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Thorny Pre-stick-ament

Introduction

Many of us have been walking through a field and looked at a tree and go, 'Wow, ouch!" This can come from just looking at a honey locust tree or by unfortunately stepping on a honey locust tree thorn. Thorny Honey Locust trees are a nuisance to farmers due to puncture issues or their ability to spread their thorns out around a certain area. For those who have had to work with these trees, it is very possible that a puncture wound was inflicted. Typical symptoms of these punctures include intense pain around puncture area, swelling, pain lasting more than a day, headache, dizziness, and possible secondary infections. Now what causes this? There is a debate amongst researchers as to the causative agent. It could be a chemical from the thorn, which would help explain the swelling, pain, and other effects. It could also be from a certain bacterium that grows in your body after the puncture. This also would explain the swelling and pain. Lastly, some say that it's your natural body defenses to the puncture wound itself. However, that doesn't necessarily explain the pain from the body part afflicted by the thorn.

Chemistry

To answer these questions, I preformed the chemical experiment for my organic research project. I managed to get some good data, but I did not get as far as I wanted.

Methodology

1. Acquire thorns and cut them up into small pieces. The small pieces will make the extraction easier and it will make the thorns easier to work with.

2. Set up a Soxhlet Extractor to preform experiment.

3. Place 10 grams of thorns in filter for the Soxhlet extractor.

4. Add 100 ml of Dichloromethane to the flask at the bottom of the Soxhlet extractor, place filter of thorns in extractor. Heat to a steady boil with the dichloromethane filling the filter of thorns. Let run for one-two days. Add more dichloromethane as needed.

5. Run resulting solution with Flash Chromatography and Gas Chromatography to determine structure of chemical.

Research suggests that honey locust thorns have phytotoxins, toxins found naturally in plants, that could be responsible for the pain and swelling.

I used a Soxhlet extractor and compared those results of extraction to those done by traditional extraction methods and found the Soxhlet to be a better method of extraction for this experiment. I found my results to be; for every 10 grams of thorns, I extracted .3g of chemicals from the thorns. The Flash Chromatography instrument yielded three results from the test. The Gas Chromatography from each test gave my molecular weights of 446, 447, and 443. My results have lead me to the conclusion that there are lipids, steroids, and possible phytotoxins in the thorns. (See Figure 1)

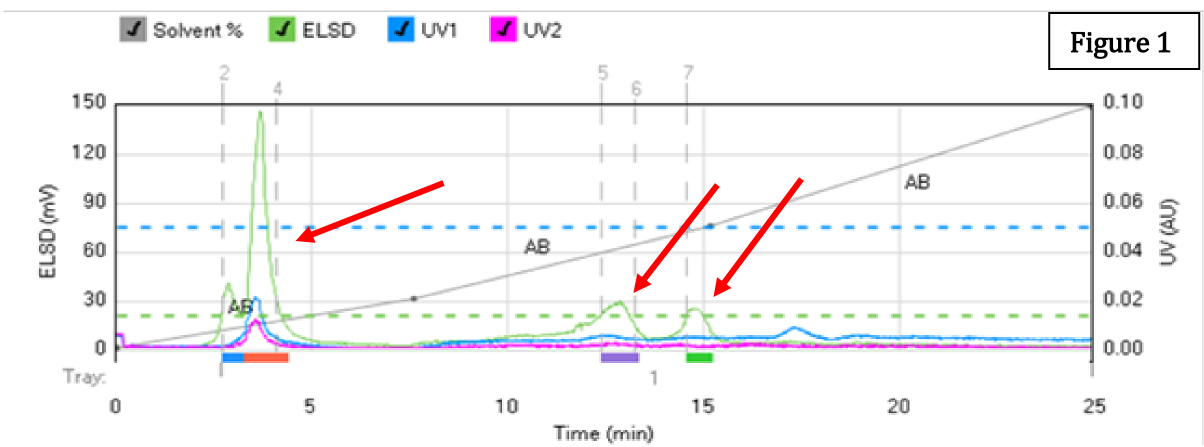


Figure 1 represents the data collected from the Flash Chromatography machine. Each one of the green peaks represent the three results discussed, the lipids, steroids, and phytotoxins.

