The Motility of Carnivorous Plants

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Contents

Abstract

The purpose of the experiment is to determine the reason for movement in carnivorous plants. These organisms are able to capture their prey through a series of subtle attractions and traps, but their motion's occurrence is the basis of this project. Procedures such as electrophoresis, the Ouchterlony method, and paraffin embedding have

been the most useful in identifying a muscle-type protein system in these plants, if one is present. The reason we used these procedures is because each of them were useful in classifying certain muscle proteins. Electrophoresis sorts proteins by specific molecular weights, an Ouchterlony identifies proteins based on an antigen-antibody reaction, and paraffin embedding allows for microscopic study of the sample while remaining perfectly in tact.

Results for each of these processes were for the most part negative, but an alternate reason for movement has been discovered. By comparison with a water-expanding fungus, it is apparent that the plants may move due to an interaction with water. When trigger hairs are activated, water pressure is relieved and the traps relax, thus capturing their prey. The cause for motility in these plants is not a muscle-type protein system, but a series of reflexes.

Introduction

Carnivorous plants are organisms that have adapted to their environment in such a way that allows them to capture and digest other living organisms, i.e. insects. Due to inadequate nutrition in their indigenous soils, they have evolved to accommodate their ecological niche. By digesting other organisms, they attain the proper nutrients that allow them to sustain life (for example, phosphorous and iron).

The species used in this experiment were the Dionaea Muscipula (Venus Flytrap), Sarracenia Purpurea (Pitcher Plant) and the Drosera Pygmaea (Cape Sundew).

There are five basic trapping mechanisms that have evolved in carnivorous plants.

These are:

- -Pitfall traps (pitcher plants), which trap prey in a rolled leaf that contains a pool of digestive enzymes and/or bacteria
- -Flypaper traps, which trap prey using a sticky mucilage
- -Snap traps, which trap prey with rapid leaf movements
- -Bladder traps, which suck in prey with a bladder that generates an internal vacuum
- - Lobster-pot traps, which use inward pointing hairs to force prey to move towards a digestive organ

All carnivorous plants are capable of devouring insects. The Venus Flytrap clamps its mouth shut, the Sarracenia engulfs the insect in a pool of water, thus drowning it, and the Drosera rolls its tentacles in and keeps the insect attached to its sappy molasses-like substance.

Last year we received three terrariums containing the three species of carnivorous plants (Dionaea Muscipula, Sarracenia Purpurea and Drosera). Each terrarium was filled with soil and peat moss. The peat moss gives the soil excess nutrients. We fed each of the plants a drosophila fruit fly once a week to prevent over feeding them. The

common misunderstanding about these plants is that they require insects to feast upon. However, they don't need anything beyond sunlight, water and nutrients. These carnivorous plants somewhat appreciate being fed. This causes for them to have an elevated alertness in their winter dormancies.

Dormancy for plants usually occurs at the end of autumn all the way through winter. They require six months of inactivity just as humans require half a day's sleep or so. Trying to break this dormancy could result in death. However, if precise conditions are met, not only will the plants be active during their dormancy, but they will be thriving and producing flowers that allow them to procreate. Without flowers the carnivorous plants are unable to reproduce. Therefore Venus Flytraps are only common in North Carolina, where these conditions are existent. The level of activity in a plant's life cycle is known as Circadian Rhythm.

Conditions that must be met include the following:

- Given sunlight
- Given distilled water every three days
- Fed once a week

- 60 wattage fluorescent light bulb eight inches overhead
- Left in a proper humidified container (the terrarium)

The ability to produce flowers meant that our plants were flourishing beyond their expected capacity. Also, we were fortunate enough to come across a peculiar fungus that our research supervisor brought into the classroom from a stroll on the beach one day. These mushrooms expand rapidly when sprayed with water. We believe that this is somehow involved with the motion of the carnivorous plants because the time in which its movement is complete is 4.6 seconds, while the closing of a Venus Flytrap's mouth is 4.2 seconds. Under a microscope the paraffin embedding of both the fungus and the plant share a similar morphology.

Initial Hypothesis

If carnivorous plants are able to move, then some sort of system involving muscular motion may be present. Our theory is that these organisms share a similar composition with human muscle systems. If

this is not the reason, then we believe a series of reflexes and water interactions cause their movement.

