The Effect of Different Food Sources on Slime Mold Growth and Memory

Abstract:

Physarum polycephalum is a species of slime mold that spends most of its life cycle as a plasmodium, a unicellular multinucleated amoeboid. This species of slime mold is well known for its spatial memory. Oats, quinoa, and barley have a lot of protein and nutrients; therefore, they were used to feed the slime mold. Other studies tested the memory or growth of slime mold. This experiment compared both slime mold growth and memory using oats, quinoa, and barley. It was hypothesized that barley would make the slime mold grow the quickest and largest due to its high protein concentration. Into 3 Petri dishes a culture of slime mold, and each food source was placed. The slime mold was then placed in a shoebox, with graph paper taped to the bottom, to make sure there was minimal lighting. After sections were cut out and placed into the remaining petri dishes with the same food sources it grew in and then placed into a Lego maze with the food sources to track the time of its memory. Barley and oats tied for overall growth after 48-hours, but barley-fed plasmodium grew much faster. It was hypothesized that the maze portion of the experiment, barley fed slime mold, would also complete the maze faster. The hypothesis was not supported. Oat grown slime mold completed the maze the fastest. The slime mold was placed into different mazes and allowed to grow for 48 hours. After 48 hours the hypothesis was concluded to be unsupported.

Introduction

Slime Mold

Physarum polycephalum, slime mold, spends most of its life cycle as a plasmodium, a unicellular multinucleated amoeboid (Ansorge *et al.,* 2017). This slime mold is referred to as the 'many-headed' slime mold due to its multiple nucleus while being one organism and its capability to grow to very large sizes (Brix *et al.,* 2017). The plasmodium is an aggregate of protoplasm with a network of tubular elements. The protoplasm is differentiated into two phases: a gel phase (ectoplasm) that makes up the walls of the tubular structures, and a sol phase (endoplasm) that flows within the tubes (Ansorge *et al.,* 2017). Tubes are made of a gel-like outer layer and interior cytoplasmic fluids. The outer layer houses an actin–myosin cytoskeleton and the cytoskeleton generate periodic contractions of tube walls (Alim *et al.,* 2017). The motion of the sol gel is driven by organized rhythmic contractions of the gel within a period of two minutes. The sol serves as a circulation system for the cell transporting nutrients and chemical signals. The tubes act as pseudopodia and enable the slime mold to navigate around its environment. As the slime mold moves it changes shape, having the ability to reconfigure within hours as a response to external conditions. As it moves over a surface, the plasmodium changes its shape and if food is placed at different points, it will put out tubes that connect these food sources (Ansorge *et al.*, 2017).

Physarum polycephalum is the species of slime mold used in this experiment. It is one of the most complex species of slime mold. Its complex behavior can be attributed to its stimulus triggers, these triggers release the signaling of a molecule, which affects the fluid flow of sol. The triggering of the molecule increases the sol's flow, which in turn generates a feedback loop that enables movement throughout the organism (Alim *et al.,* 2017). The movement of sol creates a vibration in the slime mold that mimics its movement. The plasmodium moves in a way that is consistent with amoeboid movement however its major difference between itself and amoeboids is its lack of lobopodia. The behavior and movement of slime mold can be linked back to the makeup of its cytoskeleton. The way the slime mold moves is consistent with the way the organism is made up (Döbereiner *et al.*, 2018).

Not only does *Physarum polycephalum* make smart foraging decisions but so do other species of slime mold. While *Physarum polycephalum* is the most popular species of slime mold there are other species such as, *Didymium bahiense*. *Physarum polycephalum* has long been coined as the “smart” slime mold; this has to do with the fact that other species such as *Didymium bahiense* do not have as advanced foraging abilities. Light harms slime mold, it makes it grow slower and weaker (Beekman *et al.*, 2013). While foraging *Didymium bahiense* did not react to the light. The slime mold was moving to the food much slower than usual, but it would not retreat (Beekman & Latty, 2015). Therefore, in this experiment *Physarum polycephalum* is used as it is naturally superior in making foraging decisions.

