibalism rates by actively removing competition in the form of eggs already laid from the higher quality unconditioned patches. Larvae have greater nutritional demands and they are also directly competing with other larvae, so it is expected that they would have high cannibalism rates. However, as flour quality declines it was predicted that cannibalism might increase, which was not evident in our study. This could be because flour condition was still adequate or that cannibalism rate was already at or near its maximum. Low cannibalism by males is consistent with them having different nutritional needs than females and that they have fewer fitness benefits from removing offspring if they are unable to determine paternity.

Our results are different than Sonleitner (1961), who indicated that egg cannibalism by adult *T. castaneum* was higher in conditioned than in fresh flour. Sonleitner (1961) used pairs of male and female adults that were 90 d old, which may have increased variability because of the large differences in cannibalism between males and females. He also obtained his conditioned flour from stock cultures that were several months old, so it is difficult to determine the amount of conditioning from his methods. Similar to our findings, Sonleitner (1961) did find that female *T. castaneum* adults had much higher rates of egg cannibalism than male adults. Rich (1956) also showed that this was true for *T. confusum*. Park (1935) showed that cannibalism rates of *T. confusum* adult males decreased in conditioned flour. However, Park did not quantify the amount of flour conditioning, so it is difficult to compare his results with ours. Ethylquinone in the conditioned flour produced by the beetles is a repellent and it is thus, not surprising that cannibalism of eggs by female adults would be inversely proportional to the amount of flour conditioning (beetle-days). To avoid competition, females should prefer to lay eggs in fresh flour. Sonleitner and Guthrie (1991) suggested that quinones in conditioned flour may act as a signal to stop laying eggs and to disperse to avoid high egg mortality. In our experiment, it is likely that the adult females spent more time trying to disperse from the arena and less time tunneling in the flour in the conditioned compared with the fresh flour.

Less time tunneling would decrease the chances of encountering eggs or pupae in the flour, and thus, lower rates of egg and pupal cannibalism. Oviposition also was inhibited by flour conditioning. The biggest drop in oviposition was from 0 to 75 beetle days, which fits well with the changes in observed cannibalism. This pattern would suggest that cannibalism may be related to reducing competition for offspring more than for female nutrition. Thus, increased tendency to disperse would be expected to be correlated with a decrease in oviposition, which is associated in turn with an assessment by the female that the patch is of lower quality.

Rates of larval cannibalism of eggs and pupae did appear to increase slightly with the level of flour conditioning (Figs. 1 and 2). However, the regression was not significant at the 5% level ($P = 0.09$), probably because of the considerable variation among conditioning levels. The difference in behavior between larval and adult females may be caused by the larval stages being less likely to leave the flour compared with adult females. As the amount of flour conditioning increased, egg and pupal cannibalism by larvae increased slightly. Eggs and pupae are a rich source of water and nutrients for the larvae. As nutrients decreased with the amount of flour conditioning, larvae may have increased their search for eggs and pupae to supplement the lower nutrition of the conditioned flour.

Results from this study will be used in an agent-based model of the red flour beetle that is currently under development.

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