Experiment Title
Project Toad - *The cultivation of Ganoderma lucidum in a microgravity environment for medicinal purposes upon the International Space Station*

Experiment Outline
The aim of the mission is to observe the growing patterns exhibited by Ganoderma lucidum in a microgravity environment. By observing these behaviours, we hope to evaluate the possibility of utilising the mushroom as a crop upon the International Space Station and future missions, for both food and medicinal purposes, and record the observations for future endeavours in the field of astrobiology. The experiment plan is to utilise Ganoderma lucidum due to their versatility in their growth and medicinal purposes for nutrient supplements. They are able to grow in harsher conditions when compared to other crops and can grow in controlled amounts depending on the amount of substrate and heat available to the mycelium. We predict that the design of the payload we intend to create will allow the Ganoderma lucidum to grow in an environment similar to that of Earth with the exception of the presence of microgravity. On the initial space experiment, the mushroom will not be able to be harvested. However, if successful, it would allow for the team to design a stable, self-maintaining and reproducing crop for astronauts upon the International Space Station. These mushrooms would be easily harvested through this device, and will be supplied to the astronauts as a nutritional and medicinal supplement.

School
Australian Science and Mathematics School

Presenters
Max Amerl, Jade Baker-Johnson, Julia Erceg, Bataille Foo-Brady, Giordan Staines, Huey Pretila

Teachers
Matthew Verdon

Principal
Jayne Heath

Industry/Tertiary Connections
Michael Taylor (*Flinders University, Mycologist*), Raviteja Duggineni (*University of Adelaide, ResearchSat, Aerospace Engineer*)
Introduction

Food and nutrients for astronauts are lacking in space due to a limited amount of supplies to support agricultural processes, such as the cultivation of sustaining crops. This shortage of sufficient food intake is one of the obstructions that impede efforts for further space exploration. However, the viability of alternative crops such as Ganoderma lucidum that have been utilised both in medicinal and culinary contexts provide a possibility to produce a staple crop to be used in space. By utilising the simple growing requirements of Ganoderma lucidum, this experiment allows the growth and cultivation of this mushroom highly viable. Ganoderma lucidum takes several months to reach its fruiting stage. However, this period of growth can be altered depending on the temperature of cultivation. This folio details the development and planning involved in the project.

Background

The aim of this project is to produce an experiment to send into low-orbit onboard the International Space Station. The team initially produced three ideas alongside three different principles of astronomical and astrophysical science. The initial ideas highlighted investigation of alternate silicon-based or arsenic-based life forms, the magnetic fields of liquid sodium and the spectroanalysis of the atmosphere. However, after analysing the respective contexts of each idea, the process of refinement resulted in more viable proposals such as the creation of a variable magnetic field, the analysis of weather patterns and the testing of mushroom growth in space.

Upon submitting proposals for each initial idea, feedback suggested that mushroom growth in space was more viable in comparison to the other two proposals. For instance, the variable magnetic field idea hung on the basis of producing a magnetic field through the rotation of suspended liquid sodium, which posed a risk to equipment on the ISS, on which the experiment was to be held on. The spectroanalysis, in the other hand, was far too underdeveloped to be accepted, with barely any research held regarding the mechanics of such an experiment.

The Ganoderma lucidum

Ganoderma lucidum, which is one of the many related species referred collectively as Reishi or Lingzhi mushroom, is a mushroom that natively grows in areas of East Asia. Today its cultivation relies on using hardwood logs and woodchips. Traditional Chinese medicine uses this mushroom as it is able to reduce stress and chances of cancer due to it being an immune system regulator (Benzie, Wachtel-Galor and Packer, 2011).

Ganoderma lucidum was chosen for this experiment as it has a wide range of nutrients, as well as anti-cancer, anti-fungal and antiviral properties. As well as acting as a nutrient supplement, the Ganoderma has a sufficient amount of energy and carbohydrates when compared to other species. The usage of this mushroom would be great in a space situation as they are highly beneficial for the body, in both aspects of nutrition and as a food source.

On-Ground Experiment

Introduction

The initial test experiment was aimed to observe growing variations of Ganoderma lucidum, testing variables and discovering the best conditions to plan out the final experiment. Three experiments were conducted to theorise outcomes of the final experiment. The first experiment was conducted to merely observe the growing patterns of the reishi mushroom in a cylindrical environment. The experiment was using a simple substrate and unprotected environment. The second experiment was conducted to test a sparsely oxygenated environment for the Ganoderma. It was also set up with several other sensors to test and record the suitability of a realistic environment of a sealed, low-oxygen experiment in microgravity.

