

Aflatoxin in *Aspergillus flavus* : Can peanuts give you Cancer?



Andrea Green

Andrea McLean

Paul Johnson

Rob Harper



Abstract

- *Aspergillus flavus*, a mold found in peanuts and other crops produces a carcinogenic metabolite called Aflatoxin. Aflatoxin poses a serious threat to human and animal health, as well as to agricultural economics. Many studies have been conducted on aflatoxin and its effects, and in this project, those studies have been compiled. Also, information provided in scientific journals allowed a biological circuit which shows the synthesis of aflatoxin in *Aspergillus flavus* to be created. With this information, we can discover which genes are most influential in the production of aflatoxin, and ways in which the system could be perturbed so that aflatoxin production is stopped. A computer program which simulates this circuit was analyzed and perturbed. The analysis shows that if the aflR gene is knocked out aflatoxin is still produced. But, if Acetyl Coa is taken out, the circuit is not turned on. Also, if the X gene is turned off, no aflatoxin is produced.



Introduction

- Aflatoxin is a toxic carcinogen produced in several species of *Aspergillus* molds that can grow in corn, cotton, tree nuts, and more importantly in peanuts. Due to its toxicity, if any aflatoxin is detected in a sample of peanuts, the entire load cannot be distributed to the general public. Those particular samples that do contain some amount of aflatoxin are then used as feed for livestock. Moreover, this creates an even greater problem for the farmers. Not only is it costly due to the lack in profit from peanut sales, but it is also harmful and many times deadly for the livestock. In the past, efforts to control the spread of aflatoxin have focused on post-harvest elimination of the toxin. More recently, attention has been directed towards pre-harvest solutions which include several genetic manipulation procedures. In *Aspergillus flavus*, the genes for biosynthesis are found in a cluster, regulated by a positive regulator, aflR, and a newly discovered negative transcriptional regulator. As a whole, a biological circuit was created by the group for aflatoxin B1 biosynthesis.



Methods

- There are several different theories as to the exact nature of aflatoxin and how it can be deleted or contained. Information on each of these ideas has been compiled from many different scientific journals, books, research studies, and other various publications. From this data, a biological circuit was created. A computer program was used to simulate the actual biosynthesis of aflatoxin. It was perturbed in different ways which could be possible solutions to the aflatoxin problem.
- The compiling of this information then provided a better understanding of the impact of aflatoxin in the areas of health and economics.



Dangers of Aflatoxin Contamination

■ Contamination in Livestock

- The allowable level of aflatoxin in cattle feed is 300 parts per billion (ppb).
- For other livestock the allowable level is between 100 and 200 ppb.
- Effects of the toxin include reduced growth rate, hemorrhagic enteritis, suppression of natural immunity to infection, decreased production of meat, milk, and eggs. It can also cause death in some cases.

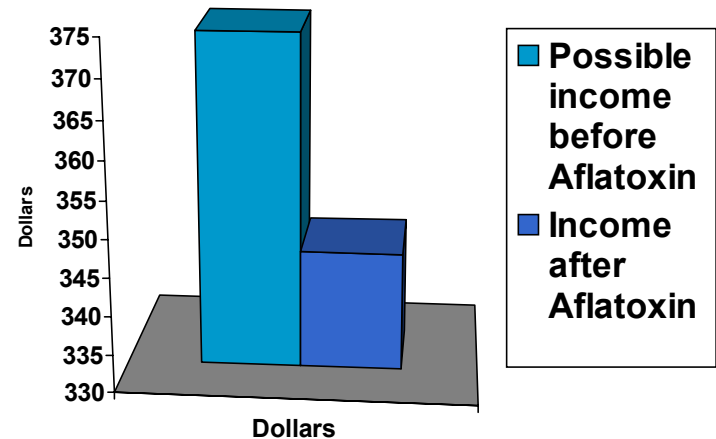
■ Contamination in Humans

- Inhaling the this toxin has been presumed to be a health hazard to humans. Such inhalation appears to be directly related to a condition known as “Farmer’s lung.” The known symptoms are irritation, fever, wheezing, breathlessness, cough, and ulcers.
- Aflatoxin has also been identified as a cause of liver cancer. **Evidence shows that it is the leading cause of liver cancer in Africa and China.**

