FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68375
Scriptwriter Name: Pallavi Sharma
Project Page Link: https://review.jove.com/files_upload.php?src=20894378

Title: Production and Testing of Moisture Behavior and Thermal Properties of Rapeseed Straw and *Ganoderma resinaceum* Mycelium Bio-Composites

Authors and Affiliations:

Francisco Ortega Exposito*, Catherina Adriana (Ilse) Rovers*

Centre of Expertise MNEXT, Avans University of Applied Sciences

Corresponding Authors:
Francisco Ortega Exposito (fa.ortegaexposito1@avans.nl)

Email Addresses for All Authors:
Catharina Adriana (Ilse) Rovers (ca.rovers@avans.nl)
Francisco Ortega Exposito (fa.ortegaexposito1@avans.nl)

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? Yes

Authors: Please create screen capture videos of the shots labeled as SCREEN, create a screenshot summary, and upload the files to your project page as soon as possible: **https://review.jove.com/files_upload.php?src=20894378**

3. **Filming location:** Will the filming need to take place in multiple locations? No

Current Protocol Length
Number of Steps: 27
Number of Shots: 55

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Ilse Rovers:** The research aims to integrate mycelium bio-composites into the construction industry by developing materials competitive with conventional options, addressing questions of performance, durability, and applicability.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.10*

What research gap are you addressing with your protocol?

1.2. **Fran Ortega Exposito:** The protocol addresses the gap in ensuring correct production and testing of mycelium bio-composites in alignment with construction industry standards, with a focus on achieving repeatable and homogeneous experimental results.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What new scientific questions have your results paved the way for?

1.3. **Fran Ortega Exposito**: The material shows performance comparable to conventional construction materials, highlighting its promise. The next challenge is addressing durability and end-of-life management, a novel consideration for biobased construction materials.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: Figures 9 and 10*

What research questions will your laboratory focus on in the future?

1.4. **Ilse Rovers**: Our focus remains on mycelium construction materials, and in the future, we will also focus on pure mycelium materials, looking at intrinsic values of the species themselves and on mycelium bio-composites. We investigate automation at an industrial scale and upscaling.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Mycelium-Based Composites Production for Thermal Insulation Applications

Demonstrator: Fran Ortega Exposito

2.1. To begin, use a sterile inoculation loop to cut a fully colonized 100-millimeter diameter Petri dish of *Ganoderma resinaceum (GAN-uh-DUR-muh- rez-ih-NAY-see-um)* medium into four equal sections **[1]**.

2.1.1. WIDE: Talent using a sterile inoculation loop to cut a fully colonized 100 millimeter Petri dish into four equal sections. NOTE: A close up is added

2.2. Transfer two sections of the colonized medium to a sterile laboratory blender cup **[1]**. Add 50 milliliters of malt extract broth to the cup **[2]** and mix it with the mycelium on agar **[3]**. NOTE: The VO is edited for the additional shot

2.2.1. Talent placing two sections of colonized agar into a sterile lab blender cup.

2.2.2. Talent pouring 50 milliliters of malt extract broth into the same cup.

Added shot: Talent mixes the malt extract broth with mycelium on agar NOTE: A close-up of the mixture

2.3. Then, pour the inoculated broth mixture into the bag containing autoclaved cellulose and water **[1]**. Massage the contents of the bag manually for 2 minutes to ensure even mixing **[2]**.

2.3.1. Talent pouring the broth mixture into a bag of autoclaved cellulose and water.

2.3.2. Talent manually massaging the bag.

2.4. Now, set the climate chamber to 30 degrees Celsius and 80 percent relative humidity **[1]** and transfer the inoculated bag to the climate chamber for 5 days **[2]**. NOTE: The VO is edited for the additional shot

2.4.1. Talent sets the climate chamber at 30 degrees Celsius and 80 percent relative humidity. NOTE: Close-up also filmed

Added shot: Talent placing the inoculated bag inside a climate chamber.

2.5. For substrate preparation, place an empty bowl or bucket on a scale and tare it **[1]**.

Then, transfer the substrate into the bucket and record its weight **[2]**.

2.5.1. Talent placing a bowl on a digital scale and pressing the tare button.

2.5.2. Talent adding substrate to the bowl and recording the weight. NOTE: Close-up of the weight at 160 grams is filmed

2.6. Next, weigh demineralized water in a 1.65 to 1 ratio relative to the substrate **[1]**. Mix the substrate and water thoroughly with the hands **[2-TXT]**. Use a cement mixer for handling larger quantities of substrate **[3].**

2.6.1. Talent measuring the correct amount of demineralized water based on the substrate weight. NOTE: Close-up of the weight.