Foraging bacteria, such as slime mold, are expected to make multi-objective foraging decisions to choose between food sources that vary in quality. They choose the ones that maximize their net energy intake (Beekman & Latty, 2010). These foraging decisions dictate where the slime mold is going to move next. It also helped to influence where the slime mold has given up searching. When slime mold is choosing between two or more different food sources it will almost always choose the one that increases the amount of energy the slime mold intakes. However , slime mold also chooses its food sources based upon safety (Beekman & Latty, 2010). Slime mold grows best in dark environments, therefore when light is present slime mold cannot grow to the best of its ability. While slime mold chooses food based on its energy content it also chooses food in the safest environment, slime mold will rarely venture out into an unsafe environment unless the food they can find in the light is the only food available or that much more nutritious than the food in the dark (Beekman & Latty, 2010).

Spatial Memory

Physarum polycephalum is well known for its spatial memory; remembering where things are in its environment for later. Memory is defined as experience-dependent modification of internal structure, in a stimulus specific manner that changes the way the system will respond to a stimulus in the future as a function of its past (Sims & Kiverstein, 2022). *Physarum polycephalum* can solve mazes, mimic the layout of man-made transportation networks and choose the healthiest food from a diverse menu even without having a brain and or nervous system (Jabr, 2013). This is called spatial memory. Spatial memory enhances an organism's navigational ability. Brainless slime mold *Physarum polycephalum* constructs a form of spatial memory by avoiding areas it has previously explored. In an experiment it was found when the agar substrate was covered in extracellular slime, the plasmodia took much longer to reach the goal compared with plasmodia that could use the presence of extracellular slime to determine where they were before (Reid *et al.,* 2012). Cues and signals can be thought of as informationbearing structures in the sense that the presence of a cue or signal raises the probability of a state of the world. This is because the cue (e.g., extracellular slime) and the state of the world (e.g., the depletion of food) stand in a relation of reliable causal covariation (Sims & Kiverstein, 2022).

While slime mold travels throughout an environment it sends out an oscillation frequency to the rest of the system (Beekman *et al.,* 2012). Also, when a *Physarum* senses food or attractant gradients, the oscillation frequencies of the individual oscillator units which are sections of the tubule network that are most proximal to the food increases, resulting in protoplasmic wave propagation within the tubule network towards the direction of the food (Sims & Kiverstein, 2022). Once it does discover a food source it oscillates strongly from the area in which the food source was found; therefore, directing all attention to the food source (Beekman *et al.*, 2012). Despite the usage of the word "memory" slime mold does not actually remember where it was before. Slime mold can use vibrations or oscillations to quickly solve problems and find food. It can take this information and remember where items are placed further in the future (Austin, 2021).

Food Sources

Oat flakes are the most standard food source for slime because of their nutritional value and the bacteria that it contains. Oat flakes' most common bacterial genera are *Pantoea*, followed by *Acinetobacter*, *Bacillus*, and *Staphylococcus* (Herranen *et al.*, 2010). All these bacteria are very beneficial. *Pantoea*, for example, has a vast number of valuable antibodies such as herbicolin, pantocid, and microcin, among others. The antibodies have a substantial amount of healing properties, due to their ability to maintain the homeostasis that the slime mold seeks out. *Staphylococcus*, another bacterial genus found in oats, is also very beneficial. The bacteria are very Gram-positive which means that they have an outer cell layer (Dutkiewicz *et al.*, 2016). Being gram-positive is important because it secretes peptides (Kumar & Rawat, 2020). Peptides assist in making protein which makes most foods nutritious, to begin with, and thus, benefits the slime mold (Hull, 2022).