In other terms, I took my thorns and took the chemicals out of them and ran tests on the chemicals inside the thorns to figure out what it is made of. It is typical to find lipids, steroids, and phytotoxins in nature. What I was happy to find is all the phytotoxins, or possible phytotoxins that could be present.

The major phytotoxin I found, that could be responsible, had a molecular weight of 447. A major component for this group is Androst-5-ene-17-carbonitrile, 4-acetoxy-17-hydroxyl-cyano-17-hydroxyandrost-5-en-4-yl acetate. (see Figure 2) The compound contains a Nitrogen group which was expected to be found. The cyano group is the one of major concern. Cyano is one of the major chemical groups in cyanide, which is very poisonous for humans. So, what does all this data mean?

Figure 2

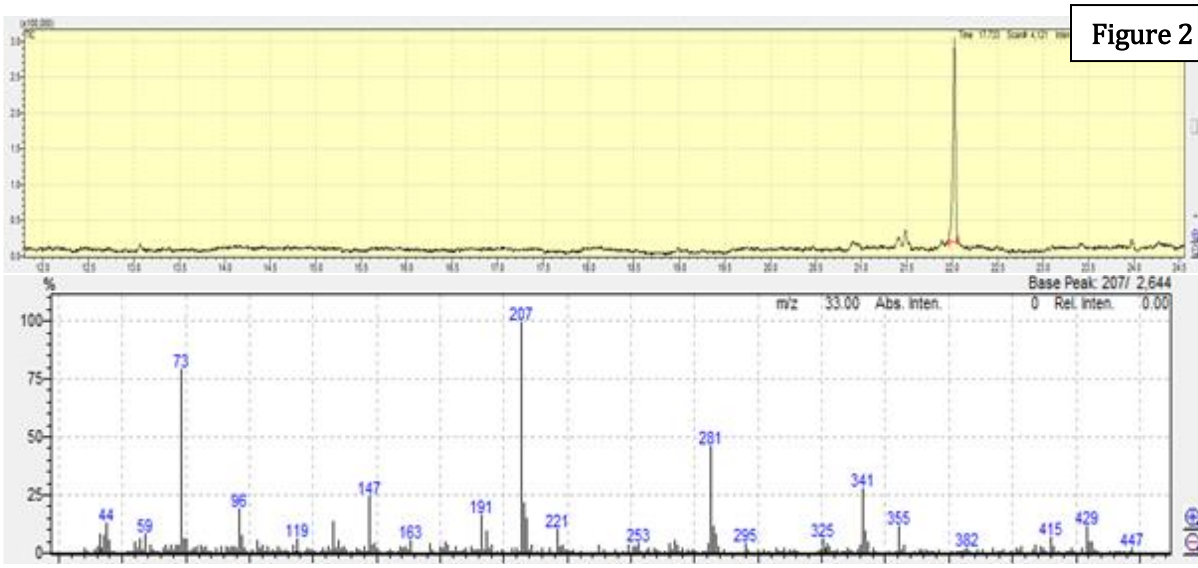


Figure 2 is the result of the phytotoxin group after being run by a gas chromatography machine. The big peak in the top picture is the chemical and the peaks on the bottom picture represent the composition of the chemical group.