Aim

To test and experiment the growing conditions and requirements of Ganoderma lucidum in a similar environment to that of which would be used in the SA Schools Space Mission final project.
Hypothesis
Ganoderma lucidum will be grown in an environment similar to that of which it will be grown during the Space Mission experiment.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Basic Method</th>
<th>Considerations (Ethical, Safety etc)</th>
</tr>
</thead>
</table>
| Initial                       | * Sterilise, prepare and accurately measure all substrates.  
|                               | * Prepare and code sensors with Arduino board    | There are several safety considerations to be taken into account when experimenting. Although Ganoderma is a safe mushroom, there are risks of inhaling the spores when opening and transferring it to substrate. Also, using 3D printers, laser cutters and Arduino boards also poses several considerations to take into accord in the development stage. When sterilising substrates, bunsen burners are used. Basic lab-safety and procedures need to be taken into consideration. |
| * Hardwood substrate         | * Create 3D printed model of on-ground experiment - troubleshoot and improve  
| * Various Grains (used for substrates)  | * Add substrate to compartments, add micillium |                                                      |
| * Air tight containers       | * Attach sensors and microcontroller              |                                                      |
| * 3D-printed model and housing| * Seal in airtight container                       |                                                      |
| * Accurate scales and measuring equipment | * Regularly check and record growth |                                                      |
| * Sensors, wires and raspberry pi (or Arduino) |                                                   |                                                      |
| Secondary                     |                                                   |                                                      |
| * 9 grams of various substrate, crushed |                                                   |                                                      |
| * 3 grams of hardwood sawdust |                                                   |                                                      |
| * Sterilisation equipment    |                                                   |                                                      |

Process

Fig. 1- process method in pictures

Growing Ganoderma lucidum
Ganoderma lucidum was shipped from New South Wales, 3 - 4 weeks into cultivation, to aid with the research and experiment of this project. The Ganoderma had already started its first stages of growth (spawn run; typically 20+ days until aided with substrate). From then, the mushroom was to be added to sterile and prepared substrate to encourage the second stage of growth (known as the antler stage) (*TC Permaculture n.d.*). If equipped with the proper conditions, the antler stage could last up to 3 weeks. From then, the Reishi mushroom enters its last stage; the primordia stage of reproduction. This is when the caps of the reishi will form - a hard woody surface facing ‘upwards’ and the spores protected underneath the cap. In a microgravity environment, this could pose some issues. As a trial, the on-ground experiment is going to force the mushroom to ‘choose’ which way is up, and whether or not the conditions are correct to reproduce. Ganoderma lucidum enters the primordia stage of growth based on sunlight exposure, temperature and oxygen/CO₂ levels. In this experiment, these factors are to be artificially prepared so that the Ganoderma mushroom is given the optimal growing conditions, therefore allowing for the growth to be controlled. The Ganoderma will be given a 5 minute oxygen intake everyday (except weekends), whilst the growth is being monitored and documented. Then will be returned into the airtight container. During the first stage of the Ganoderma growth, it consumes a large amount of oxygen. This stage, however, will not be completed inside the airtight container. After the Ganoderma is entering its second stage, it will be added to the air tight container, wired to the sensors and sealed shut. Having no additional exposure will also keep the moisture levels of the environment constant or fairly regular (*Taylor, 2017*).

Modelling the Housing
The container that is to house the Ganoderma lucidum was created using Autodesk 2017, a computer design and 3D printing programme. This compartment not only had room for substrates and spawn but an extra layer had room to fit an Arduino board, sensors and wires. After many trials and tests, the current design of the on ground experiment was made. It was created for the purpose of the on-ground experiment; weighing only approximately 180g. Having a compartment within the weight constraints of the SA Schools Space Mission allowed the group to be able to test as if it were to be conducted under the experimental parameters. With the addition of sensors and reishi spawn, the experiment weighed under 280g. The created box has been
The hypothesis of the space experiment (refer to page 6).

indicates program. cap stage, entering Mission Space formation the Ganoderma would be sealed in to the box;

From the form. available artificial they out oxygen to caps side growing through in experiment the within holes initial of substrates of Ganoderma predicted. Through the sterilised been sterilisation; the microbial destruction of the container, any mould before minimal grounds, variables), grew stage the spawn spawn primordial stage the weeks. coffee chance experiment with (conducted original a substrate, boiling reishi the and the funnel buchner. By vacuuming.

boiled small dried was added ml to the mixture then the the before minutes, being drying a bunsen sterilisation; above. the and of the was first substrates. measured substrates the sterilisation main the of the scales, measured about gains of growing and mixture of substrates and was then sterilised.