Materials

1. 1.5 millimeter microfuge tubes
2. Microfuge
3. Micro-pipette
4. Sonicator
5. Vortex
6. 12% Acrylamide Gel
7. Carnivorous Plants
8. Meat Samples for comparison
9. Carolina Brand: Protein Extraction Buffer
10. Protein Extraction Buffer for plants (Solution I)
11. Protein Extraction Buffer for plants (Solution II)
12. Ice
13. Paraffin
14. Xylene
15. Histological Cassette
16. Boiling Water Bath
17. Specimen Vial
18. 10% Formulin/Formaldehyde -Tweezers
19. Plastic Containers
20. 70% Alcohol
21. 80% Alcohol
22. 95% Alcohol
23. 100% Alcohol
24. Small Aluminum Pan
25. Knife
26. Slides
27. 95% Ethanol
28. Slide Holder

29. 500 mL Beaker
30. #1 Filter Paper
31. Thymerisol Crystal
32. Humidified Chamber
33. Vacuum Pump
34. Blue Transfer Pipette Tip
35. Anti-calmodulin

Procedures

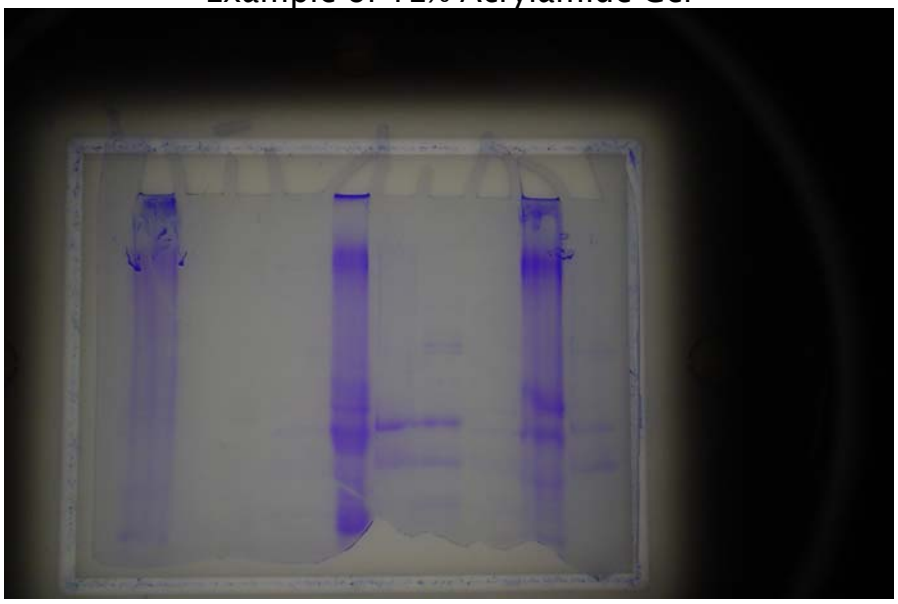
Electrophoresis:

Electrophoresis is a procedure that identifies proteins according to molecular weight. It's similar to filtering out particles of sand in a permeable pan: within the gels, the larger proteins are located on the top, while the smaller proteins are deposited more towards the bottom. Agarose gel is used on a grander scale, while acrylamide gel is a denser version that displays the smaller proteins in a more efficient manner. An

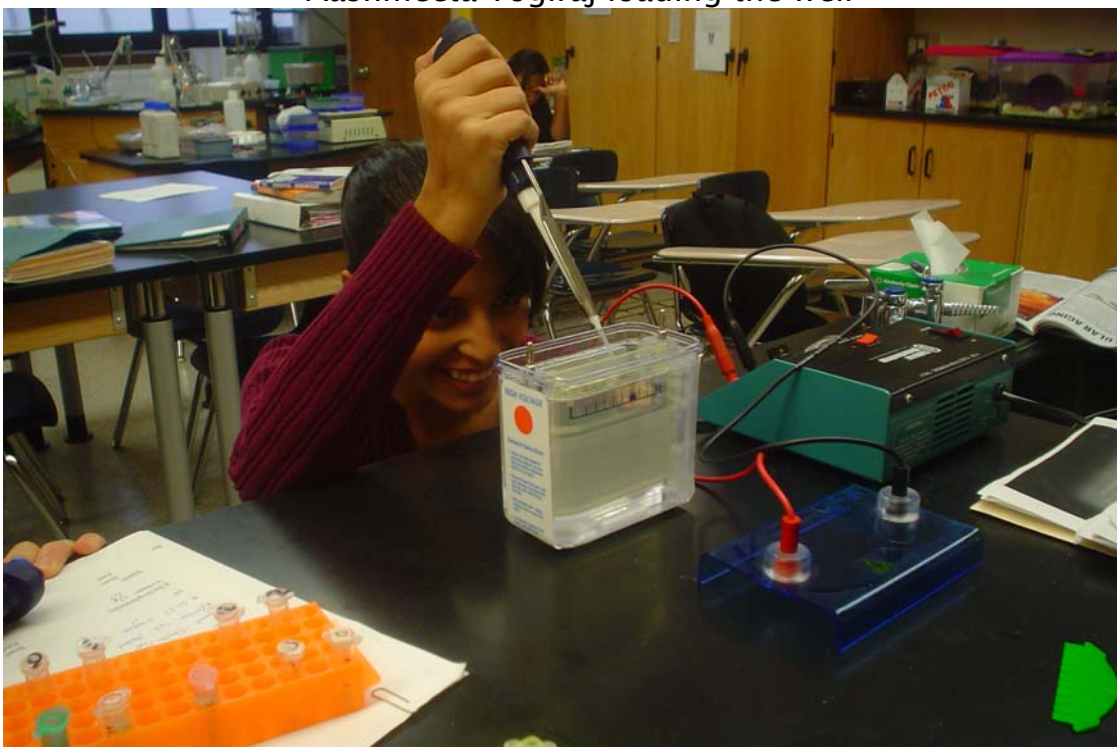
electrical current is generated by a power source, which creates the bands. Muscle proteins that allow humans to move include myosin (weighed at 200,000 kilodaltons) and actin (weighed at 45,000 kilodaltons).

