Behavioral responses of *Tribolium castaneum* to mycotoxin contaminated wheat

Adriane Fifield[1,2], Alison Gerken[3] and Erin Scully[3]

[1]Department of Entomology, College of Agriculture, Kansas State University
[2]Department of Biology, College of Arts and Sciences, Kansas State University
[3]USDA-ARS Center for Grain and Animal Health Research, Manhattan KS

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Abstract

Red flour beetles are major pests of stored grains throughout the world. Since the females can lay up to 300-400 eggs in their lifetime, an infestation can get out of control quickly (Brown et al, 2009). Although ingestion of stored product insects is not associated with any major health risks to humans or animals, infested products can have a pungent odors and are often unsuitable for consumption (Smith et al, 1971). To understand what may attract the beetles to a food source, we tested to see how they would react to mycotoxin deoxynivalenol (DON), a common mycotoxin found in stored grains that have been contaminated with mold (Sobrova et al, 2010). Red flour beetles routinely feed on grain that has been contaminated with mold; however, it is unknown if red flour beetles follow volatile cues from mold to find food sources. This research is to find out if red flour beetles are attracted to wheat that has been contaminated with the mycotoxin deoxynivalenol (DON). The results show that the male beetles avoided the mycotoxin and that the females show neither an attraction or an avoidance of the volatile. Ultimately, identifying compounds that attract or repel stored product insects can help us develop novel behaviorally based-strategies to prevent insects from locating food sources.

Purpose

Red flour beetles routinely feed on grain that has been contaminated with mycotoxins and may use odors associated with these mycotoxins to locate food sources. The purpose of this experiment is to determine whether or not red flour beetles are attracted to food that has been contaminated with deoxynivalenol (DON), which is a common contaminant of stored wheat.

Questions and Hypotheses

Question: Are red flour beetle adults attracted to wheat that has been contaminated with the mycotoxin deoxynivalenol (DON)?

Hypothesis: DON will be attractive to red flour beetle adults.

Study System

Tribolium castaneum (red flour beetle) is a cosmopolitan pest of stored grains found that causes significant yield losses throughout the world. This insect is commonly found in flour mills, but also feeds on a variety of products including pet food, bird seed, cake mix, dried museum specimens, and pasta. These insects are particularly problematic because a single female beetle can lay anywhere between 300-400 eggs during her ~ six month life span. Thus, stored products can become quickly infested. Red flour beetles use volatile chemical cues to locate suitable food sources for colonization. The identity of many of these volatile compounds has been determined, but it is currently unknown whether volatiles derived from mold-infested grain, which is routinely colonized by stored product insects, could serve as attractants for these beetles. Fungi that contaminate grain often produce mycotoxins that inhibit growth or feeding of competitor organisms; however, red flour beetle is known to feed on contaminated mold and may actually be attracted to mycotoxins and have resistance to these toxins.

https://www.ars.usda.gov/plains-area/mhk/cgahr/spieru/

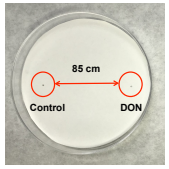

https://www.uni-bielefeld.de

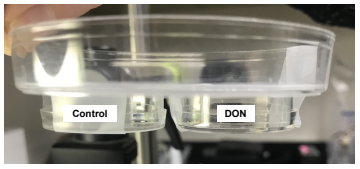

https://www.agric.wa.gov.au

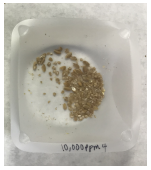
Methods and Experimental Design

1.Red flour beetle pupae were collected and sex was determined. Pupae were kept in 24-well plates until adults emerged, after which they were starved for a period of 4-7 days prior to behavioral analysis.

2.Starved adults were placed into a 90 cm petri dish that was lined with filter paper. Two holes were drilled into the bottom of the dish using a 7/64 inch drill bit. 3.5 cm petri dishes containing food were placed directly below each of the holes in order to minimize visual cues and ensure that beetles had to rely solely on volatile cues to make decisions. Food contaminated with 10,000 parts per million (PPM) DON was placed on one side of the dish while control food was placed on the opposite side of the dish.