2.6.2. Talent mixing substrate and water thoroughly with hands.

2.6.3. Shot of the cement mixer and pouring content

2.7. Then, place the hydrated substrate mixture into autoclavable bags **[1]**. Place the bags in an autoclave and run a cycle at 121 degrees Celsius for 25 minutes **[2]**.

2.7.1. Talent transferring the mixed substrate into autoclavable bags.

2.7.2. Talent loading the bags into an autoclave and starting the cycle at 121 degrees Celsius for 25 minutes. NOTE: An Additional close-up shot is filmed

2.8. For substrate inoculation, using a scale, weigh the spawn to be 10 percent of the total wet weight of the substrate and water **[1]**. Pour the weighed spawn into the bag containing the sterilized substrate **[2]**.

2.8.1. Talent weighing the appropriate amount of spawn using a digital scale. [Speed up the weighing until 100 grams].

2.8.2. Talent pouring the spawn into the bag of substrate. NOTE: Only the pouring is filmed and then the close-up of 2.8.1.

2.9. Then, seal the bag using a heat sealer or tape **[1]** and shake or massage the bag gently for 2 minutes to distribute the spawn evenly **[2]**.

2.9.1. Talent sealing the inoculated bag with a heat sealer.

2.9.2. Talent gently massaging the sealed bag for 2 minutes.

2.10. For moulding, weigh the mold on a scale **[1]**. Fill it with the inoculated substrate and spread it evenly to create a flat surface **[2]**.

2.10.1. Talent placing an empty mold on the digital scale and pressing the tare button.

2.10.2. Talent filling the mold with inoculated substrate and spreading it evenly to create a flat surface.

2.11. Afterward, cover the mold with perforated foil **[1]** and secure it in place with tape **[2]**. Place the filled and covered molds in a climate chamber set to 25 degrees Celsius and 80 percent relative humidity for 7 days **[3-TXT]**.

2.11.1. Talent covering the filled mold with perforated foil.

2.11.2. Talent taping the foil securely to the mold.

2.11.3. Talent placing the molds into the climate chamber and setting the temperature and humidity parameters. **TXT: Inspect for uniform mycelium and even surface whiteness**

2.12. After approximately 7 days of growth, carefully remove the sample from the mold **[1].** Place it on baking paper in an oven set to 65 degrees Celsius for 24 hours to absorb moisture and prevent sticking **[2]**.

2.12.1. Talent removing the grown sample from the mold. Also: removing the sample from the climate chamber towards the LAF. NOTE: 2.11.1 and 2.11.2. are filmed in a single shot

2.12.2. Talent placing the sample on baking paper and loading it into an oven set to 65 degrees Celsius. NOTE: Close-up of setting the oven.

2. Material Testing of Mycelium-Based Composites

3.1. After calibrating the heat flow meter software, input the basic data and specimen description into the software **[1]**. Set the upper and lower plate temperature as required **[2]**. ~~Use the built-in thickness gauge to measure the sample thickness **[3]**~~. NOTE: The VO is edited for the deleted shot

3.1.1. SCREEN: SCREEN-3.1.1---Startpage-HFM

3.1.2. SCREEN: Scene-1.2---Startpage-thermal-conductivity-+-table.mkv

~~3.1.1. Talent using the built-in thickness gauge to measure the sample.~~ NOTE: Not filmed as this is not a physical step, but part of the machines procedure]

3.2. Then, place the mycelium-based composite sample in the heat flow meter and close the door **[1]**.

3.2.1. Talent positioning the sample in the heat.

3.3. Select the previously performed calibration **[1]**. Then, left-click to tick the **load setpoint** at 2.1 kilopascal, and click the **Start** button to initiate the test **[2]**.

3.3.1. SCREEN: SCREEN-3.3.1---Selecting-calibration.mkv

3.3.2. SCREEN: SCREEN-3.3.2---Load-setpoint

3.4. To measure specific heat capacity, place the mycelium-based composite sample inside the heat flow meter and close the door securely **[1]**.

3.4.1. Talent loading the sample into the heat flow meter, with the sample properly centred and closing the door.

3.5. Set the values specified in the set point table **[1]** and select the previously performed empty stack calibration **[2]**. Left-click to tick the **load setpoint** at 2.1 kilopascal, then click the **Start** button **[3]**.