The sterilisation process of the substrates was completed in 3 main steps. The first was preparing the substrates, as explained above. The second was the first step of sterilisation; boiling and drying the substrates. Using a bunsen burner, beaker and tripod, the substrate mixture was added to the small beaker with 175 ml of water. It was then boiled for ten minutes, before being dried using a buchner funnel and the hydraulic pressure vacuuming. By boiling and drying the substrate, this would give the reishi mushrooms a higher chance of fruiting within the next 2 weeks. From observing the original experiment (conducted with coffee grounds, spawn and minimal constant variables), the spawn grew mould before reaching its primordial stage. The second stage of sterilisation included Ultraviolet light treatment of the container, equipments and substrates. This would have destroyed any extra microbial life they may have been present in the substrate mixes. The sterilised substrates were then mixed with Ganoderma lucidum spawn, and added to the already 3D printed container and then sealed in an airtight container.

Predictions
Through the proper use of sterile substrates and spores, it is predicted for the Ganoderma lucidum spores to reach their Primordia antler stage within weeks of being added to the experiment box. These initial mushroom antlers should grow through the holes in the side of the box; growing out towards the available oxygen and artificial light. From then, the caps should form. If they were to form in a completely sealed environment, it would mean the Ganoderma would be a viable experiment to use in the SA Schools Space Mission program. The cap formation indicates that the Ganoderma is entering its reproductive stage, which would support the hypothesis of the space experiment (refer to page 6).
Results of on-ground experiment

Table of Results - Record of observations from several days of the experiment process.

<table>
<thead>
<tr>
<th>Date</th>
<th>Photos</th>
<th>Observations</th>
</tr>
</thead>
</table>
| Wednesday (6/09/17) | ![Photos](Image 18x724 to 205x775) | Today we set up the second ground experiment. We predict that the most effective substrate is going to be the wheat, as it is high in nitrogen. In the centre of each substrate is a small amount of Ganoderma lucidum mycelium which should start to consume the substrate within a few days.

The substrates were sterilised using a two-step process, as stated above. Our sterilisation process may have not been the ideal method for Ganoderma lucidum, however we are fairly sure that the process we undertook would sterilise the substrate enough.

The design of the box we added the substrate too allows it to grow off to the side: which can be observable from the outside containment. |
| Monday (11/09/17)  | ![Photos](Image 75x176 to 334x639) | The results collected today show the mycelium successfully consuming the substrate, in each of sectioned areas of the box. We observed that all of the substrates had successfully allowed the mycelium to grow. So far, the oats have proved most efficient in terms of growing the mycelium. However, this may be because oats ended up with the least amount of substrate after the sterilisation process. The mycelium may have grown in larger amounts because it consumed most of the substrate, thus showing more of the growth when viewed from the opening of the box.

Another observation of today’s growth was that the type of mycelium density and covering varies from grains. This is because of the properties of each grain (nitrogen content, density etc.) or the process of the sterilisation method. |
| Monday (18/09/17)  | ![Photos](Image 454x726 to 579x775) | Over the weekend the mycelium seemed to completely consume the substrate. The different substrates were indistinguishable; a large difference from seven days ago. As similar to last week, the oat substrate section was still a lot lower than the other sections.

When viewed from the side of the device (where the mycelium was directed to grow towards), the wheat had been the most successful. The holes in the side of the box had assisted the growing of the mycelium towards the oxygen and light supply the most successful in the wheat.

The observations of today show that wheat would probably be the most fruitful in terms of substrate for Ganoderma lucidum. With a mixture of wheat and hardwood sawdust (3:1), the mycelium will grow the most successfully. |

Results (so far)

From observations of the experiment so far, it is supported that Ganoderma lucidum grows more successfully in a wheat and sawdust substrate (3:1). This success rate may be because of several different factors. Wheat is very high in Vitamin E, as well as phosphorus, magnesium, iron and zinc. Ganoderma possesses all of these minerals in higher concentration (compared to standard mushrooms), as well as being high in carbohydrates. The presence of these minerals in Ganoderma lucidum is one of the reasons it has been chosen for this experiment over other species of mushroom. Another reason for choosing Ganoderma lucidum is it high levels of carbohydrates and anti-fungal, anti-cancer and antiviral properties due to its Polysaccharides and Peptidoglycans.

