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ABSTRACT

This publication is the second of a series of seven supplementary investigative materials for use in secondary science classes providing up-to-date research-related investigations. This unit is structured for grade levels 7 through 9. It is concerned with food value of liquified cellulosic agricultural wastes: paper, straw, corn cobs, sawdust and food processing plant wastes. This guide provides students with the opportunity to study fungi (mushrooms) and the effect they have on common waste material found in and around the home and school. The first part of this guide describes: (1) materials needed; (2) preparing nutrient solutions; (3) sources for spawn; and (4) suggested reading. The second part provides students with background information and one laboratory investigation. The information consists of: (1) materials needed for a four-student team; (2) procedures; (3) questions for thought; (4) extending the investigation; and (5) suggested readings. (HM)

science study aids

2

mushrooms:
nature's recyclers

Developed by Bill McConnell, a science teacher at Redwood High School in Larkspur, California. Mr. McConnell prepared the manuscript in cooperation with Dr. Ralph H. Kurtzman, Jr., scientist of the Agricultural Research Service, U. S. Department of Agriculture, at the Western Regional Research Center, Berkeley, California.

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TO THE TEACHER

This Science Study Aid (SSA) is structured for grade levels 7 through 12. It is based on research conducted by ARS scientists at Berkeley, California, seeking the development of new processes to improve the feed value of lignified cellulosic agricultural wastes. The waste materials include paper, straw, corn cobs, sawdust, and food processing plant wastes.

A primary purpose of such research is to develop techniques employing the action of bacteria and fungi on various cellulosic waste products to convert them into protein for food and feed. One project deals with the growth, nutritional requirements, and improved flavor of the edible oyster mushroom, Pleurotus ostreatus.

This SSA will provide students with the opportunity to study fungi and the effect they have on common waste material found in and around the home and school. They will conduct their studies in much the same way research is conducted in ARS laboratories.

This SSA uses the inquiry method to help develop meaningful group or class discussions. Also, extensions of the investigation are provided for students who show special interest and for more able learners. Most activities can be carried out using standard laboratory equipment or improvised equipment found in the home, supermarket, or hardware store.

The material will be of particular value in general science and biology courses during studies of plants, energy cycles, nutrition or ecology. An essential part of this SSA is the background material. Students should receive the background information a few days prior to the laboratory investigation. This will allow sufficient time for you and your students to survey the suggested reading material in the SSA, and for students to complete any readings you assign from textbooks. Students may work individually or in teams as they carry out the activities in the Science Study Aid.

MATERIALS LIST

For your convenience, the materials needed to perform the Investigation in this Science Study Aid are listed below. The list gives the quantities needed for each student.

STUDENT MATERIALS

shredded paper
straw
large pans in which to mix paper and straw
spawn - Pleurotus ostreatus
1 one-pint jar
forceps
spoons or glass stirring rods

TEACHER MATERIALS

(for a class of 36 students)

300 ml dark molasses
18 g Ammonium Nitrate (NH_4NO_3)
6 g Ammonium Sulfate $(NH_4)_2 SO_4$
6 g Monobasic Potassium Phosphate KH_2PO_4
5700 ml water
1 large mixing beaker

PREPARING NUTRIENT SOLUTIONS

You will need about 2 liters of nutrient solution for a dozen pint-size jars packed with the straw and paper mixture. Make the solution by mixing together:

100 ml dark molasses
6 g Ammonium Nitrate NH_4NO_3
2 g Ammonium Sulfate $(NH_4)_2SO_4$
2 g Monobasic Potassium Phosphate KH_2PO_4
1900 ml water H_2O

SOURCES FOR SPAWN

Order two weeks in advance from:

*American Type Culture Collection
12301 Parklawn Drive
Rockville, Maryland 20852

Order: Pleurotus ostreatus
#9415, #9424, or #9427

Order gray Pleurotus ostreatus spawn from the following companies:

*The Kinoko Company
P. O. Box 6425
Oakland, California 94621

*Somycel U. S. Inc.
P. O. Box 476
Avondale, Pennsylvania 19311

*King Mushroom Company
P. O. Box 607
Moses Lake, Washington 98837

*Old World Mushroom Company
833 West 17th Street
Costa Mesa, California 92527

*Fee charged (minimum quantity and price vary)