As mentioned before slime mold also prefers oats because of their nutritional value. Proteins, fibers, and carbohydrates are what make a food nutritional in the first place. Oats have a substantial nutritional value since it has a vast amount of each. For example, when considering 100 grams of oats, it has 16.9 grams of protein, 10 grams of dietary fiber, and 67.70 grams of carbohydrates (U.S. Department of Agriculture, 2020). Each aspect of nutritional value (protein, carbohydrate, dietary fiber) is very significant in its way. Protein is used to repair cells and since slime mold is a single-celled organism, protein is essential so its whole form can stay strong and healthy. Carbohydrates are also important because they are necessary for structural and energy-storage purposes. They are made of sugar molecules that help an organism, such as slime mold, have energy and be able to continue (Parker *et al.*, 2016). Fiber is a type of carbohydrate that most organisms cannot completely digest. Its structure does not allow them to be digested which gives organisms more energy and keeps them fuller for longer. All these components are essential for slime mold and can be found in other grains as well.

Quinoa and barley are good examples of grains that have a similar amount of or more nutritional components/than oats. 4.4 grams of protein, 16.0 grams of fiber, and 39.4 grams of carbohydrates are in every 100 grams of quinoa. According to these statistics by the U.S. Department of Agriculture, quinoa has less protein and carbs but more fiber. Barley on the other hand has even better statistics. Barley has 12 grams of protein, 17 grams of fiber, and 135 grams of carbs. Although barley has less protein than oats, it has almost double the amount of fiber and carbs. Barley also has the same main beneficial bacterial genus as oats, *Pantoea* and has additional bacteria such as *Paenibacillus* and *Pseudomonas* that are similarly advantageous. (Rahman *et al.*, 2018). Taking these components into consideration, we hypothesized that barley would be a better food source to use when experimenting with and researching slime mold because it not only has more fiber and carbohydrates than oats but contains the same and additional bacteria that can make it advantageous. We also hypothesized that barley fed slime mold would complete the maze faster than oat and quinoa fed slime mold.

Implications

With the increase of harmful bacteria, such as *E. coli*, *Salmonella*, and *Listeria*, due to the increase of garbage and waste, slime mold may be more important than one may realize, slime mold is a very significant organism in the food chain. It is a decomposer and recycler of nutrients and one of the main players when it comes to decomposing bacteria and releasing nutrients. Slime mold is very important and very endangered in some parts of the world. In the British Isles slime mold is an endangered animal (Casadevall, 2005). The endangerment of slime mold in the British Isles is harmful because it can cause the bacteria formed by excess waste to go unchecked. According to a UK government website, the UK isles generated 222.2 million tons of total waste in 2018. The waste undoubtedly brought in several unwanted bacteria. Slime mold can help decompose the unwanted bacteria and help its useful nutrients to come out. If the hypothesis presented in this experiment is correct, barley may help grow slime mold quicker and larger. Barley is hypothesized to help grow the slime mold faster due to its favorable bacteria and higher fiber and carbohydrates. Slime mold that is grown quicker and larger can then be presented to waste abundant places and help break down harmful bacteria (Heilmann-Clausen, 2001). The more efficiently grown slime mold wouldn't only help places where slime mold is endangered such as the British Isles, but also help other places too. For example, the United States famously has an excess amount of waste. In 2018 it was reported that 292.4 million tons or 4.9 pounds per person per day (Miller, 2021). If slime mold was introduced directly to the waste or garbage that piles up in the landfills, it could help break down the harmful bacteria

there. Slime mold can help with the global waste crisis by decomposing harmful bacteria in waste. Additionally, Slime mold is a very important creature as it is a significant decomposer, nutrient recycler, as mentioned before, and food source to larger animals. Determining if its nutrient intake helps us understand more about this obscure creature which may help to further scientific and mathematical discoveries.