From these results, the substrate requirements for the space experiment can be hypothesised. The wheat allowed the mycelium to grow and feed the most successfully. When mixed with the hardwood sawdust, sterilised wheat substrate would be a good choice for the next experiment test. Also, the size requirements of the growth in the apparatus and amount of mycelium substrate shows results that could be used for the test as well.
The Space Experiment

The Design
We intend the payload to be constructed from materials specified in the table below. The payload box will have an outer layer consisting of aluminium 6061 insulated with Divinycell H foam and additional foil if required. The electronic systems within the payload include temperature, humidity, barometric pressure, moisture, carbon dioxide, and potentially radiation sensors in addition to small cameras, photodiodes, (LDR), LEDs, and an accelerometer. These sensors will be run from a suitable microcontroller with telemetry capabilities to transmit data to Earth. All electronic hardware will be integrated into the payload structure, which will consist of a 3D printed container housing the mushroom growth units, a separate electronic hardware compartment, and a growth chamber for fruiting mushrooms where growth measurements will be taken. Sensors will be integrated into both the mushroom growth units and the growth compartments for fruiting mushrooms.

<table>
<thead>
<tr>
<th>Material</th>
<th>Information</th>
<th>Justification</th>
</tr>
</thead>
<tbody>
<tr>
<td>6061-Aluminium</td>
<td>6061 is commonly used for structural components, and is easily cold worked and formed in the annealed condition. <em>(Typical Applications, 2017)</em></td>
<td>Aluminium 6061 is used for heavy duty structures requiring high strength-to-weight ratios. *(Typical Applications, 2017)*The material has a density of 2.70g/cm² and a tensile strength varying from 124-290 MPa. It provides high strength with a low weight. This material is therefore highly suited to use in constructing the payload because it has a high durability.</td>
</tr>
<tr>
<td>Polycarbonate</td>
<td>Polycarbonate is an incredibly useful plastic for applications requiring transparency and high impact resistance. <em>(Polycarbonate done right, 2010)</em></td>
<td>With a density ranging from 1.20-1.22g/cm² Polycarbonate is quite light. The material also has a tensile strength of 55 to 75 MPa, meaning it is strong despite its low density. We will 3D print the inside structures of the payload out of this material, as it is light and has a moderate tensile strength.</td>
</tr>
<tr>
<td>Divinycell H Foam</td>
<td>Divinycell H provides excellent mechanical properties and low weight. It is widely used and has a proven track record in virtually every application area where sandwich composites are employed. <em>(Divinycell H, 2017)</em></td>
<td>Divinycell H Foam is especially suitable as insulation at low or cryogenic temperatures <em>(Divinycell H, 2017)</em>. Given its incredibly low density and its low absorption of water it would serve as a suitable insulation for the space experiment design.</td>
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Initial Prototype Design of the Space Experiment
The initial prototype design consists of 4 components. The first is the frame; which is designed to enable the aluminum shell and Divinycell H. foam to be attached to the outside of the frame. The second is the inner housing *(see fig - 2)*; which has a bottom compartment to store the electronics and sensors and two separate cells to grow the Ganoderma. The third is the side wall *(see fig - 3)*; which will go on both sides of the inner housing and the fourth is the lid *(fig - 4)* which fits on the top and fully encloses the experiment. The inner housing and it’s walls will be able to slide into the frame once fully constructed.
Limitations

The proposed experimental apparatus does not allow the accurate measurement of the mass of growth rate of the mushrooms in orbit, presenting as an initial limitation of the project. Despite this, it is possible to quantify the mushroom growth using grids to approximate surface area in both the x and y planes of growth allowing later approximation of the volume and mass of the mushroom growth. Additionally, a range of limitations also arise from the absence of ventilation for the experiment. As a result of there being only a fixed amount of oxygen in the sealed apparatus, there will be no extra oxygen intake throughout the lifetime of the experiment. This restricts the growth of the mushrooms with respect to the fact that they rely on oxygen intake to grow considerably. This limitation can be mitigated by completing further ground experiments to determine the effect of small amounts of oxygen on mushroom growth, allowing the determination of appropriate conditions to maximise mushroom growth. Further limitations associated with the sealed apparatus result from gradually increasing levels of carbon dioxide in the experiment container over extended time periods. The effect of this limitation can again be mitigated by completing ground experiments to determine optimal growth conditions in a space environment. Given the extreme nature of a space environment it is also necessary to consider major limitations associated with heat loss, which can greatly affect the growth of the mushrooms. To minimise the effect of this limitation on the experiment it is possible to include thermal insulation in the payload structure to maintain consistent temperatures of 10 to 15 degrees Celsius. With a payload weight limit of 300 grams careful consideration will be required to ensure that sufficient insulation is available without exceeding specified weight limits.