Economic Impacts In Georgia

- Georgia farmers could possibly make \$373.88 if Aflatoxin did not infect their crops.
- In reality, Georgia farmers make \$345.77 dollars per acre per year.
- Aflatoxin contamination causes an average loss of \$28.06 per acre per year.
- Farmers face a 7.5% loss in production due to Aflatoxin contamination.

Economic Impact of Aflatoxin





Social Costs of Aflatoxin Contamination

- Less nutritious food accompanied by higher costs
- Exposure to serious health problems including cancer in humans
- Time and money must be spent researching and educating the public
- Increased regulations of trade with foreign countries
- Loss of productive livestock due to death and milk contamination
- Losses in the peanut market



Environmental Conditions that Promote the Growth of Aflatoxin

- Aflatoxin requires a certain environment in which it grows the best. Such an environment takes place when temperatures are between 80° and 100° F. Another factor that can contribute to its growth are periods of drought, which cause a grain moisture between 17%-20%. However, when grain moisture is above 30%-50% aflatoxin will not grow.
- The growth of *Aspergillus flavus* is promoted when damaged has been done to the peanut shell. Damage can be done due to insects, birds, mites, hail, early frost, heat, and drought stress.



Current Detection and Control Methods

■ Detection

- One test that is done to detect aflatoxin is the black light test. This black light test allows for the visual inspection of the peanut sample.
- Rapid screening procedures are also performed which include fluorometric iodine rapid screening and minicolumn tests.
- Other detection methods include thin-layer chromatography, gas-liquid chromatography and high-pressure liquid chromatography.

■ Control

- The main control process of aflatoxin is known as ammoniation. Ammoniation is a process where the sample that is contaminated with the aflatoxin is treated with ammonia which allows for the detoxification of the sample. Thus, most but not all of the aflatoxin is removed.
- Strains of *Aspergillus flavus* which do not produce aflatoxin are introduced in the field to provide competition for toxin producing strains. This “competitive exclusion” is done so that the amount of toxic strains are minimized in the field.