Novelty:

This experiment aimed to test the effects of three different food sources, quinoa, oats, and barley, on slime mold growth and memory. Previous studies tested the slime mold memory using only oats or tested the growth of slime mold using only oats. For example, a study done in 2019 by Subash and associates, measured the information transfer of *P. polycephalum* during food choice of oats and only agar. This experiment tested both slime mold growth and memory using various food sources (oats, quinoa, and barley). Oats are the standard food for slime mold due to their nutrient content and its bacteria, but quinoa and barley may be good or even better alternatives. Both barley and quinoa possess similar nutrient content compared to oats. Barley also has the same and additional bacteria as oats. Along with having the same bacterial content as oats, barley has more fiber and carbs than oats. With all this information combined the hypothesis came to light that slime mold may grow better under barley as supposed to under oats. Most other slime mold experiments only use oats and do not even consider other food sources, but this experiment did and tested how different food sources may help slime mold be a better forager too.

Materials and Methods

Experimental Setup:

There are a lot of purchases to make and safety measures to take before the start of the experiment. The nutrient agar which is extremely vital to have for this experiment was purchased from Carolina Biological. The three food sources that were used in this experiment were quinoa (*Chenopodium quinoa Willd*.), barley (*Hordeum vulgare*) and oats (*Avena sativa*). The oats were obtained from Carolina biological, and the quinoa and barley were purchased from Amazon. For the lab safety measures, first we tied our hair back and put on goggles, gloves, and aprons. Next, Clorox wipes were used to wipe down the countertops that were used to do the experiment. The surface was allowed to dry before continuing onto the next step. The agar solution was made with 2 grams of agar powder and deionized water, which after being autoclaved, was placed in the bottom of 15 sterilized Petri dishes so that the entire surface was coated with a thin layer of agar. It was then put upside down in the fridge to cure.

Measuring Slime Mold Growth:

Slime mold (*Physarum polycephalum)* was purchased from Carolina biological. 15 pre-prepared Petri dishes with agar were used to grow the slime mold. The slime mold was grown according to table 1.

Group	Petri Dish	Food Type	Amount of Food Fed (Pieces)	Sample Size
A	1-5	Quinoa	20	5
B	6-10	Barley	10	5
C	11-15	Oats	10	5

Table 1: Treatment of Slime Mold Groups

The food was placed in each dish, according to Table 1. After treating the slime mold according to Table 1, the slime mold was left to grow for a full three days. The slime mold was put into a shoebox to block out light and monitored for its growth. After the slime mold grew for those three days, it was measured using the number of squares the slime mold passed on the graph paper. It was measured using the radius of the graph paper circle and how much of that radius was covered (Ex: the full radius of the graph paper is 6 cm (6 squares), but the slime mold had only passed 3 cm (3 squares), therefore, the slime mold grew 3 cm). An iPhone 11 camera took pictures of the slime mold and graph paper to show the measurements and the measurements were also recorded on a labeled google doc to later be placed on a graph for the results. Next the fully grown slime mold was taken from the Petri dishes depending on what they were fed, quinoa, barley or oats were then put in a maze, and it was recorded how long it took to find its food in minutes.

To find out the measurements of the slime mold graph paper was obtained. A piece of graph paper was obtained from Staples (the store) with each square on the paper being 1cm^2 (in terms of area) and the Petri dishes were placed on top of the graph paper to be outlined. The graph paper needed to be taped onto the bottom of the shoebox so the measurements could be accurate. Scotch tape was obtained from Staples (the store) and small pieces were cut in order to tape the paper to the bottom of the inside of the shoebox. After collecting the measurements with a ruler (centimeters), the slime mold was removed from the Petri dish using a serialized micro spatula to scrape up the slime mold's pseudopods. The sub-cultured pieces of slime mold were placed into the maze after being measured with the three treatment sources**.**