2018 Plan / timeline

Creating such a large project requires adequate time management skills to ensure that goals are completed and requirements are made. A rough outline of the end of this year and next year has been created month by month to help prioritize tasks. The timeline shows what would happen if Project Toad was selected to take part in the SA Schools Space Mission. It’s difficult to create a plan far into the future, especially when the experiment might not go ahead depending on who is selected. If Project Toad is selected, this is how we would structure our time:

<table>
<thead>
<tr>
<th></th>
<th>2017</th>
<th>HOLIDAYS</th>
<th>2018</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sept</td>
<td>Oct</td>
<td>Nov</td>
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<tr>
<td>Create presentation</td>
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<tr>
<td>Continue ground experiments</td>
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<tr>
<td>Meet with aerospace engineer</td>
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<tr>
<td>Create more design drafts</td>
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<tr>
<td>Do more ground experiments</td>
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<tr>
<td>Cultivate mushrooms</td>
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<tr>
<td>Source materials</td>
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<tr>
<td>Create prototypes</td>
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<tr>
<td>Review prototypes with expert</td>
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<tr>
<td>Create a proposal (if needed)</td>
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</table>

*Timeline is subject to change
Professional help and industry partners

Dr Michael Taylor - Mycologist at Flinders University.

michael.taylor@flinders.edu.au
http://www.flinders.edu.au/people/michael.taylor

Qualifications

- PhD in Environmental Biotechnology, School of the Environment, Flinders University, South Australia. Thesis title: Microscopic and molecular study of multi species biofilms containing Legionella. (2011)
- Honours Degree of Bachelor of Biotechnology, Flinders University, South Australia, Australia. Thesis title: Green fluorescent protein (GFP) tagging of Legionella longbeachae isolated from commercial potting mixes.
- Degree of Bachelor of Biotechnology, Flinders University South Australia.

Honors, awards and grants

- Three Minute Thesis competition, University finalist (2011)
- Australian Society for Microbiology, Becton Dickinson prize for best student presentation of novel microbiology research (2009).

Dr Taylor has played an important role in supporting the team. His work is based at Flinders university, which is where the ASMS is located. He has since started up his own mushroom farm and is the only Mycologist who specialises in mushroom growth. Dr Taylor has spent some time with helping support this experimental plan by sharing his knowledge and idea's to help contribute to the project. Some idea's Dr Taylor has contributed to are the water consumption and storage configuration of the mushroom as well as maintaining CO₂ levels in the experiment. He has also taught the team how to cultivate the mushroom spores on petri dishes. He has spent a large amount of time with the team, in the laboratories at the Australian Science and Mathematics School, where the team is located.

Raviteja Duggineni - University of Adelaide

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https://www.researchgate.net/profile/Raviteja_Duggineni

Qualifications

- Feb 2016 - Dec 2017–Master's Student, University of Adelaide · Aerospace Engineering Australia.

Raviteja has met with team to discuss the design aspects of the experiment. This includes the material the casing will be made out of, the design of the experiment as well as what area's of science we should be focusing on. Raviteja has been working with Giordan Staines on the design of the on-ground experiment, as well as the test design for the space experiment. Not only did he introduce the team to titanium 3D printing, but he assessed our design and gave feedback on what we should improve on. From the first meeting with RaviTeja, the engineers of Project Toad have worked on improving the on-ground experiment design. The improvements will allow for the future experiments to be plausible within the given parameters. Raviteja offered to be Project Toad's industry partner, to which the team accepted his offer. The full extent to which the terms and conditions of being an industry partner are yet to be decided and agreed upon by the Department for Education and Child Development (DECD). DECD are funding the project so the guidelines must be created by them.

Up until this point in time Project Toad's only industry partner is Raviteja. The team plans to get in contact with more companies, if the experiment is selected. Building relationships with people and companies can be an immense help and provides support whether that be with advice and help or with using facilities and materials.
References


Taylor, M 2017, pers. comm., Various Dates.