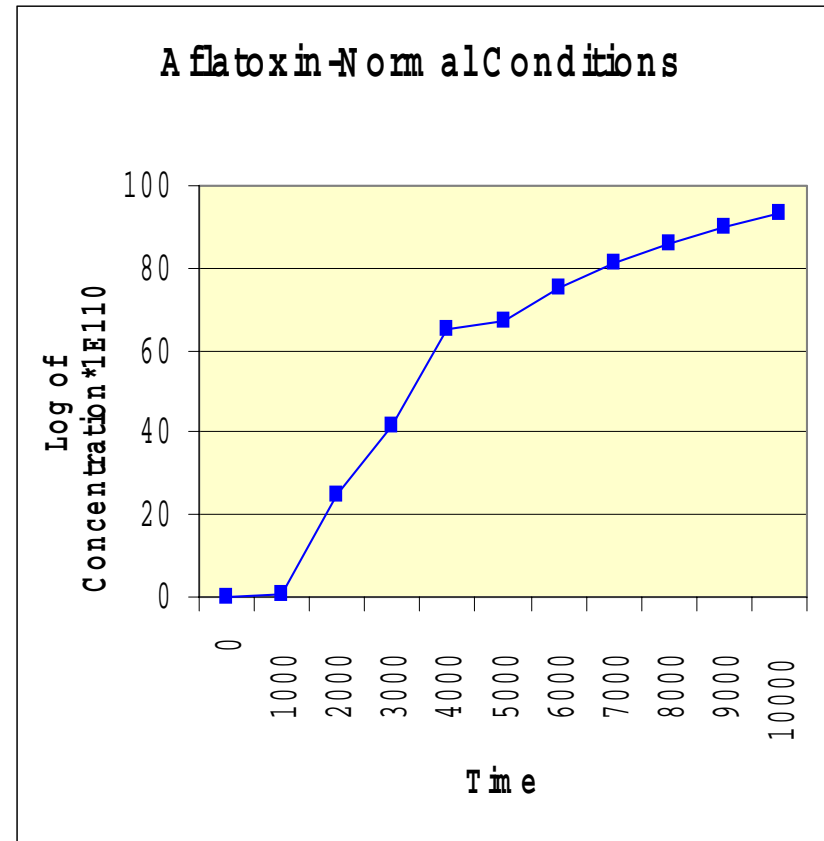


Circuit Outputs-Introduction

- The computer simulation of aflatoxin biosynthesis was done four times.
- The first simulation was the circuit under normal conditions: Acetyl Coa present, all genes present.
- In the second simulation, aflR1 gene was disabled, to analyze this effect.
- The third simulation tested the effects of removing Acetyl Coa from the environment where aflatoxin is produced.
- Finally, a fourth simulation removed the X gene, which creates an enzyme which changes O-methyl sterigmatocystin into Aflatoxin B1.
- Note: In the first two simulations, the output concentrations of the simulation were too small for the computer to recognize. In order to graph these concentrations, the original outputs were multiplied by $1E110$, and the log of these numbers were taken to reduce the range.

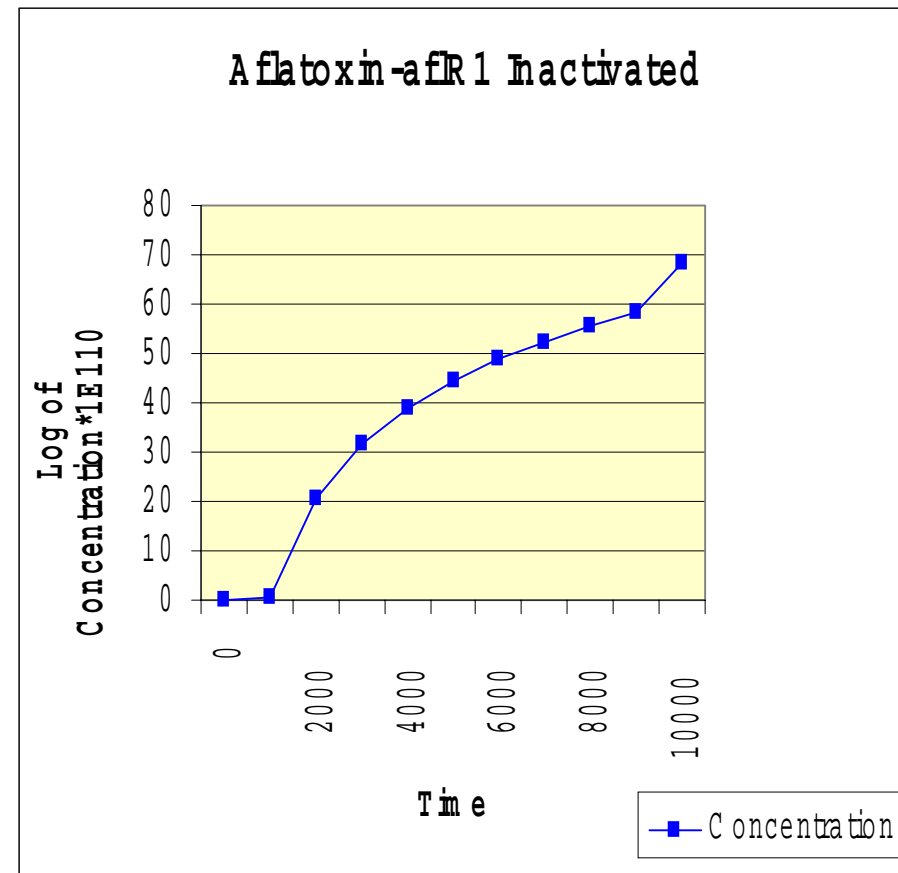
Circuit Output 1: Aflatoxin Production-Normal Conditions

- This graph shows the rise in concentration of aflatoxin over time.
- In this run, the concentration of Acetyl Coa was set to 1, and all genes were present.