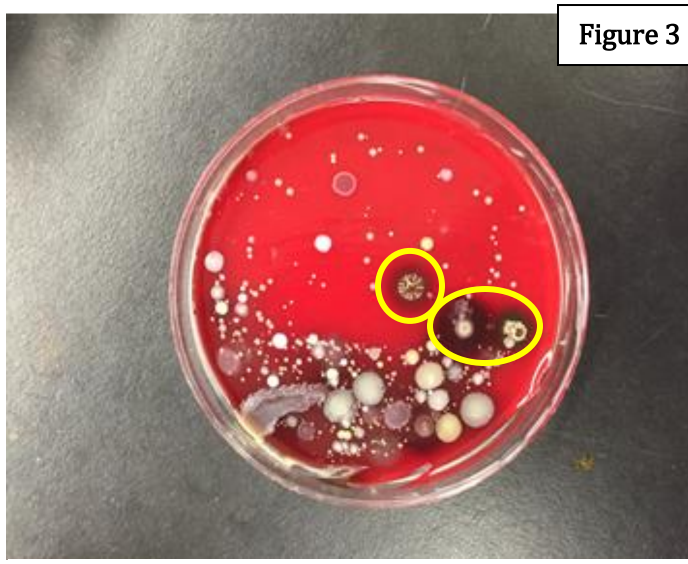
Call to Action

Well with a little more research, we could see if the cyano group is responsible for the pain and swelling. This information could help us find a way to combat these effects and provide relief for those who were punctured by these thorns. Another thought to consider is that when you observe honey locust trees in nature, typically you will not see large animals around them. Which brings to question the idea of pheromones, from these chemicals, in the trees that also deter animals. While small animals will use those trees for protection, most tend to stay away from them. If this is the case, is it the phytotoxins that emit these pheromones to deter animals along with the thorns? If so, we can then synthetically recreate these pheromones to use as an organic pest deterrent.

Bacteria

The other part of this experiment is to see if it is a bacterial issue. I ran another experiment to see that kinds of bacterial growth we would expect to see. (See Figure 3) I expected to see bacterial growth commonly exposed to environmental conditions, but I was specifically testing for bacteria that grew in anaerobic as well as aerobic environments. I also used a Blood Agarose gel plate to test hemolytic properties. Hemolytic tests are good test to determine if a bacterium can grow in blood. If it grows on the plate, then there is a good chance in can grow on, or in, human blood. I was not able to distinguish all the different kinds of bacteria and figure out what each one was; however, I did have many different species growing on the gel plates with a few having hemolytic properties.

Figure 3



This Blood Agarose Gel plate shows growth in 37 degrees Celsius in an oxygen environment. The areas in the yellow circles did show hemolytic properties that could indicate that it can grow in blood.

I also ran an experiment, using the same bacteria, to determine if they could grow in an environment without oxygen. This experiment is important because for bacteria to grow in our bodies, the bacteria must be able to grow without oxygen. *Figure 4* shows the results from the oxygen deprived environment. I had five colonies that formed but only two different species of bacteria that were hemolytic.

I took samples from both species and looked at them under a microscope to determine the morphology, the shape, of the two-bacterial species. The alpha hemolytic colony, the one with the yellow rings in *Figure 4*, was a Streptobacillus bacteria. I ran out of time to determine the exact name of the bacteria, but I know the bacteria is gram positive and appears rod-like. I am calling it a Streptobacillus bacterium because of the way it grows in chains versus clumps. (See Figure 5) The beta hemolytic bacterial colony, the one that has the large black arrows from Figure 4, looked almost like a Diplobacillus that is Staph orientated. This means both bacterium have thick cell walls, but the alpha hemolytic group grows in clumps or clusters. This is also a gram-positive bacterium that appears rod-like. (See Figure 6)

Figure 4

The yellow circles represent an alpha hemolytic bacterial colony. This means they did not grow much in the gel plate, but there was growth. The two black arrows are pointing to two beta hemolytic colonies showing great growth. This gel plate was grown at 37 degrees Celsius.