Oyster mushrooms have been grown on both fresh and dry straw. The substrate material in this SSA requires a mixture of straw and paper. Either fresh or dry straw can be used. Cut it into 3-to-4 inch lengths or chop in a blender. Two sheets of newspaper (23" x 30"), or the equivalent from magazines or other scrap paper, cut or shredded in 1/4-inch widths, are required per pint jar. Students could prepare the straw and paper, but you can save time by doing the cutting with a paper cutter prior to beginning the investigation.

If your spawn culture arrives growing on an agar slant, the following procedure is recommended for developing adequate grain spawn cultures for your classes:

1. Cook rice, wheat, or other whole grain until tender. The rice or grain must not stick together in a mass! Drain well!
2. Fill 4-oz. jars with the cooked grain. (One cup uncooked rice will fill 5 or 6 4-oz. jars.)
3. Autoclave at 15 psi for 1/2 to 3/4 hour. Allow the jars to cool to room temperature (68° F.). Larger jars require longer sterilization.
4. Using aseptic technique, transfer the mycelium of Pleurotus ostreatus to the sterilized grain. Culture the jars at room temperature for two weeks prior to carrying out the investigation in this SSA. Every other day, shake the bottles to mix the enclosed grain.

WARNING WARNING

The mushrooms that will be grown in these investigations are to be considered as research materials. Under NO circumstances are they to be eaten.

SUGGESTED READING FOR THE TEACHER

J. Agr. and Food Chem., Block, S. S., Tsao, G., Lunghwa, H., 1958, "Production of Mushrooms from Sawdust", Vol. 6, pp. 923-927.

Mycologia, Eugenio, Cesaria P., and Anderson, Neil A., 1968, "The Genetics and Cultivation of Pleurotus Ostreatus"., Vol. LX, No. 3, pp. 627-634.

Fr. Minn. Agr. Expt. Sta. Bull, Kaufert, F.

H. 1936, "The Biology of Pleurotus corticatus", 114, 35 p.

Mushrooms and Truffles, Singer, R., 1961, Leonard Hill Books, Ltd., London.

FILMS

Microbiology, Part II - Fungi, No. 6, 1961, (Sound, color, 27 min.), purchase or rent, AIBS, McGraw Hill.

You may wish to write to the following address for information on up-to-date films and film strips:

American Mushroom Institute
P. O. Box 373
Kennett Square, Pennsylvania 19348

TO THE STUDENT

BACKGROUND

How would you like to grow some mushrooms and, at the same time, help solve part of the pollution problem?

Scientists of the U. S. Department of Agriculture's Agricultural Research Service (ARS) at the Western Regional Research Center (WRRC), Berkeley, California, are doing some exciting things researching the problem of the effect of fungi and microbes on common waste products. One project deals with the growth, nutritional requirements, and improved flavor of the edible mushroom, Pleurotus ostreatus (Jacquin) Fries. In addition, cellulosic wastes such as corn cobs, waste paper, sawdust, and straw are being used to grow this fungus (mushroom). As this research proves successful, another step toward higher agricultural yield with little increased land use will be made. These mushrooms provide better protein than most vegetables and can be grown in a very small area as compared with other food plants. At the same time, many of the common waste products found around the home and at school are being used for the growth substrate (the material on which the plants grow). Sound exciting? Well, it is! Can you envision people throughout the country some day growing their own highly nutritious mushrooms in their homes using their waste paper and old magazines to grow on? Maybe you will be one of the first to do so.

Mushrooms -- the best known of the fungi -- are found in fields, woodlands, and orchards. They appear suddenly after spring and autumn rains. We seldom see the vegetative mushroom plant. It is a structure called a mycelium and is composed of many white thread-like structures called hyphae, which spread out through the soil or the wood of a decaying stump or log. Digestive enzymes, like those in your intestinal tract, are secreted by the hyphae and break down organic substances in the host and convert them to nutrients that can be absorbed and used as nourishment.