3.3 Making the Maze and Memory Measurements

There were 3 identical mazes that were built for each food source to individually be placed into it. The mazes were made of Lego pieces. Saran wrap was placed on the bottom of all 3 mazes to prevent liquid from going through the open spaces in between the Lego pieces. Then the saran wrap was put on the mazes, 2 grams of agar was mixed with 100 ml of deionized water and placed into the autoclave for 20 minutes. After being in the autoclave an oven mitten was put on by one of the group members and the agar was removed from the autoclave still covered to prevent contamination. When the agar cooled down enough, a thin layer of said agar was poured into the maze and left in the fridge for a day. The maze was taken out of the fridge the next day and it was placed on a clean counter in the school lab and the fully grown slime mold was placed on one end of the maze and the grains of the food source was placed on all the dead ends of the maze. Saran wrap was placed on top of the maze to limit contamination and to limit the slime mold from going outside of the maze when looking for the food sources. Then those said wrapped mazes containing the food sources and slime mold were placed in a dark, room temperature drawer to limit lighting so that the slime mold did not die and would be monitored every day until it can find its food. For each day it was monitored one of the group members checked on the slime mold and used an iPhone 11 camera to take a picture of its progress and later when the searching for food process finished, the number of days it took to find the food was recorded in a spreadsheet.

Data analysis

The results were collected through pictures, taken by a smartphone, of the smile mold growth every 24 hours for two days after they were first placed in the petri dishes with their food source, quinoa, barley or oats. There was a piece of graph paper on the bottom of the environment where the petri dishes sat to measure the growth of the smile mold in total area (cm). The pictures were used to visually see the growth of the slime mold at three different time periods of growth. The area the slime mold covered was counted or measured every day and recorded in a spreadsheet. The recorded measurements were then placed into a line graph through the usage of excel. The graph compared the slime mold growth to time. The line graph was used to show the numerical difference in growth between the three groups, quinoa, barley and oats.

The results regarding the maze were collected through pictures, taken from a smartphone: like how they were collected regarding the growth of the slime mold when fed with different food sources. First, the slime mold was collected from each petri dish, one slime mold culture was collected from barley fed slime mold, one was collected from oat fed slime mold, and one was collected from quinoa fed slime mold. They were then placed into Lego mazes. The mazes were disinfected, wrapped with saran wrap, and filled with agar before placing the designated slime mold (fed with either oats, barley, or quinoa) into the maze. The maze was then placed in a dark cabinet and untouched for 2 days or 48 hours. The maze was then removed from the cabinet and pictures of the slime mold were taken.

Results

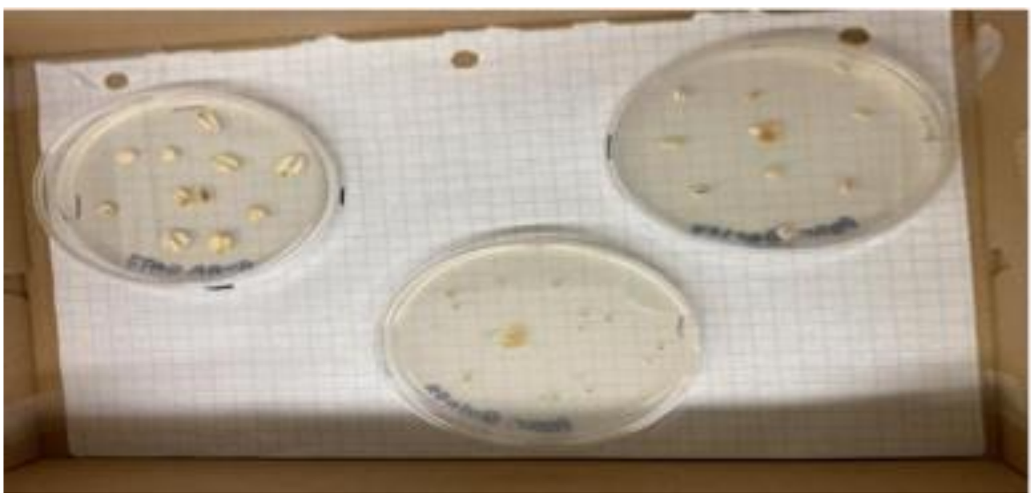

Figure 1: When the slime mold was first placed into the Petri dishes. There was no growth yet.