Four percent agarose would be used to identify myosin. Twelve percent acrylamide should be used to identify actin. If carnivorous plants move due to the interaction of the actin-myosin complex and calcium, then under an electrophoresis gel, the extraction's molecular bands should be apparent.

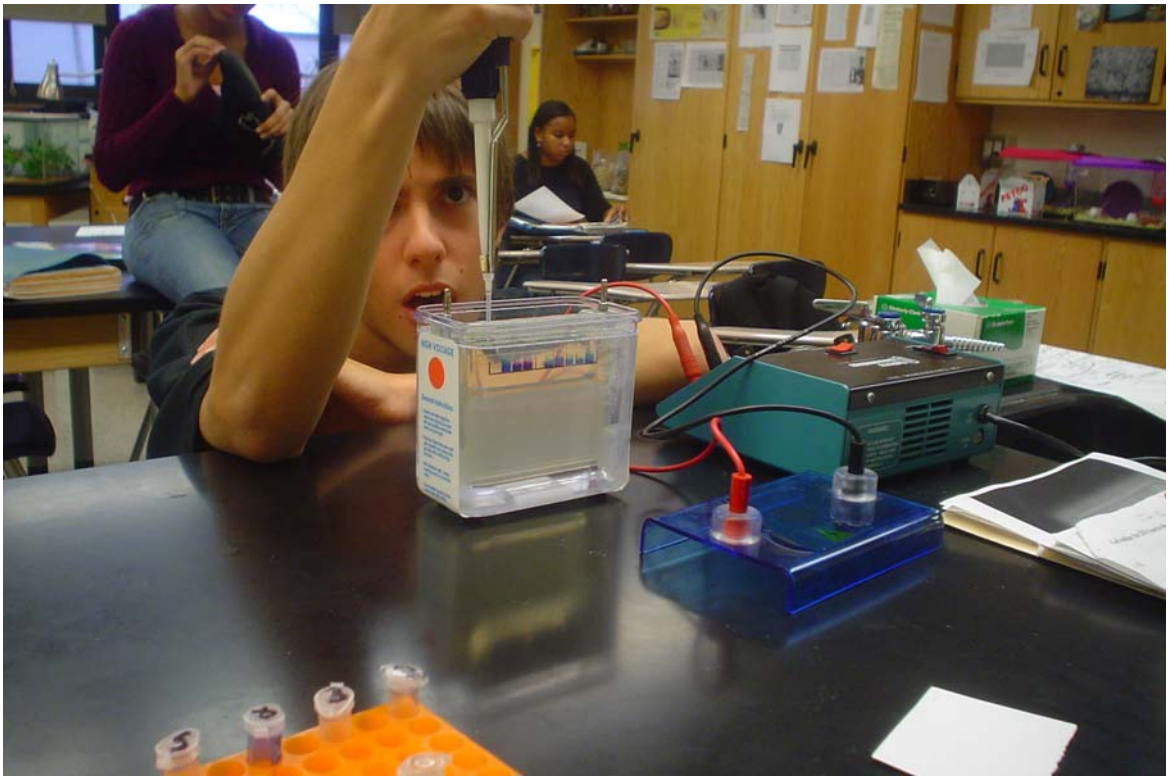
Example of 12% Acrylamide Gel



Aashmeeta Yogiraj loading the well



James Abbate loading the well



G-Bioscience Protein Extraction:

1. Allow solution I to come to room temperature.
2. Set up a boiling water bath.
3. Weigh out .06 g and place into a 15 mL centrifuge tube.
4. Add 300 uL of solution I and grind the tissue on ice until you get a homogonous suspension.
5. Add 25 uL of solution II.
6. Vortex for 30 seconds to mix completely.
7. Place tube into a boiling water bath for 30 seconds.
8. Repeat heating vortexing-process until solution is clear.
9. When extraction solution is clear, continue to heat the sample for 10 minutes.
10. Centrifuge for 5 minutes at 12,000 RPM at 4°C. Spin for 2.5 minutes.
11. Transfer all of supernatant into labeled 1.5 mL tubes and store at -20°C. Discard pellet.