At certain times, especially during the spring and autumn, small knobs develop on the mycelium. These consist of masses of tightly packed hyphae. The knobs or buttons develop into the familiar spore-bearing structure we recognize as a mushroom (Fig. 1). The mature mushroom consists of a stalk, or stipe, which supports the umbrella-shaped cap, or basidiocarp. After emerging from the soil, the cap opens out, in some cases, leaving a ring, or annulus, around the stipe at the point where the cap and stipe were joined (Fig. 2). However, your mushrooms will not appear exactly like the ones described. They will look like the mushroom in Figure 3.

FIG. 1

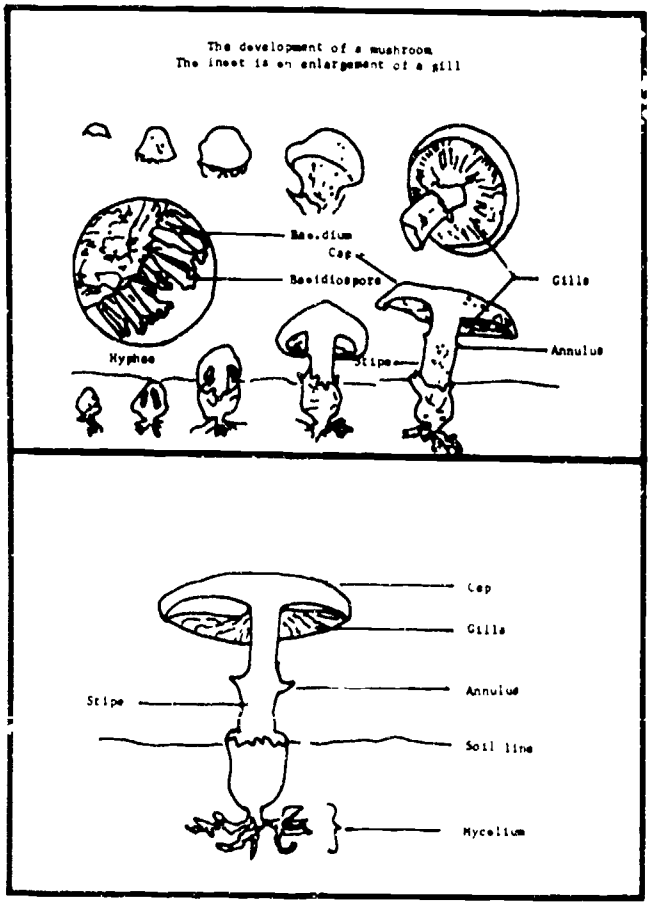


FIG. 2

Most mushrooms have numerous plate-like gills on the undersurface of the cap, or basidiocarp. On the surface of each gill and extending all around it are many small basidia, each of which bears four basidiospores, or reproductive structures. A single mushroom may produce as many as ten billion spores. These spores drop from the basidia when mature and are carried by air currents and the wind. Each spore can form a new mushroom mycelium if it happens to land in a place favorable for growth; if not, the spore dies. Some basidiospores are black, while others are pink, yellow, white, or brown.

Do you think that reference materials in your room or library might give you a better understanding of the characteristics and life cycles of the fungi? Chances are, your teacher has assigned readings on the subject from the textbook and you may wish to review this material. Obviously, there are a number of questions that you can think of and your review of the available literature should help you answer these questions.

Like ARS scientists, you will now consider a research problem dealing with the action of fungi on straw and waste paper. Finally, a successful research program always uncovers new questions which lead to new ideas along with schemes to test these ideas. The careful and informed researcher always will generate more questions than his present investigative activities can answer.

How about you? Will your work lead to new and more interesting ideas related to the study of the fungi?

