Effects of 15-Acetyl-deoxynivalenol (15-ADON) and Diacetoxyscirpenol (DAS) Mycotoxins on *Tribolium castaneum*

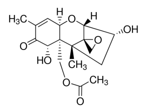
Mollie Toth[1,2], Valerie Nguyen[1,3], and Erin Scully[3]

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

Abstract

Two major threats to stored products in the United States and throughout the world are stored product insects and contamination from mycotoxins, which are secondary metabolites produced by fungi, such as *Fusarium* spp. (Glenn, 2007). Although stored product insects are not associated with negative health impacts when consumed by humans or animals, mycotoxins can negatively impact fertility, reproductive health, growth and development, particularly in livestock (Brake, 1999). Unfortunately, it is difficult to remove mycotoxins from contaminated products and most products have to be destroyed. However, stored product insects routinely feed on products contaminated by mold and may posses detoxification enzymes that could be exploited by the food industry to degrade mycotoxins. To determine whether *Tribolium castaneum* (red flour beetles) may have resistance to mycotoxins commonly encountered in stored products, such as 15-acetyl-deoxynivalenol (15-ADON) and diacetoxyscirpenol (DAS), we performed bioassays using 10-fold serial dilutions of both purified toxins independently and measured life history parameters, including mortality rates, growth rates, and progeny production. These parameters were compared to insects fed on a control diet to determine whether mycotoxins negatively impacted red flour beetle adults. This allows for an understanding of how individual mycotoxins influence the insects rather than their combinations seen in naturally contaminated mold (Guo, 2014). The results showed no significant impact on any of the life history parameters from the 15-ADON mycotoxin; however, the DAS showed a significant impact on growth rates and progeny production. Furthering the study of the resistance of red flour beetles to mycotoxins may allow us to discover novel enzymes that could be used to degrade mycotoxins contaminating grain, allowing it to be used as animal food and reducing post-harvest losses.

15-acetyl-deoxynivalenol (15-ADON)



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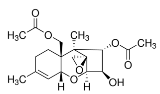
Diacetoxyscirpenol (DAS)



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Purpose

The purpose of this research is to determine whether red flour beetles are resistant to the mycotoxins DAS and 15-ADON.

Questions and Hypotheses

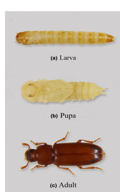
Question: Does exposure to the mycotoxins DAS or 15-ADON impact mortality, growth, and progeny production of red flour beetle adults?

Hypothesis: Both of the mycotoxins, DAS and 15-ADON, will have minimal effects on mortality, growth, and progeny production in red flour beetles.

Study System

The red flour beetle originates near Australia and is one of the most common pests of stored food grains around the world. They are most often found in the southern states of the United States due to a more northern distribution of its close relative, the confused flour beetle. They are known to have long life spans of up to three years. The red flour beetles feed on damaged grains and are external feeders that feed on the surface on their food sources. For this reason they prefer products, such as flour, crackers, pet food, and bird seed, over whole grains. Notably, these insects often encounter and feed on products contaminated by molds and thus, they may have physiological mechanisms in place to detoxify mycotoxins (15-DON and DAS) that may be encountered during feeding. It is thought that insects have coevolved with mycotoxin producing fungi to protect against predation and allowing for resistance (Niu, 2010).

http://animalia-life.club/sweet-animals-saturday-1.html

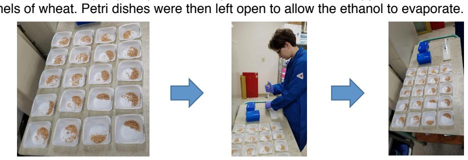


https://www.researchgate.net/figure/Different-life-stages-of-red-flour-beetle-Tribolium-castaneum_fig2_309145658

Methods and Experimental Design

Both the DAS and 15-ADON mycotoxins were purified and prepared using dilutions for the bioassays. Both were prepared using serial dilutions ranging from 1 to 1,000 parts per million (PPM) using ethanol. Controls were also prepared with 100% ethanol for both mycotoxins for reference. The solutions were vortexed until well mixed and all mycotoxin was dissolved.

After preparing the, solutions they were each applied to four replicate petri dishes for each concentration containing 2.0 grams of cracked wheat (n=4). 50 µL of the respective solutions were applied and mixed to coat the surface area of all the kernels of wheat. Petri dishes were then left open to allow the ethanol to evaporate.

