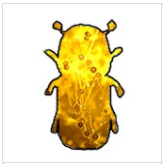

Nov 20, 2020

Rearing Ambrosia Beetles in Media

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1 Works for me dx.doi.org/10.17504/protocols.io.bnupmevn

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

The purpose of this protocol is to produce a supply of living beetles in various developmental stages in the laboratory.

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

DOI

dx.doi.org/10.17504/protocols.io.bnupmevn

DOCUMENT CITATION

James Skelton, Sawyer Adams, Andrew J. Johnson 2020. Rearing Ambrosia Beetles in Media. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bnupmevn

CREATED

Oct 23, 2020

LAST MODIFIED

Nov 20, 2020

DOCUMENT INTEGER ID

43631

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The protocol is particularly useful for the production of pupae, which can serve to develop aposymbiotic adults, when removed from the gallery and reared in the absence of the fungus.

Beetle Rearing Media Recipe modified from: Cruz, L. F., Rocio, S. A., Duran, L. G., Menocal, O., Garcia-Avila, C. D. J., & Carrillo, D. (2018). Developmental biology of Xyleborus bispinatus (Coleoptera: Curculionidae) reared on an artificial medium and fungal

cultivation of symbiotic fungi in the beetle's galleries. Fungal Ecology, 35, 116-126.

Makes ~ 25 tubes

60 g of wood flour
15 g coarse sweetgum sawdust
20 g agar
10 g sucrose
5 g corn starch
5 g casein
5 g yeast extract
1 g Wesson salt mixture
0.35 g streptomycin
5 mL 95% ethanol
2.5 mL wheat germ oil
500 mL distilled water

Mix solid ingredients, then incorporate water. Autoclave mixture for 30 min. Add Streptomycin to ethanol and vortex. Add strep/ethanol mixture to media, and mix thoroughly to re-suspend settled ingredients. Pour 15-20 mL media into each 50 mL Falcon® tube, working aseptically in flow hood. Loosely cap tubes and leave to dry 1 week in flow hood.

When the media is fully dry, scratch the surface of the media with a sterile scalpel or other sterile pointy object so that the beetles have something to grip. The tubes may be pre-inoculated with the beetle's preferred fungus 3-4 days before placing the beetles in the tubes. Place a small square of preferred fungus onto the media surface and leave the lid slightly unscrewed. This ensures a higher success rate and prevents the media from being colonized by unwanted fungi.

Once you have collected the beetles, examine them in a filter paper lined petri dish under a microscope and use forceps to remove as many mites as possible. If mites remain on the beetle, they can take over the tube of media, as well as spread it to the other tubes. Place one female beetle in each tube. To prevent mites traveling between tubes, place the falcon tubes upright and spread out in a rack that is lined with insecticidal paper. They can also be placed in a rack which is submerged in a soapy water bath so that the tops of the tubes are still above the water, but this method is less space efficient. The beetles also prefer the dark, so an upside down opaque bin can be placed over the Falcon tubes. The beetles should then be left alone for approximately 28 days for Xyleborus ferrugineus. Xyleborus affinis needs around 26 days to catch the pupae before they mature into adults. They can be checked periodically for mites and overgrowth of fungi. Completely mite infested tubes should be disposed of to prevent them from spreading to other tubes. The presence of frass at the surface of the media is a good sign that the beetles are doing okay.

To extract the pupae, lay out some paper towels to protect the work surface, and prepare 2 petri dishes: one for adults and one for pupae. Place a round cut of paper towel in the bottom of the petri dish and wet with one drop of water. Cut off the tip of end of the Falcon tube with a box cutter VERY CAREFULLY. It helps to roll the Falcon tube slightly while cutting. You can then push the end of a paintbrush through this hole to push the media out of the opening of the Falcon tube. If the media is too squishy to remove initially, use a scalpel to separate the edges of the media from the tube and try again. Once the media is removed, use a scalpel to cut away small pieces of media until you reveal a segment of a gallery. A paintbrush may be used to remove pupae and large larvae, and soft touch forceps can be used to remove adults and place them into their respective petri dishes.

The extracted beetles can be used to rear multiple subsequent generations of beetles. To remove mites and contamination, the beetles can be dipped for a few seconds in a bath of 50% ethanol, and then for a few seconds into sterile water. Put 4-5 females and at least 1 male into a pre-inoculated media tube. If only females are used, there is a chance that the brood will only consist of males if the females have not been fertilized, but only females can be used if no males were produced in the first generation. Repeat the rearing process used for the first generation.

Citation: James Skelton, Sawyer Adams, Andrew J. Johnson (11/20/2020). Rearing Ambrosia Beetles in Media. https://dx.doi.org/10.17504/protocols.io.bnupmevn