3.Beetles were placed in the center of each arena and were allowed to move freely for a period of 5 minutes. Behavioral responses, such as time spent near each food source, duration of time spent near each food source, and total distance traveled near each food source, were video recorded using a digital camcorder (HDR-XR520V; Sony Corporation, New York, NY) and scored using Ethovision XT (Version 8.0, Noldus Information Technology, Wageningen, The Netherlands). Five males and five female red flour beetles were tested and the position of the DON contaminated food was periodically switched. Zones consisting of 4 cm diameter circles were drawn around each food source are were and were labeled control and volatile zones respectively.

4.GLIMMIX (SAS Institute, Cary NC, version 9.4) was used to test for differences between males and females in terms of time spent near each food source, duration of time spent near each food source, and total distance traveled near each food source.

Results

No major differences were found between average number of visits to the zones containing the control and the DON contaminated grain in either males or females (Figure 1). Similarly, no differences in average time spent in zones containing DON contaminated grain or control grain were noted between the sexes (Figure 2). However, males spent more time away from the zone containing the DON contaminated grain (Figures 3 and 4).

Figure 1

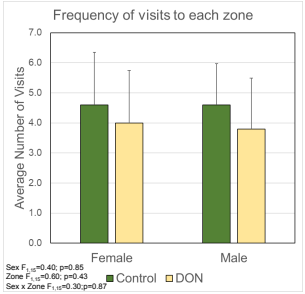


Figure 2

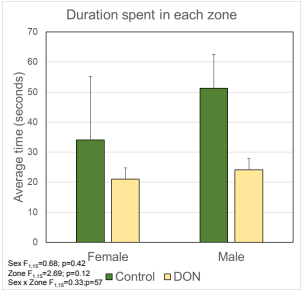


Figure 3

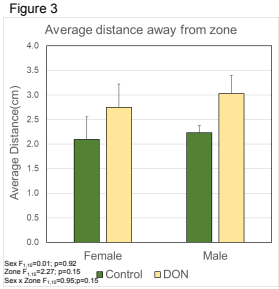


Figure 4

Conclusions

In these experiments, red flour beetles did not seem to be attracted to or repelled by DON in terms of number of visits to zones containing control or DON contaminated food or the duration of time spent in each zone. However, male beetles tended to avoid the food contaminated by the DON compared han females. This finding could suggest that DON might not be attractive to male beetles and may even serve as a repellent, but more experiments are needed to confirm this result.

Future Directions

For many of these experiments, there was a lot of behavioral variation between individual insects because the recordings could not all be performed on the same day due to time constraints. Analyzing all individuals on the same day would potentially reduce behavioral variation because environmental factors, such as light, temperature, humidity, and air flow, can influence behavioral responses and can vary from day to day, even in controlled laboratory environments. Additionally, analyzing more individuals (n=20 or n=30) would help us provide additional statistical power that would potentially allow us to detect differences between groups of insects. Behavior varies tremendously between individuals so larger numbers of replicates are often required for these experiments.

References

Brown SJ, Shippy TD, Miller S, Bolognesi R, Beeman RW, Lorenzen MD, Bucher G, Wimmer EA, and Klinger M. The red flour beetle, *Tribolium castaneum* (Coleoptera): A model for studies of development and pest biology. *Cold Spring Harbor Protocols*. 2009;8, pdb-emo126.

Smith LW, Pratt JJ, Ni I, and Umina, A P Baking and taste properties of bread made from hard wheat flour infested with species of *Tribolium*, *Tenebrio*, *Trogoderma* and *Oryzaephilus*. *Journal of Stored Products Research*. 1971;6(4), 307-316.

Sobrova P, Adam V, Vasatkova A, Beklova M, Zeman L, Kizek R. Deoxynivalenol and its toxicity. *Interdiscip Toxicol*. 2010;3(3), 94-9.

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