Ouchterlony:

In an Ouchterlony, the protein extraction(s) should react with the set antibody. For example, if one is to test for calmodulin in a certain sample, they would load one of the wells in the agarose gel with anti-calmodulin. The presence of the protein would be displayed if a curving band of antigen-antibody interactions developed within the gel.

Subbing Slides:

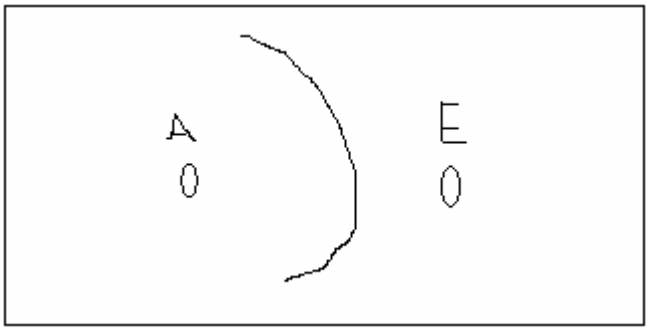
- Clean slides by soaking with dilute HNO3 for 10 minutes at room temperature.
- Wash slide for 20 minutes with running tap water.
- Rinse/soak slides with 95% ethanol.
- Wash slides three times with diluted H20.
- Paint on subbing solution.
- Place slides in slide holder overnight at 37°C to 40°C.
  Subbed slides should be stored at 4°C.
- Subbing solution (0.25% gelatin in diluted H20)
- Weigh out 0.63 g gelatin.
- Place into a 500 mL beaker containing 250 mL diluted H20.
- Warm gelatin into solution, then add one thymerisol crystal.
- Filter immediately using a funnel and #1 filter paper.
- Paint onto slides (see above).

The Ouchterlony Assay:

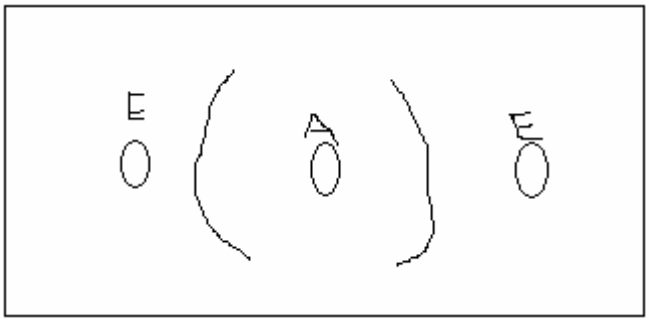
1. Transfer 3mL of molten 1.5% agarose using pipette and place on slide. Spread carefully.
2. Wait 10 minutes for it to gel in humidified chamber.
3. Place pipette in boiling water so it does not plug.

4. Poke two holes in gel after cutting 8 mm. off a blue transfer pipette tip.
5. Attach a vacuum pump to the end of the pipette tip to make the wells (holes).
6. Antibodies should be placed in one well, and the extract in the other. Antibodies diffuse through the agarose in all directions; as well the protein extracts (antigens). The antibody is anti-calmodulin.
7. It will diffuse over night at room temperature. A similar pattern would test positive for calmodulin.
*You can store slides at 4°C in the refrigerator overnight until ready for use.

Key:
Protein Extract: E
Antibody: A



← 1 Extract



← 2 Extracts

Enzyme-Linked Immunosorbent Assay:

This procedure is a super-sensitive method that offers identification of a protein with adequate precision.

Buffers for ELISA:

1. PBS (10mM NaPO4/0.15M NaCl pH 7.5)
2. Na2HPO4: (142 g/mol) (0.01 mol/L)= 1.42 g/L
3. NaCl: (58.44 g/mol) (0.15 mol/L)= 9 g/L

- Add to 800 mL dH2O
- Adjust to pH 7.5 with HCL or NaOH
- Bring up to 1 L
- PBS +0.1% T-20
- Add 100 uL T-20 (non-ionic detergent) to 100 mL PBS pH 7.5

REMEMBER: When using the peroxidase system, never use NaN3 as a preservative.

<u>ELISA Procedure</u>:

- Add 200 uL of extract diluted in 0.05M Carbonate Buffer pH 9.6.
- Cover with parafilm and incubate overnight @ 4°C.
- Wash 2x
  - Flick plate to remove extract than blot onto paper towels
  - Add 400 uL of PBS-Tween-20
  - Incubate at room temperature for 3 minutes
  - Flick plate blot onto paper towels
- Block: (yellow tube: normal rabbit serum) Save 3 mL for Ab dilution
  - Mix 1-4 drops of Rabbit Serum/10 mL PBS-Tween-20
  - Add 200-300 uL per well
  - Cover with parafilm, incubate 15 minutes at 37°C

- Wash 1x
- Primary Antibody:
  - Dilute antibody 1/500 to 1/1000 in PBS-Tween-20
  - Add 200 uL/well