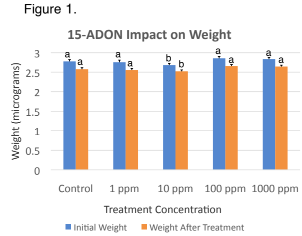


10 adult red flour beetles, sifted from their original rearing media, were placed in each petri dish after recording their initial individual weights. The petri dishes were then sealed using parafilm for protection and placed in an incubator at 24º C and 64% relative humidity. Mortality and presence of offspring were recorded each week and their weights were recorded biweekly (total assay length = 1 month). ANOVA was used to test for differences in mortality and progeny production between controls and individuals exposed to 15-ADON or DAS and a mixed linear model was used to test for differences in weight between the controls and the individuals exposed to the mycotoxins. Replicate dish and replicate beetle were treated as random effects and date and treatment were treated as fixed effects. Post hoc tests were performed using Tukey's HDS. Both analyses were performed using the R statistical package (version 3.3.1).

Results

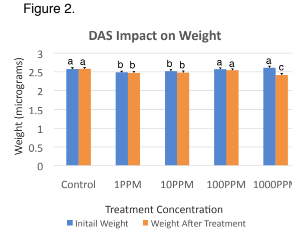
No mortality was observed in any of the treatments for either 15-ADON or DAS (data not shown). In addition, no major changes in weight were observed for 15-ADON (Figure 1). This result is expected because no major impacts on these life history parameters were observed in red flour beetles exposed to deoxynivalenol (DON), which is structurally similar to 15-ADON (see Valerie Nguyen's poster). Progeny production was also not impacted by the presence of 15-ADON (Figure 3). However, the DAS mycotoxins significantly impacted growth rates and progeny production at 1,000 PPM (Figures 2 and 4).

Figure 1.



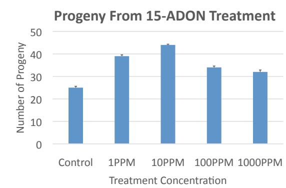
$\chi^2_{Date*Treatment}=0.03$; $p_{Date*Treatment}=0.85$.

Figure 2.



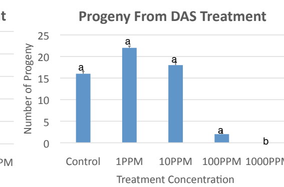
$\chi^2_{Date*Treatment}=9.45$; $p_{Date*Treatment}=0.05$.

Figure 3.



$F_{4,15}=1.02$; p=0.43

Figure 4.



$F_{4,15}=7.56$; p=0.00

Conclusions

The data collected from these experiments shows that the DAS mycotoxin has a significant impact on the *T.castaneum* weight and progeny production at high concentrations, while 15-ADON showed no significant effects. This suggests that the red flour beetles are unable to metabolize excessive amounts of the mycotoxin DAS at a quick enough rate to avoid the negative impacts on their health. This also shows *T. castaneum* can tolerate some mycotoxins while others may have negative impacts on this species and possibly other insects. This could be due to resistance to the mycotoxin, enzymes that degrade the mycotoxin, or the presence of symbiotic bacteria that produce enzymes that metabolize the toxin. Further research will need to be conducted to determine why the DAS mycotoxin had more impacts on red flour beetle life history health than the 15-ADON.

Future Directions

If further research were to be conducted, many different directions could be taken. For example, testing the response of red flour beetles to DAS in higher concentrations would be helpful to determine if it would create larger impacts on life history and it would also allow us to determine the lethal dose (LD_{50}). In addition, both mycotoxins could also be tested on *T. castaneum* larvae to determine the susceptibility of this life stage. Due to the fact that larvae take in larger quantities of food compared to adults to satisfy their growth rates, it is possible that this life stage would ingest more mycotoxin compared to the adults and may show more susceptibility. It is also important to determine if other insects are susceptible to DAS like the red flour beetles. Many of these options could be prepared in a similar set up with minor changes such as adding larger concentrations of the mycotoxin or changing the subjects from adult red flour beetles to larvae or other common crop pests.

Other options for future studies could include investigating why the insects are susceptible to DAS, but not DON or its derivatives by looking at the frass before and after the treatments to help determine how the mycotoxins may be metabolized in their systems.

References

Brake, J., Hamilton, P., & Kittrell, R. (1999). Effects of the tricothecene mycotoxin diacetoxyscirpenol on fertility and hatchability of broiler breeders. Poultry Science, 78(12), 1690-4.

Glenn, A. (2007). Mycotoxigenic Fusarium species in animal feed. Animal Feed Science and Technology, 137(3), 213-240.

Guo, Z., Döll, K., Dastjerdi, R., & Karlovsky, P. (2014). Effect of Fungal Colonization of Wheat Grains with Fusarium spp. on Food Choice, Weight Gain and Mortality of Meal Beetle Larvae (Tenebrio molitor). PLoS One, 9(6), E100112.

Niu, G., & Berenbaum, May R. (2010). Toxicity of Mycotoxins to Insects and Underlying Molecular and Biochemical Mechanisms, ProQuest Dissertations and Theses.

Acknowledgements

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