3.5.1. LAB MEDIA: Table 1.

3.5.2. SCREEN: SCREEN-3.5.2---Empty-stack-calibration.mkv

3.5.3. SCREEN: SCREEN-3.5.3--Setting-the-load-and-starting.mkv: 00:00-00:05, 00:20-00:25

3.6. For Moisture absorption and desorption analysis, prepare a water box filled to one-quarter of its height with water **[1]**. Use three strips of plastic tape to suspend the mycelium-based composite sample above the water without touching it **[2]**. Then, measure the sample using a vernier calliper **[3].** NOTE: The VO is added for the additional shot

3.6.1. Talent filling a water box to one-quarter height. NOTE: Close-up of water height and sample positioning height is filmed

3.6.2. Talent positioning the sample using three plastic tape strips so it remains suspended above the water surface. NOTE: Wide angle shot

Added shot: Measuring with vernier calliper

3.7. At specified intervals of 0, 0.5, 1, 2, 4, 8, 24, and 48 hours, remove the sample from the water box, weigh it on a scale **[1],** and measure its dimensions quickly to minimize moisture loss **[2]**. After each measurement, return the sample to the water box and close the lid **[3]**.

3.7.1. Talent removing the sample at each interval and placing it on a scale.

3.7.2. Talent quickly measuring dimensions of the sample.

NOTE: There is an alternative shot “Talent measuring with other talent noting down the dimension measurements” filmed

3.7.3. Talent returning the sample to the water box and sealing it with a lid.

3.8. After 48 hours, take the sample out of the water box **[1]** and place it in a climate chamber set at 25 degrees Celsius and 40 percent relative humidity **[2]**.

3.8.1. Talent removing the sample from the box after the final interval. NOTE: Wide-angle shot

3.8.2. Talent placing the sample in a climate chamber with the specified settings. NOTE: Filmed wide-angle & close-up of the settings

3.9. For compressive strength measurements, use a vernier calliper to measure the dimensions of the mycelium-based composite sample, following standard procedure **[1]**. Place the sample in the universal testing machine, positioning it centered above the lower compression plate **[2]**.

3.9.1. Talent measuring length, width, and height of the sample using a vernier calliper.

3.9.2. Talent placing the sample carefully between the compression plates in the universal testing machine.

3.10. Then, turn on the universal testing machine **[1]** and start the operating software to ensure proper connection **[2]**.

3.10.1. compression of sample computed with screen. NOTE: The shot is modified and an additional shot of sample being compressed is filmed

3.10.2. SCREEN: SCREEN-3.10.2---Starting-Horizon-software.mkv: 00:06-00:13

3.11. In the software, search for **ISO 29469** *(I-S-O-Two-Nine-Four-Six-Nine)* using the search tool. Right-click the listed standard and press **Edit Method** to proceed **[1]**. Press the **Start Experiment** button in the universal testing machine software to start the compression test **[1]**.

3.11.1. SCREEN: SCREEN-3.11.1---Library.mkv

3.11.2. SCREEN: SCREEN-3.11.2---Test-start.mkv

3.12. To condition fresh samples, suspend them in the water box for 24 hours as previously described **[1-TXT]**. Transfer unused, humid samples to an oven set to 50 degrees Celsius with fan ventilation for 12 hours **[2-TXT]**.

3.12.1. Talent placing additional samples in the water box, suspended using tape. **TXT: Repeat the measurement and compression steps for the humid samples**

3.12.2. Talent placing humid samples into an oven with fan ventilation set at 50 degrees Celsius. **TXT: Repeat the compression testing for the post-dried samples**

NOTE: Close-up of the settings is filmed

3.13. To assess water repellence, add 9 milliliters of water with 1 milliliter of blue dye in a 20-milliliter Erlenmeyer **[1]** and mix thoroughly **[2]**.

3.13.1. Talent pipetting 9 milliliters of demi water with a graduated 10 ml pipette and 1 milliliter of blue dye with a 1 ml micropipette into a 20 milliliter erlenmeyer. NOTE: Different close-ups of measures (9 ml and 1 ml and whisking).

3.13.2. Talent mixing the solution with a whisker.

3.14. Then, using a thin felt-tip pen, divide the surface of the mycelium-based composite sample into four quadrants **[1]**.

3.14.1. Talent drawing with felt pen quadrant lines on the sample surface as shown in the reference figure.

3.15. Using a micropipette, measure 100 microliters of the dyed water **[1]**. Place one droplet on a flat surface in each of the four quadrants **[2]**. Using a tripod-held camera aligned at eye level with the top surface of the sample, take a photograph of each droplet **[1]**.

3.15.1. Talent measuring out 100 microliters of blue-dyed water with a micropipette. NOTE: Close-up of 100 ml on micropipette.