    - Incubate overnight at 4°C, 2-4 hours @ room temperature, 1 hour @ 37°C
- Wash 3x
    - Biotinylated Anti-sheep IgG: Blue Bottle
    - 1 drop/10 mL normal serum or PBS-Tween-20
    - Add 200 uL/well
    - Incubate overnight at 4°C, 2-4 hours @ room temperature, 1 hour @ 37°C

- Wash 3x
- ABC (Orange Label)
    - Mix 1 drop A with 2 drops B
    - Let stand at room temperature for 30 minutes
    - Add 200 uL per well, incubate at room temperature 30 minutes

- Wash 3x
    - Mix Subtrate, add 200 uL per well: Stop with 50 uL 1N H_2SO_4, read at 450nm (yellow)

Paraffin Embedding:

Paraffin embedding was useful in observing cellular morphology. By preparing samples of the carnivorous plants in paraffin wax, we were able to analyze structures that have not been sheered. Paraffin serves as a preserving substance that allows cellular structure to stay in tact.

By comparison with a water-expanding fungus, it is apparent that the plants may move due to an interaction with water. When trigger hairs

are activated, water pressure is relieved and the traps relax, thus capturing their prey.

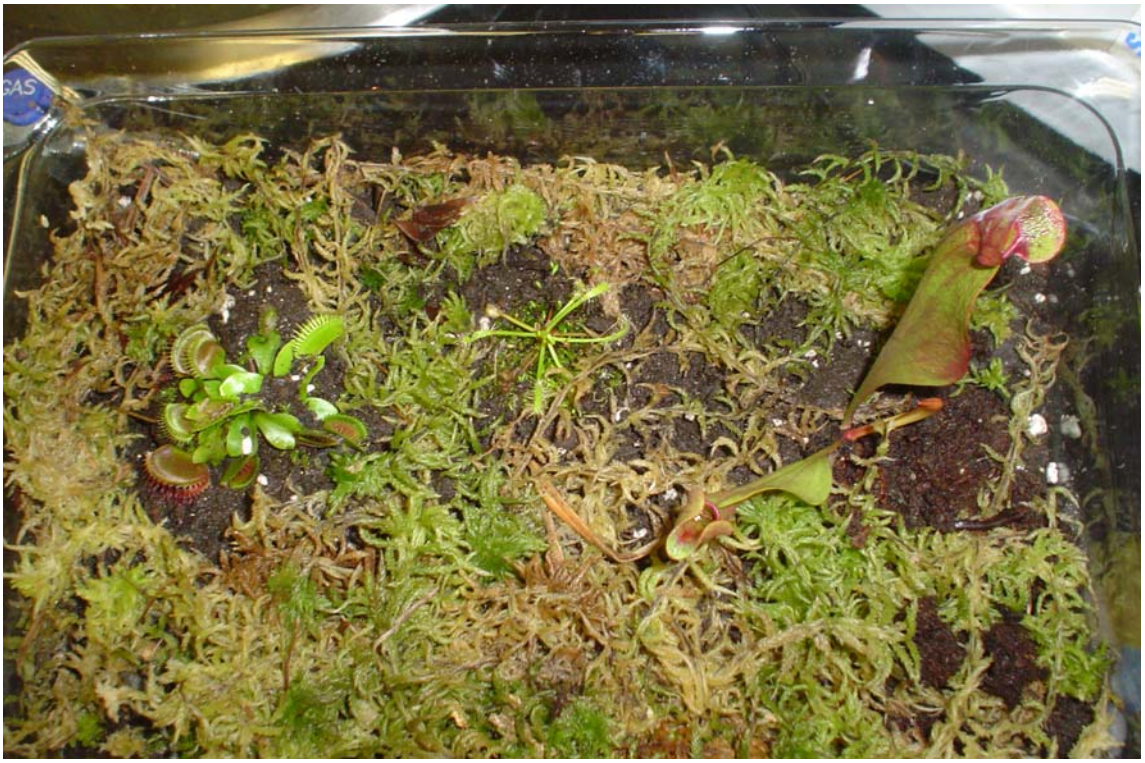
Procedure:

- Take sample and place in histological cassette (tissue holder).
- Place in 10 mL specimen vial, filled with 10% formaldehyde.
- Ex: Dionaea Muscipula (Venus Flytrap) and fungus should be placed in two separate labeled vials with the formulin/formaldehyde filling at least five times the amount of the sample's volume.
- Store at room temperature.
- The following day, pick up the cassette that holds the sample with tweezers and rinse it out. Leave in running tap water under the faucet overnight, and it will gradually rinse out the formaldehyde.
- On the third day, place sample(s) in a plastic container with 70% alcohol for 30 minutes.
- Place sample in a separate container with 80% alcohol for 30 minutes.
- Place sample in yet another container with 95% alcohol for 60 minutes.
- Place sample in a container with 100% alcohol overnight.
- On the fourth day of the experiment, place sample in Xylene for 60 minutes.
- Place sample in a container of a 1 to 1 Xylene-Paraffin mixture. Xylene is an organic solvent for paraffin. Leave sample in for 60 minutes.
- Place sample in a container that has 100% Paraffin at 60°C overnight. 60°C is paraffin's melting point. Only when it is a liquid can it do its job.
- On the fifth day, take the sample out of the histological cassette and place it in a small aluminum pan, with molten paraffin traces still on it. Wrap it up and place it in the refrigerator. The sample is now embedded in paraffin and samples can be cut with a knife

whenever necessary. The molecular structure is left in tact under this procedure, allowing for accurate measurements.