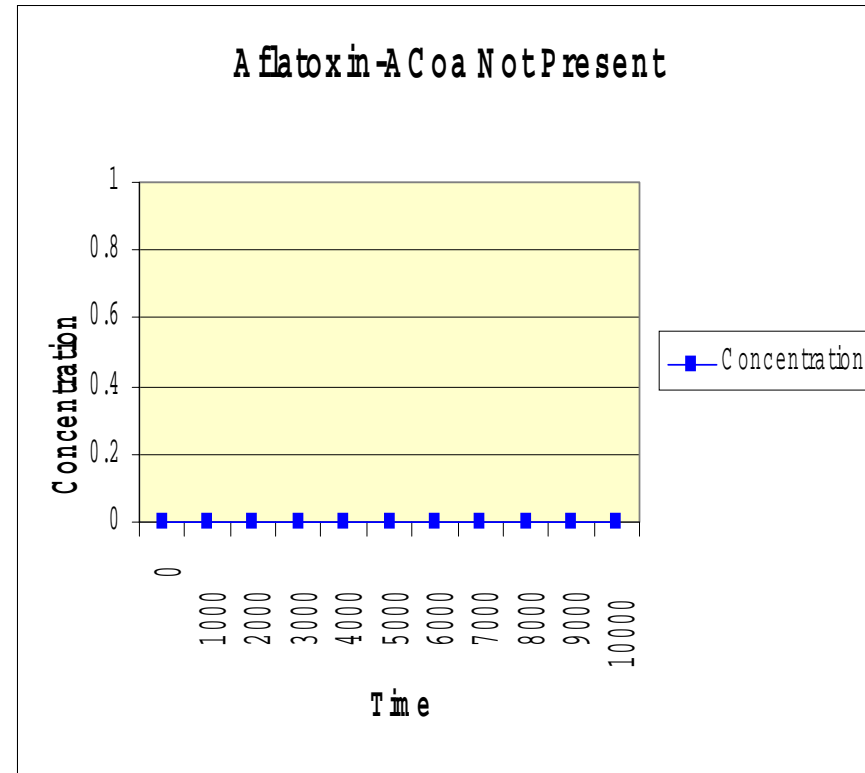
Circuit Output 2: Aflatoxin Production-aflR Gene Inactivated

- The graph shows the effects of inactivating the aflR regulatory gene.
- Without active aflR, the concentration of aflatoxin is decreased, but still produced.



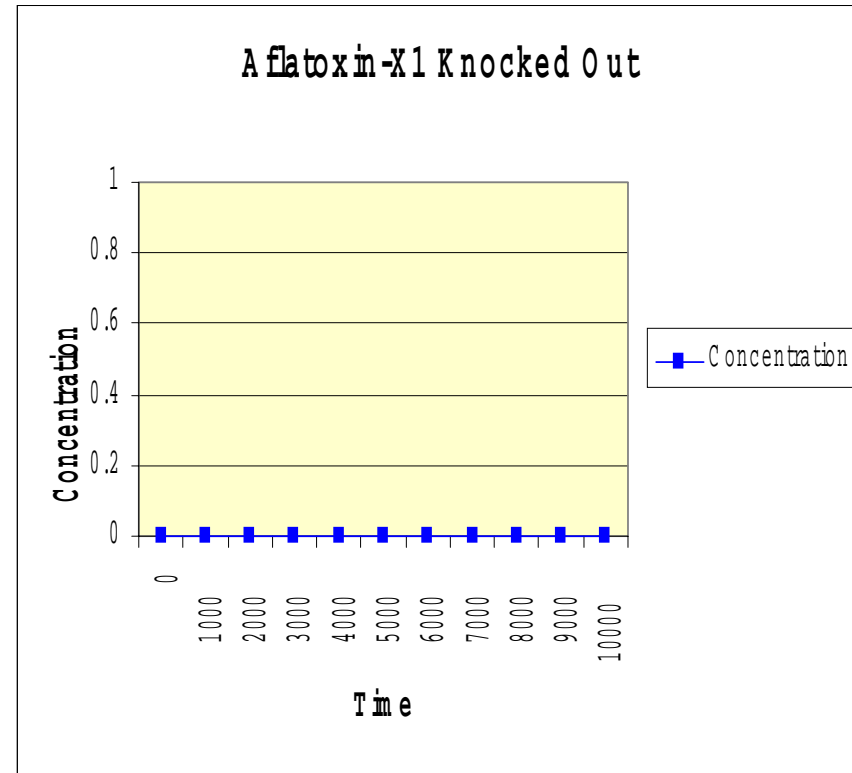
Circuit Output 3: Aflatoxin Production-Acetyl Coa Removed