Photo Credit: D. Kemmett

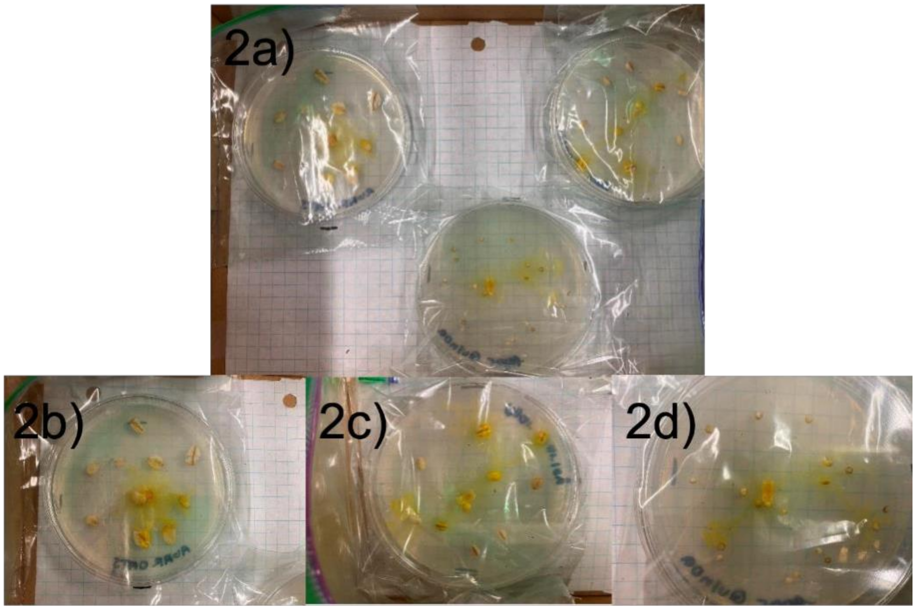

Figure 2: 2a) This is after 24 hours of growth. 2b) Oats had covered about 17 squares, this gives it a surface area of 6.12 cm^2. 2c) Barley had covered about 30 squares, this gives it a surface area of 10.8 cm^2. 2d) Quinoa had covered about 19 squares, this gives it a surface area of 6.84 cm^2.