DatA

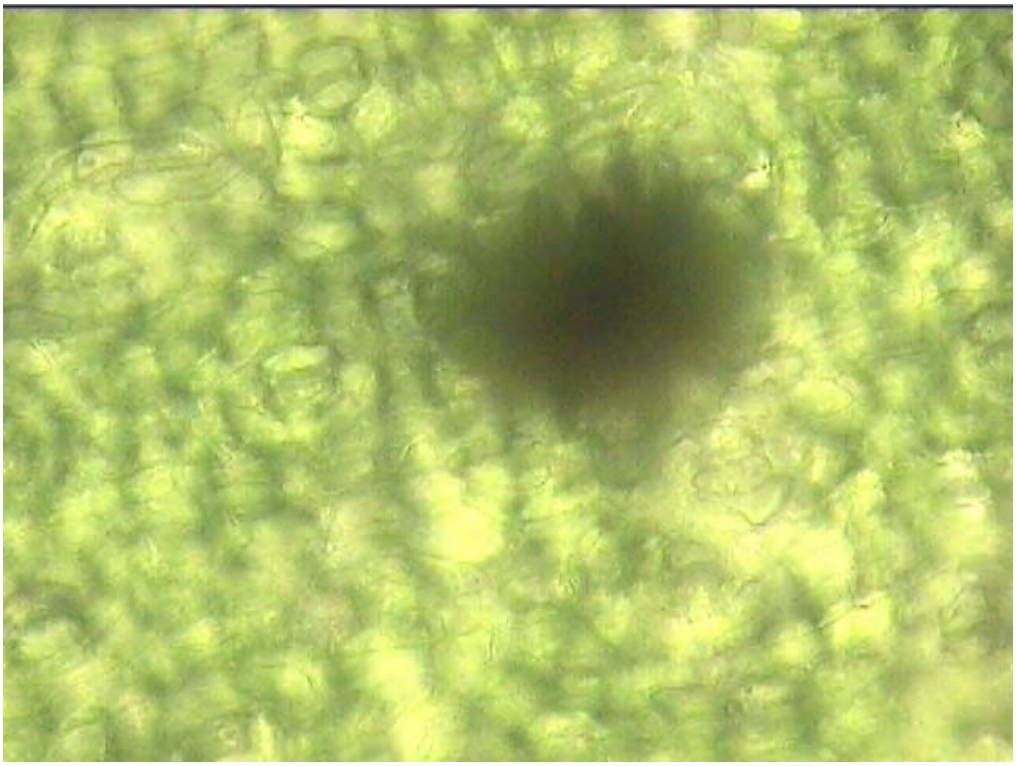
This is our terrarium when we first received it:



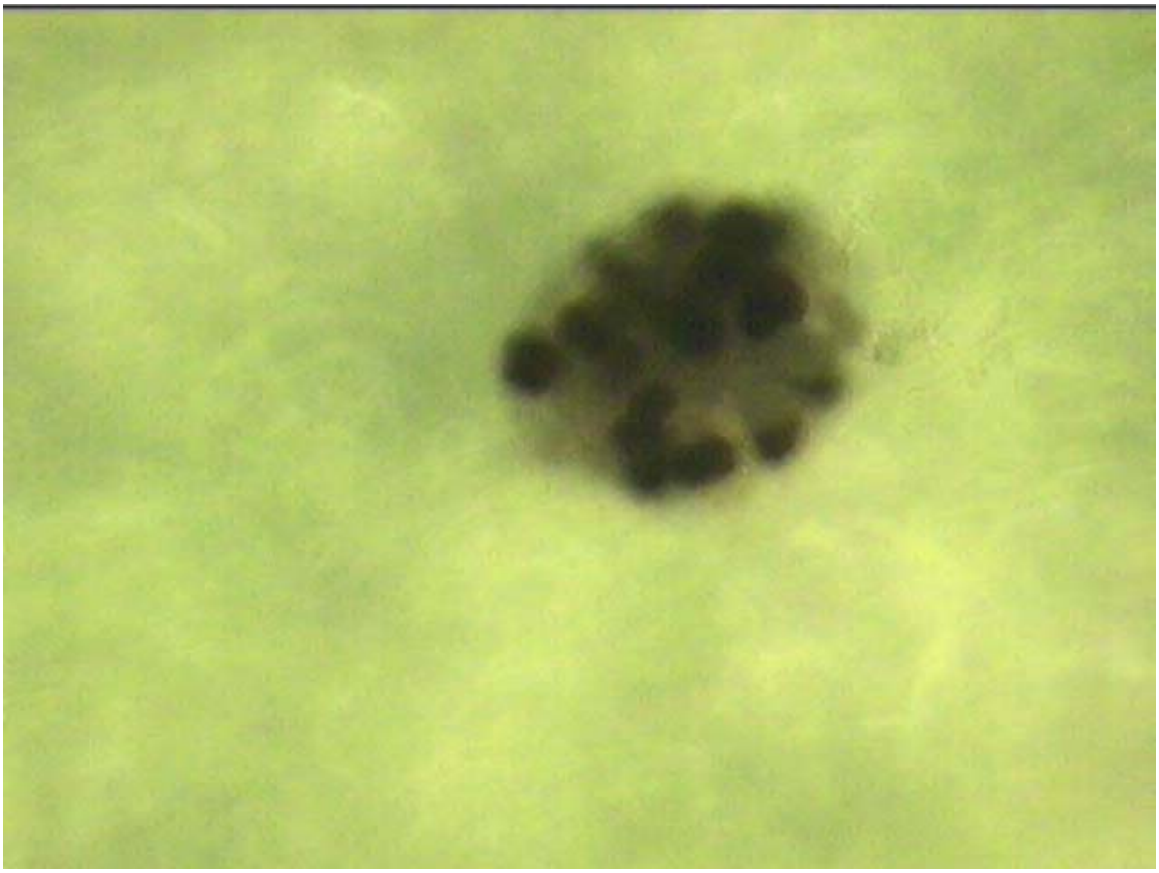
After three months, the plants flourished spectacularly (and they are still growing):

Digital Microscope Capture of a Control Plant

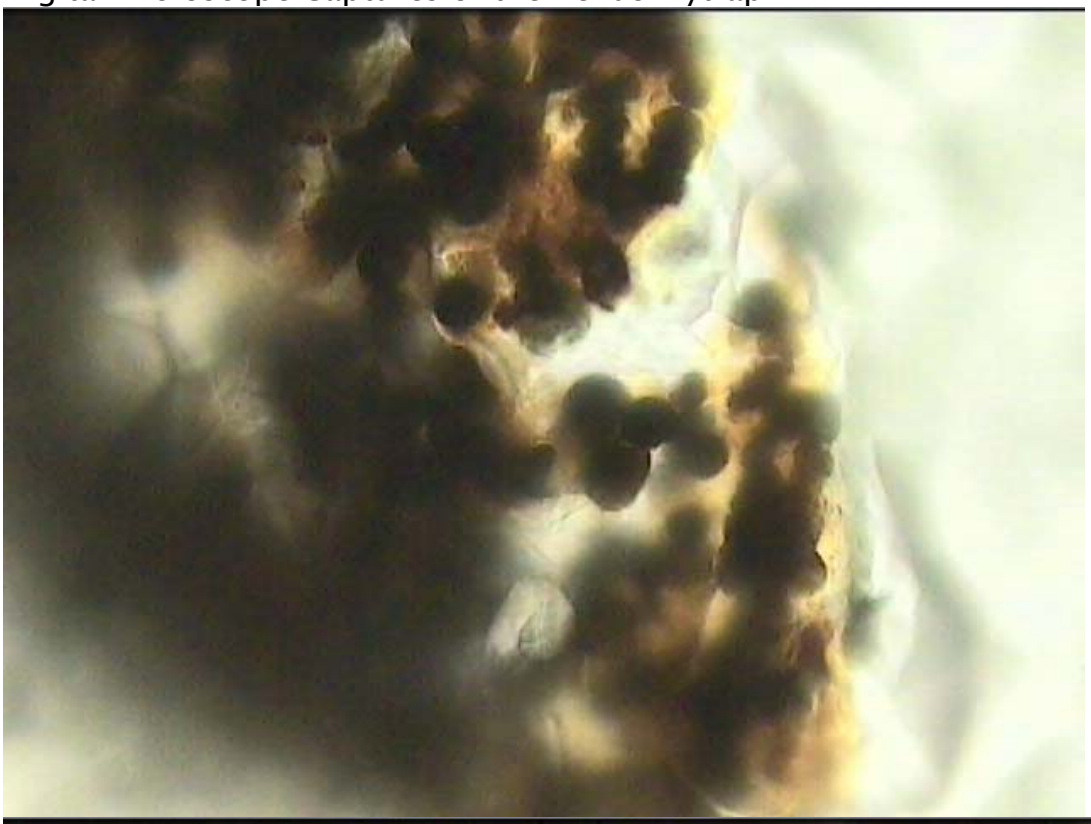
Digital Microscope Capture of a Control Plant's Stomata