3.15.2. Talent placing one droplet in each of the four quadrants on the sample surface. NOTE: close-up is filmed

3.15.3. Talent positioning a camera on a tripod at eye level and capturing close-up images of the four droplets. NOTE: Still needed in the shot while editing: a schematic drawing of droplet on surface with angle lines

Results

4. Results

4.1. Samples that passed visual inspection showed a white, uniform surface with smooth texture and no discoloration or flaking **[1],** matching the expected appearance for adequately grown mycelium-based composites **[2].**

4.1.1. LAB MEDIA: Figure 4.

4.1.2. LAB MEDIA: Figure 5.

4.2. Visibly non-adequate mycelium-based composite samples exhibited signs of contamination, including multicolored surface patches such as green, black, yellow, and blue **[1],** as well as areas with uneven texture and flaky or overgrown regions **[2].**

4.2.1. LAB MEDIA: Figure 6.

4.2.2. LAB MEDIA: Figure 7.

4.3. A third example of poor-quality MBC showed a loosely packed structure with visible straw particles and incomplete mycelium coverage **[1].**

4.3.1. LAB MEDIA: Figure 8. *Video editor: Highlight the straw fibers protruding from the sample and the white mycelium unevenly distributed across the panel.*

4.4. The average thermal conductivity of the samples remained consistently low at 0.0367 watts per meter-kelvin**,** confirming adequate insulation properties **[1].**

4.4.1. LAB MEDIA: Table 2. *Video editor: Highlight the final average value row for "Thermal Conductivity."*

4.5. The average compressive strength was highest in the post-dried condition at 24.99 kilopascals **[1],** followed by the dry state at 21.02 kilopascals **[2],** and lowest under wet conditions at 14.85 kilopascals **[3].**

4.5.1. LAB MEDIA: Figure 9. *Video editor: Highlight the bar labeled "Post-dry" showing 24.99.*

4.5.2. LAB MEDIA: Figure 9. *Video editor: Highlight the bar labeled "Dry" showing 21.02.*

4.5.3. LAB MEDIA: Figure 9. *Video editor: Highlight the bar labeled "Wet" showing 14.85.*

4.6. A clear relationship was observed between density and compressive strength. The wet samples showed the highest density and the lowest strength **[1],** while post-dried samples exhibited lower density and the highest strength **[2].**

4.6.1. LAB MEDIA: Figure 10. *Video editor: Highlight the data point labeled "Wet" at high density and low strength.*

4.6.2. LAB MEDIA: Figure 10. *Video editor: Highlight the data point labeled "Post-dry" at lower density and higher strength.*

- **Ganoderma resinaceum**
  - Pronunciation link: https://www.howtopronounce.com/ganoderma-resinaceum How To Pronounce
  - IPA (American English approx.): /ˌgænəˈdɜːrmə ˌrɛzɪˈneɪsiəm/
  - Phonetic spelling: GAN-uh-DER-muh rez-ih-NAY-see-um
- **Mycelium**
  - Pronunciation link: https://www.merriam-webster.com/dictionary/mycelium Merriam-Webster
  - IPA: /maɪˈsiːliəm/ Cambridge Dictionary+1
  - Phonetic spelling: my-SEE-lee-um
- **Autoclave**
  - Pronunciation link: https://dictionary.cambridge.org/dictionary/english/autoclave Cambridge Dictionary
  - IPA: /ˈɔːtəˌkleɪv/ (US also /ˈɑːtəˌkleɪv/) Cambridge Dictionary
  - Phonetic spelling: AW-tuh-klayv
- **Thermal Conductivity**
  - Pronunciation link: (there's no single word link, but "thermal" and "conductivity" separately in reliable dictionaries)
  - IPA: /ˈθɜːrməl kənˌdʌkˈtɪvɪti/
  - Phonetic spelling: THUR-muhl kon-duhk-TIV-ih-tee
  - Meaning: It's a measure of how well (or how fast) a material conducts or transfers heat through it. Encyclopedia Britannica+1
- **Specific Heat Capacity**
  - Pronunciation link: (again, separate parts are in dictionaries; "specific heat capacity" is a phrase)
  - IPA: /spəˈsɪfɪk hit kəˈpæsəti/
  - Phonetic spelling: spe-SI-fik heet kuh-PAS-uh-tee
  - Meaning: The amount of heat (energy) required to raise the temperature of 1 unit mass of a substance by 1 degree (Celsius or Kelvin). Wikipedia+1

Compressive Strength

- Pronunciation link: (same: “compressive” and “strength” separately)
- IPA: /kəmˈprɛsɪv strɛŋkθ/
- Phonetic spelling: kum-PRES-iv STRENGKTH
- Meaning: The maximum compressive load (force per area) that a material can withstand before failing (crushing, breaking) under compression. The Engineering Choice+1