Photo Credit: D. Kemmett

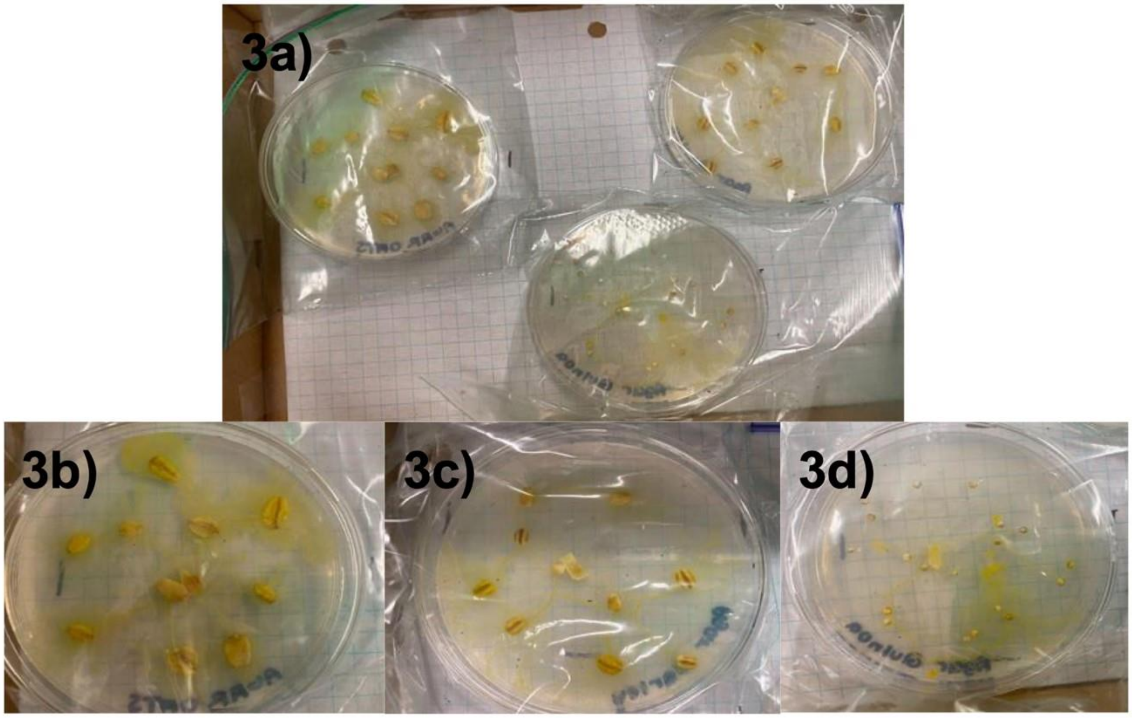

Figure 3: 3a) This is after 48 hours of growth. 3b) Oats cover about 55 squares, this gives it a surface area of 186 cm^2. 3c) Barley covers about 55 squares, this gives it a surface area of 18 cm^2. 3d) Quinoa has covered about 37 squares, this gives it a surface area of 13.32 cm^2.

Photo Credit: D. Kemmett

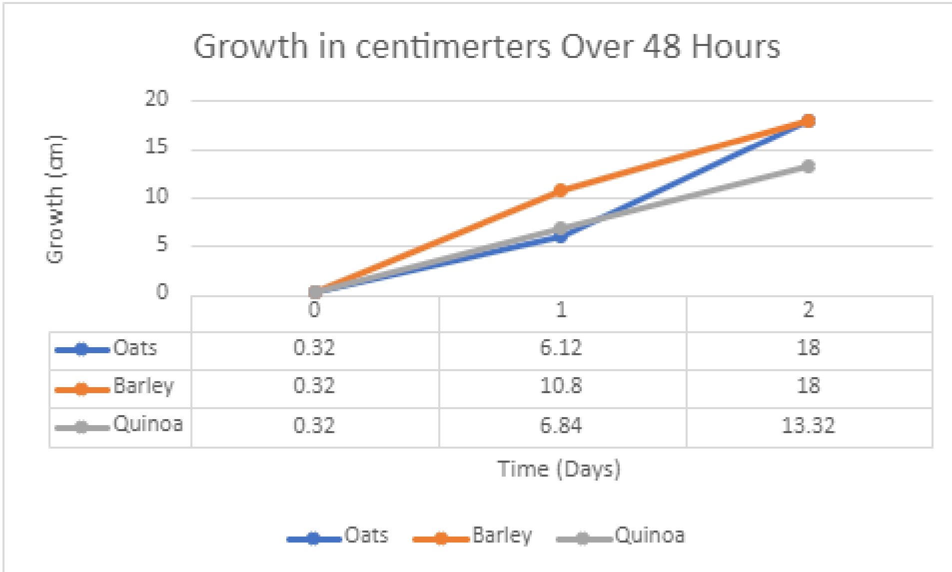

Figure 4: Barley, represented by the orange, grew at a much faster rate than both oats, represented by the blue line, and quinoa, represented by the gray line. As seen in the graph, barley grew more rapidly than both quinoa and oats and tied with oats for overall growth after 48 hours in a Petri dish. While barley and oats did tie after 48 hours, after 24 hours barley had already covered more surface area than oats.