Figure 5

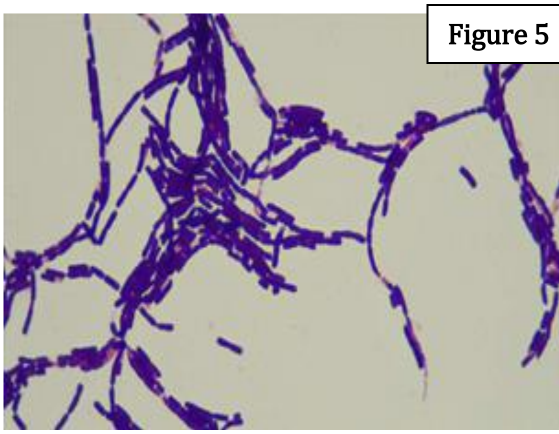


Figure 6

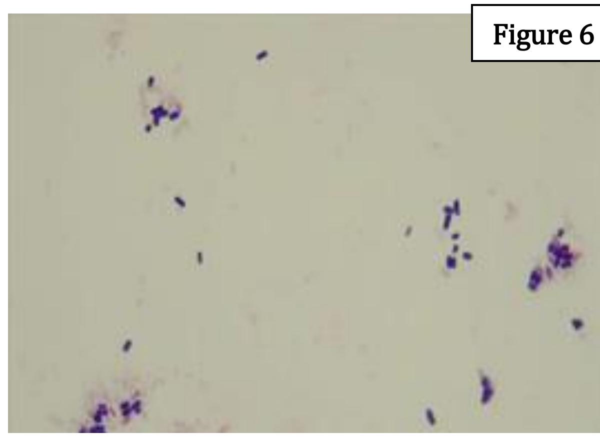
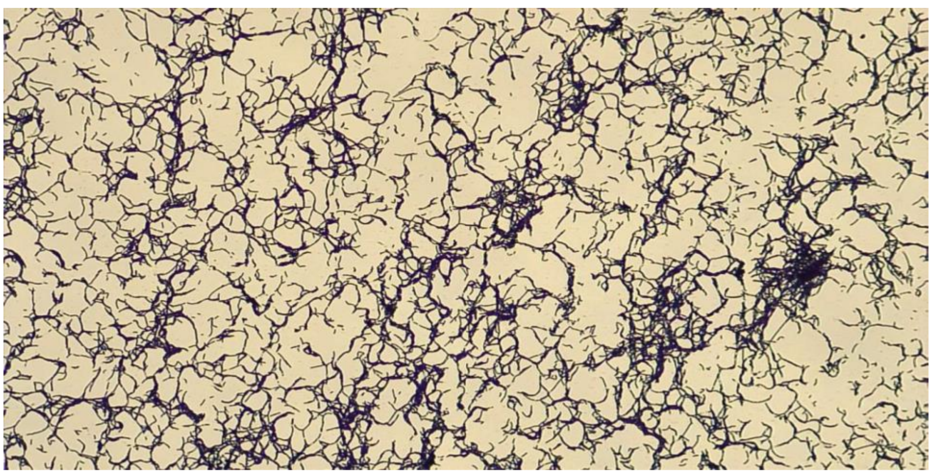


Figure 5 is the sample of the Streptobacillus bacterium from the alpha hemolytic colony. Figure 6 is the sample from the beta hemolytic group, with an appearance like those of Diplobacilli.

Call to Action

Now, what can we do with this information? If bacteria is the cause for the pain and swelling of the puncture area, can we make an antibiotic that acts as a preventative against this bacteria? Granted more research is needed to distinguish specifically what bacteria could be the causative agent, but we do know that there is a bacterium that could be the causative agent. We also know that it can grow in blood and that there are a few kinds that will. The next step will be to produce an antibiotic that counters the bacteria there now.



This is a picture of the alpha hemolytic group at 40x. This is an interesting bacterium that would be amusing to figure out.

References:

- All the pictures and research were either taken by the author or performed by the author in an unpublished research project. Many thanks to Dr. Jerry Easdon for guidance and many thanks to Dr. David Zimmerman for cultures to grow the bacteria.
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