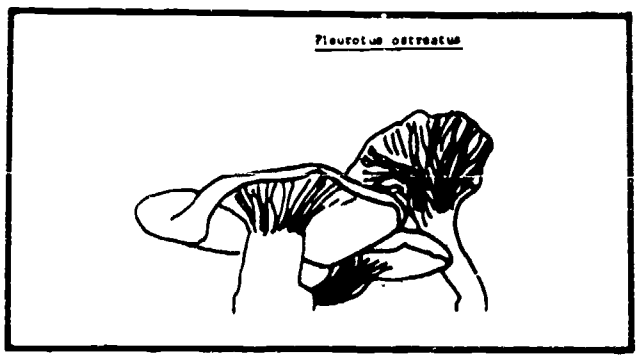


FIG. 3

INVESTIGATION 1 - THE GROWTH STUDY OF THE OYSTER MUSHROOM

As you progress through this Investigation, plan your time schedule so all initial laboratory activities can be finished during the allotted time. This is a long-range laboratory study requiring a number of observations over the next four to eight weeks. Observe the growth activities each day and, when you have made your observations, record this data in your notebook. When your mushrooms have reached maturity, consider the questions at the end of the Investigation. Do you think your observations and the data from your notebook will assist you when answering these questions?

MATERIALS

1 pint jar per student
shredded paper
straw
nutrient solution (provided by teacher)
spawn - Pleurotus ostreatus (provided by teacher)
autoclave or 21-quart pressure cooker
forceps
spoons or glass stirring rods
large pans to mix paper and straw

PROCEDURE 1 (First Day)

1. Place previously cut (long thin pieces approximately 1/4" wide), waste paper (old magazines or newspapers) in a large pan. Then add an equal amount of straw and mix thoroughly.
2. Slowly add the nutrient solution provided by your teacher. Mix the solution thoroughly with the paper and straw mixture using a spoon or glass stirring rod. Add only enough nutrient solution to moisten the paper and straw. Pour off any excess that collects in the bottom of the pan.
3. Transfer enough straw and paper mixture into a pint canning jar to fill it 2-1/2" from the top. Place the lid on the jar with the

inside lid upside down (it must not seal) and give it to your teacher. Your teacher will now autoclave (sterilize) your jar in a pressure cooker and return it to you tomorrow at the beginning of the laboratory period.

PROCEDURE 2 (Second Day)

1. When you receive your jar, remove the lid carefully to permit as little contamination as possible. Get the "spawn" from your teacher and, using clean forceps, place the grain just under the surface of the paper and straw mixture (Fig. 4). Carefully mix the spawn into the substrate. Your jars have now been spawned by putting in grain which has the fungus (mushroom) growing on it. Label your jar with your name and the current date.
2. Replace the lid loosely on your jar. Be sure that the lid is not fastened tightly. Leave it loose! Store your jar in a cool place in the laboratory out of direct sunlight.
3. Design a data table on which you can record observations of growth activities in your jar during the next four to eight weeks. Table I suggests how you might do this. The suggested data table is designed for bi-weekly observations; however, you may wish to add more space and make observations daily.
4. After approximately 3 weeks, replace the jar lid with a loose-fitting plastic bag. Keep the mixture moist, but not wet.
5. Review and think about the data you have recorded during each observation over the past four to eight weeks. Then write up the results of your investigation by taking into consideration the following questions.

QUESTIONS FOR THOUGHT

1. During any part of the period for observation and study of your jar, did you notice any color changes in the substrate (paper and straw mixture)? If so, what color changes took place and how might you explain them?
2. After "spawning" your jar, when did you first notice the appearance of a mycelium? (How long after the appearance of mycelium did the reproductive bodies (buttons) appear? How long after the appearance of the buttons did full-grown mushrooms form?
3. Considering the questions in number 2, can you suggest ways to accelerate (to speed up) the growth of this fungus plant? Explain.
4. After 3 to 4 weeks, examine some of the paper and straw mixture and describe any physical change in the substrate. What control would you use to base your observations on?
5. Relate your investigation to the fact that most small branches and leaves disappear naturally in a forest or woodland.
6. Do your mushrooms have the same shape as canned or fresh mushrooms purchased in a supermarket? If they appear different, describe the differences.
7. During the growth and study of your oyster mushrooms, was your culture successful? If so, can you suggest reasons for your success? If not, try to explain what might have gone wrong during the experiment.