5a) 5b) 5c)

Figure 5: The three mazes represent the different mazes with different food sources. Each maze was given 48 hours to grow. 5a represents the slime mold in the maze with oats as a food source. 5b represents the slime mold in the maze with barley as a food source. 5c represents the slime mold in the maze with quinoa as a food source. The starting point is marked by the green dot and the ending point is marked by the red dot. None of the mazes reached the marked ends from their marked starting points, though oats got the closest. The quinoa maze also grew mold during the process and the quinoa itself sprouted.

Discussion and Conclusions

The data showed that when slime mold was presented with barley compared to oats and quinoa, it grew faster. Barley also grew larger compared to quinoa. This data collection supports our hypothesis because while barley and oats did tie for overall growth after 48 hours, barley grew much faster than oats as shown in figure 2. The hypothesis stated that barley would make slime mold grow faster and larger because of its carb, fiber and bacteria content, which is what happened. Due to the tie, it is suspected that if the slime mold was given a larger environment, barley would have overgrown oats.

The data also showed that when the slime mold was in the maze with oats compared to barley and quinoa, it found the most amount of food and spread throughout the maze the most. This data collection does not support our hypothesis because while the slime mold in the maze with barley did grow it covered less distance than the slime mold in the oats maze and ate less of the barley in the barley maze according to figures 5a and 5b. Additionally, the slime mold in the quinoa maze died and the quinoa also sprouted according to figure 5c. The hypothesis stated that the barley fed slime mold in the maze would complete the maze faster due to its bacterial and nutritional content. Due to the humidity conditions and the type of food source the oats ended up being the most fed on and supported the slime mold growth the most as well.

Slime mold is used by researchers to study optimal foraging strategies (Adamatzky, 2012). By using barley instead of oats, researchers may have an easier time growing slime mold. Barley could be used as the standard food for slime mold rather than oats. Although the experiment is useful in nature, it is not without error. Possible errors could be that while transporting the dark enclosure for the slime mold the pieces of barley, oats, and quinoa sitting on the Petri dish could have shifted and moved around. Another limitation was, growth was only able to be recorded through time-stamped pictures rather than a recording. Also as stated before the Petri dishes were too small to see the full growth that the slime mold could have grown making it difficult to accurately compare the difference in growth. Finally, there was not enough time to test how the smile mold would have behaved once it came to the maze. Our next steps would be to test the slime mold's growth in a maze to discover how long it takes for the slime mold to find its food, depending on the food source. However, the current research can be implemented by feeding slime mold barley instead of oats for faster results in other experiments. The last limitation involving the slime mold growing in the petri dish is that the quinoa sprouted and started growing too, therefore, a limitation is the humid and moist climate in the dark cabinet. There are also limitations involving the maze part of the study. Even though the Lego pieces were thoroughly washed and the saran wrap that was placed over and around the maze was sterile, mold (not slime mold but regular bacteria mold) still grew in the agar, also the slime mold that was grown with the quinoa died and the most mold grew on that maze as well as the quinoa sprouted in the maze as it did in the petri dish. An overall limitation for making the agar is that there was no access to an autoclave and a microwave had to be used in order to make the agar which could have caused the bacteria to form in the agar.

Overall, barley appeared to be a better source of food compared to the standard oat. In this experiment, barley presumably outperformed oats because of several factors. Firstly, barley had a higher concentration of fiber and carbohydrates, making it more nutritious which all organisms look for in their food. Additionally, barley contains the same bacteria found in oats which is also what makes oats attractive to slime mold in the first place. Barley has everything oats have and more thus making it more appealing to the single-celled organism, slime mold.

Future Directions

After conducting the experiment, there will be some changes that need to be made in order to obtain more accurate results. First off, the petri dish will be taped down to limit the

amount of movement of the food and the growing slime mold. Another aspect that will be changed is the size of the shoebox and the Petri dishes to a bigger size so that the slime mold can cover more area when it grows. Some additions to the experiment can be the addition of more food sources or changing the food into essential oils.

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