- This graph shows the possible outcome of biosynthesis if there is no Acetyl Coa in the cell.
- In the absence of Acetyl Coa, aflatoxin will not be produced.



Circuit Output 4: Aflatoxin Production-Gene X Removed

- This graph shows the effect of inactivating or removing the X gene. The X gene is responsible for producing the an enzyme necessary to produce aflatoxin.
- If the X gene is removed, no aflatoxin will be produced.





Circuit Output: Conclusions

- The results of the simulations show that only some elements of the circuit are necessary to the production of aflatoxin.
- While knocking out the aflR gene decreases aflatoxin production, the decrease is not significant enough to rule out that peanuts would be safe.
peanuts would be safe.
while terminating the production of aflatoxin, is not a feasible solution.
. The cell must have Acetyl Coa for respiration. In this manner, *ergillus flavus* would be killed, and the risks of altering the vironment would be to great.
nt would be to great.
and then remove the X gene. Without the X gene, aflatoxin would not uld not be produced. Furthermore, all of the other components in the athway would still be active just in case they serve a purpose in the



Conclusions

- Because aflatoxin poses such a global threat not only to our agricultural economy but to our health as well, it is important that further studies be done in order to solve the problem. Current control and prevention methods can only slightly decrease the presence of aflatoxin contamination. Certain studies done on aflatoxin show that drought and heat provide a suitable environment for the growth of aflatoxin. Moreover, the biological circuit for the biosynthesis of aflatoxin has been partially created and is continually updated. Therefore, a possible solution to the problem could be found in the genetic manipulation of the biological circuit. Several possible alterations could be done, but we have found that the most effective and feasible manipulation would be to knock out the X gene, the last gene in the cluster. In this manner, only aflatoxin is removed.



References and Acknowledgements

■ References

- The Texas A&M Aflatoxin Resource. <<http://plantpathology.tamu.edu/aflatoxin/regulatory.htm>>
- ARS National Programs. “Aflatoxin Control Through Targeting Mechanisms Governing Aflatoxin Biosynthesized Crops.” “Costs of Aflatoxin to the Farmer, Buying Point, and Sheller Segments of the Southeast United States Peanut Industry.” “No More Peanut Roulette.” <<http://www.nps.ars.usda.gov>>
- Cheatle, Thomas, Steven Gagliardi, and Craig Llewellyn, et al. Journal of Food Protection. “The Occurrence of Aflatoxin in Peanut Butter from 1982-1989.” volume 54. Pgs. 627-631. August 1991.
- “Approaches to Elimination of Aflatoxin Contamination in Peanuts.” <<http://www.isnar.org/iita/publib/phnews2/ph-st1.htm>>
- “Georgia Fact Sheet” <<http://www.ers.usda.gov/StateFacts/GA.htm>>

■ Acknowledgements

- We would like to thank Peter Horanyi for his time and effort in helping to find the information and collect the necessary research that was used to complete the project.
- We would like to thank Dr. Jonathon Arnold for his help in the construction of the biological circuit.
- We would like to thank all of the scientists that have worked in this particular area of study and who have collected the data that we used in our project.