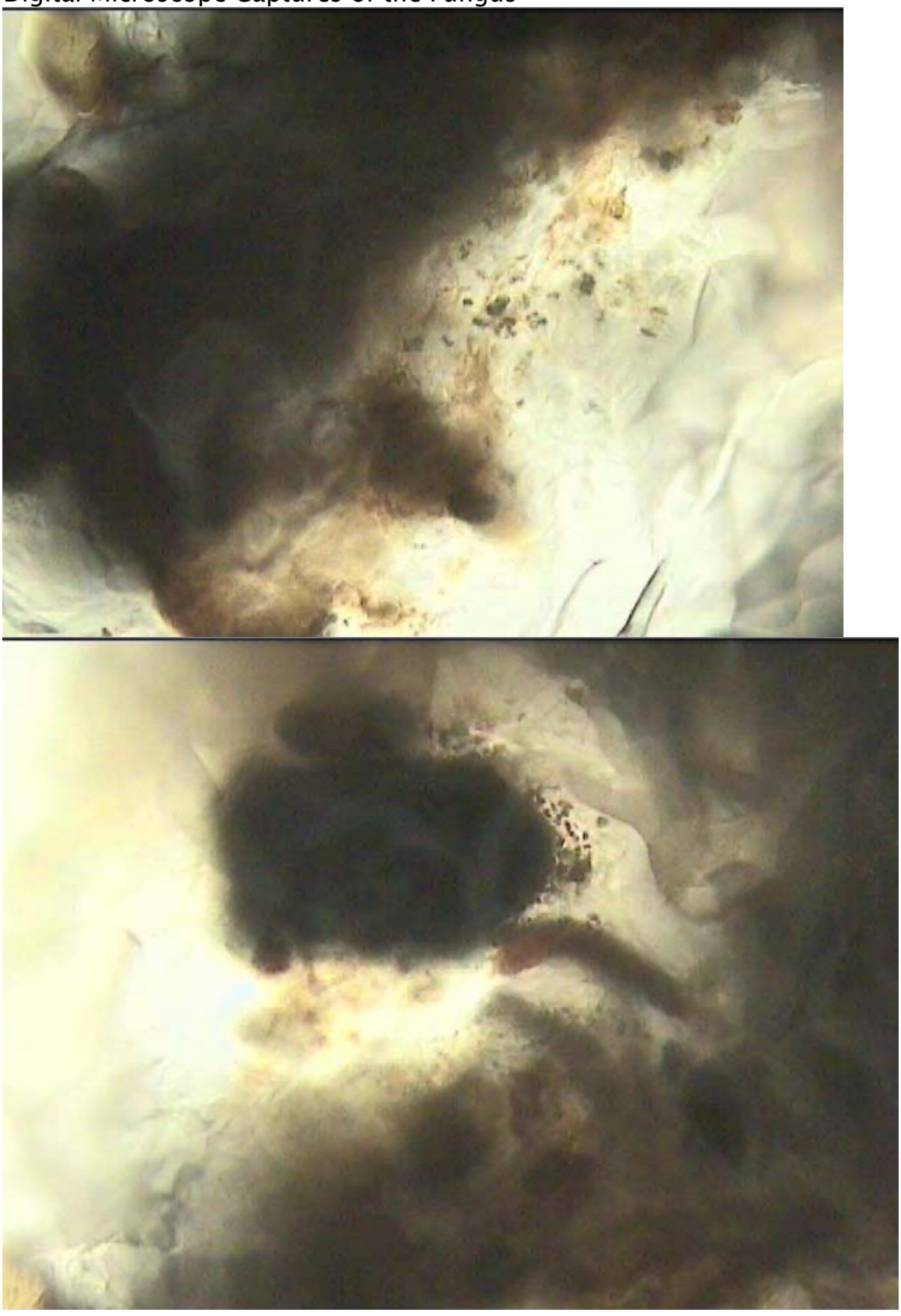
Digital Microscope Capture of a Control Plant’s Guard Cells



Digital Microscope Captures of the Venus Flytrap



Digital Microscope Captures of the Fungus



Results

Although there is still much room for speculation, we have composed three more solid hypotheses as to why these plants can move:

- Cells in an inner layer of the leaf are very compressed. This creates tension in the plant tissue that holds the trap open.
- Mechanical movement of the trigger hairs puts into motion ATP-driven changes in water pressure within these cells.
- The cells are driven to expand by the increasing water pressure, and the trap closes as the plant tissue relaxes.

Our plants grew so much that we had to move them into a larger terrarium for them to thrive. Over the summer, we're going to have to build an even larger environment so that they can comfortably grow.

Future Work

Next year, we are going to look further into the expanding fungus aspect of the experiment. We are curious as to why those mushrooms rapidly grow like a sponge with the absorption of water. We strongly believe that this reaction is connected to the movement of the carnivorous plants. The morphology will be the primary aspect of our experiment next year; we've also made arrangements to use an electron microscope in our studies.

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