EXTENDING THE INVESTIGATION

1. Design an experiment using a variety of substrate mixtures such as chopped corn cobs, leaves, sawdust, and different types of grain.
2. Design an experiment to test the effect of variable humidity, temperature, and light on the growth of the oyster mushroom. In addition, you might consider using different nutrient solutions or changing the concentrations of the nutrients used in this SSA.

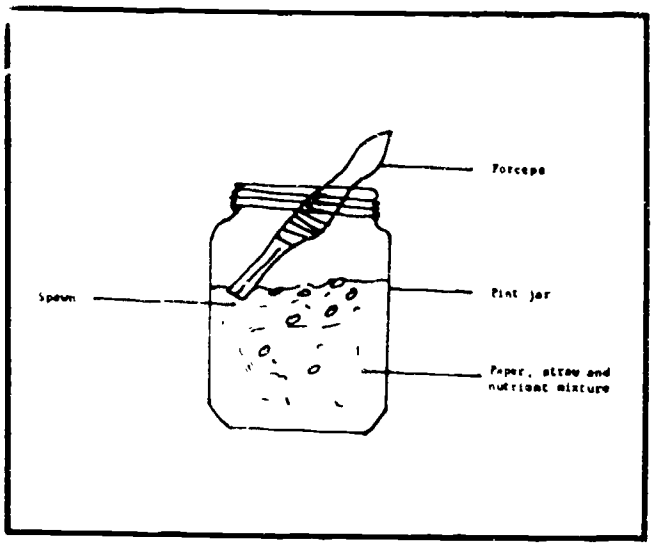


FIG. 4

3. Design an experiment to test the yield by weight of oyster mushrooms grown on cellulosic wastes with that of some food plant like lettuce grown in soil.

Common Edible Mushrooms, Christensen, Clyde M., 1943, C. T. Branford Co., Newton Centre, Mass.

Molds and Men, Christensen, Clyde M., 1951, U. Minn. Press, Minneapolis, Minn.

Illustrated Genera of Wood Decay Fungi, Ferguson, Charles, 1963, Burgess Publishing Co., Minneapolis, Minn.

The Story of Mosses, Ferns, and Mushrooms, Sterling, Dorothy, 1955, Doubleday and Co., Inc., Garden City, N. Y.

SUGGESTED READINGS FOR STUDENTS

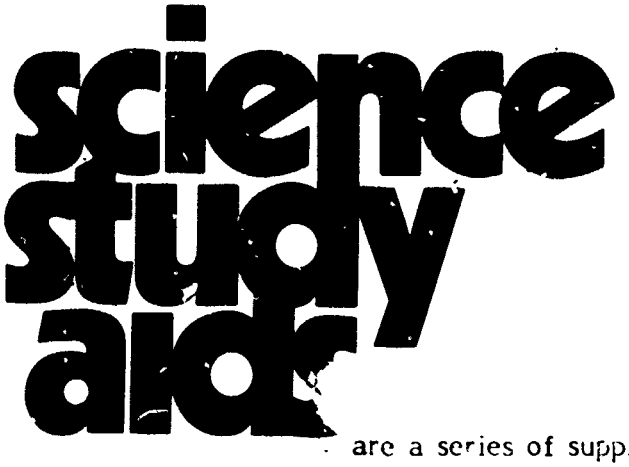
Mushroom Growing Today, Atkins, F. C., 1956, 3rd Ed. Macmillan Co., New York, N. Y.

Sc. Am., Bonner, J. T. 1956, "The Growth of Mushrooms", 194 (5) 97.

TABLE 1

OBSERVATIONS ON GROWTH OF OYSTER MUSHROOMS

Date	Check When They Appear			Notes on Observations
	Mycelium	Buttons	Caps	



are a series of supplementary investigative materials for use in secondary science classes, grades 7 - 12. The materials are based on federal and private research programs. They are written by secondary science teachers working with scientists at research facilities throughout the country. Before being published, they are tested in the laboratory and in classrooms of cooperating teachers.

Several times during the year, new SSA's are developed. If you want to be notified of their availability, request that your name be added to Wordwork's mailing list. Because we cannot provide enough copies for students, we have designed SSA's so that teachers can easily reproduce the student portion for their classes.

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