Chapter 1

Investigating Fungi Which Cause Rot and Decay

John A. Johnson

Forestry Department
University of New Brunswick
Fredericton, N.B. E3B 6E1

John A. Johnson is a native of New Brunswick, Canada, and received his B.Sc. and M.Sc. from the Biology Department at the University of New Brunswick in Fredericton. He is currently working on his Ph.D. in forest pathology and is a lecturer at the Saint John campus of U.N.B. His research involves antibiotic and toxin production by fungi found inside conifer needles and the potential for evergreens as a source of novel compounds produced by the fungi within.

Reprinted from: Johnson, J. A. 1990. Investigating fungi which cause rot and decay. Pages 1–13, *in* Tested studies for laboratory teaching. Volume 11. (C. A. Goldman, Editor). Proceedings of the Eleventh Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 195 pages.

Contents

Introduction

On land, fungi are important decomposers and major recyclers of nutrients. In forest ecosystems the decomposition of forest litter is essential to nutrient recycling. The humus in the lower litter layer and the soil are very much a product of the complex relationships between the carbon sources (dead plant and animal tissue) and organisms that decompose these compounds. Fungi are able to decompose material as rigid as wood and reduce it to a soft almost paper-like substance.

Fungi have been included in many groups over the years since the detailed study of them began. Initially they were studied by botanists because they were often found associated with plant habitat or the plants themselves. For many years fungi were considered "lower" non-photosynthesizing plants, in fact some plant pathology textbooks written since 1980 still consider them as such. Mycologists, in general, recognize fungi as belonging to their own kingdom and this has been accepted by biologists in other areas as well.

The kingdom Fungi is a large and diverse group. Fungi possess many characteristics which provide ways to categorize those organisms of interest. Some are parasites, others pathogens, and still others are saprophytes. Within these groups are the various classes: Ascomycetes, Basidiomycetes, and Deuteromycetes. Fungi are capable of taking on many shapes and fill many niches. Within the groups mentioned above are fungi which eat everything from birch trees to leather, and crude oil to kerosene.

As fascinating as fungi are, this introduction is not meant to provide a comprehensive overview of mycology. Instead it emphasizes the diversity of fungi. Having done this, the next step is to begin looking at one particular aspect of fungi found in forests everywhere. This exercise introduces the role of fungi that cause rot and decay in the forest ecosystem and, in particular, the study of white rot fungi both in the forest setting and in the laboratory.

The first thing to know about white or brown rots is that the fungus itself may be virtually any color, the name comes from the color of the wood as a result of the activity of the fungus (this will be explained in more detail further on). The fungi being considered are members of the basidiomycetes, most often recognized by the large fruiting bodies (conks) associated with them.

Fungi are expert at digesting and decomposing nutrient sources not available to many other organisms. Competition for nutrient material comes from bacteria, insects, and other fungi. External digestion using enzymes excreted from the hyphae and the subsequent ingestion through

absorption of compounds is a short description of how many wood digesting fungi operate. The hyphae ramify throughout the material after initial colonization by spores or hyphal fragments.

Fungi which eat woody plant material are found in forests everywhere. In fact, fungi which cause rot and decay are essential for the maintenance of the balance of new dead plant material, soil formation, and carbon cycling. Such fungi are saprophytes since they act on dead wood material. It is estimated that 40–60% of woody plant material is made up of cellulose, while lignin constitutes between 20 and 30% (Hudson, 1980). Cellulose and lignin are carbon rich compounds and it is vital to ecological cycles that this carbon be made available.

Different forest types have different characteristics that help or hinder the decomposition of dead wood material. The fast growing and abundant trees found in tropical locations have rich humid environments and the fungi are able to keep up with decaying various wood sources. Pine forests on the other hand are poor sites, with low pH and are difficult sources of cellulose and lignin for fungi.

The rot-causing fungi are often concerned with the **cellulose** and **lignin** compounds of the forest (Figure 1.1). Specific enzymes suited to breaking bonds in the long cellulose chains as well as the difficult to get at lignin structures allow these compounds to be broken up into shorter chains and the carbon becomes available for the fungi to use. This happens on a vast scale in our forests. It has been estimated that 90% of the energy in a mature forest flows along the decomposition pathway (Figure 1.2) and 10% is grazed as living material (Odum, 1975). Clearly this is an important aspect of the forest ecosystem.

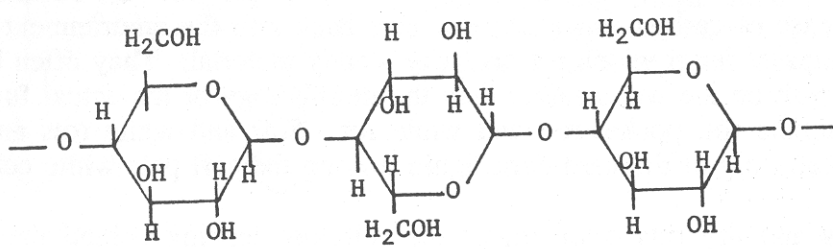

Figure 1.1. Chemical structures of cellulose and lignin.

Figure 1.2. Simplified scheme of energy flow in a forest. Approximately 10% is used by grazers (insects, etc.) and 90% by decomposers.

Decomposition is important in the forest in order to prevent vast build-ups of dead material, and for the release of carbon and other elements back into the environment (Figure 1.3). There are several groups of fungi which rot or decay woody material. They often have common names based on the result on the wood rather than the morphology of the actual fungus. Examples are: soft rot, dry cubicle rot, pocket rot, and white rot. Soft and white rots are often related since white rots typically digest the hard dark lignin leaving the soft pale-white cellulose behind, hence white soft rot.

Brown rots and dry rots often digest the cellulose leaving behind the brown lignin which eventually dries and cracks in a brick or cubical fashion. While all of these rots affect dead woody material in the forest they can also affect wood in service, such as posts, poles, and wood structures like steps and floor joists. Some forest pathogens also employ the same or similar enzymes while infecting and digesting trees. These fungi, as well as many others, are in great abundance in North American forests.

This exercise can be adapted to suit almost any region where trees are available in groups or small stands. Wooded lots, small stands on school property, and local parks are all suitable sites; a backyard may also provide the necessary components to utilize some aspect of the role of fungi in forest ecology.

This exercise will provide information on what to look for and do in a general way, and will be approached in three parts, each giving a list of important points and any materials and methods required. The three parts are: Out Among the Trees, Laboratory Demonstrations, and Student Activities. Any one of these parts can be used alone or in any combination as desired. Some of the information and tasks may fit into an existing laboratory or be followed-up by an additional laboratory.

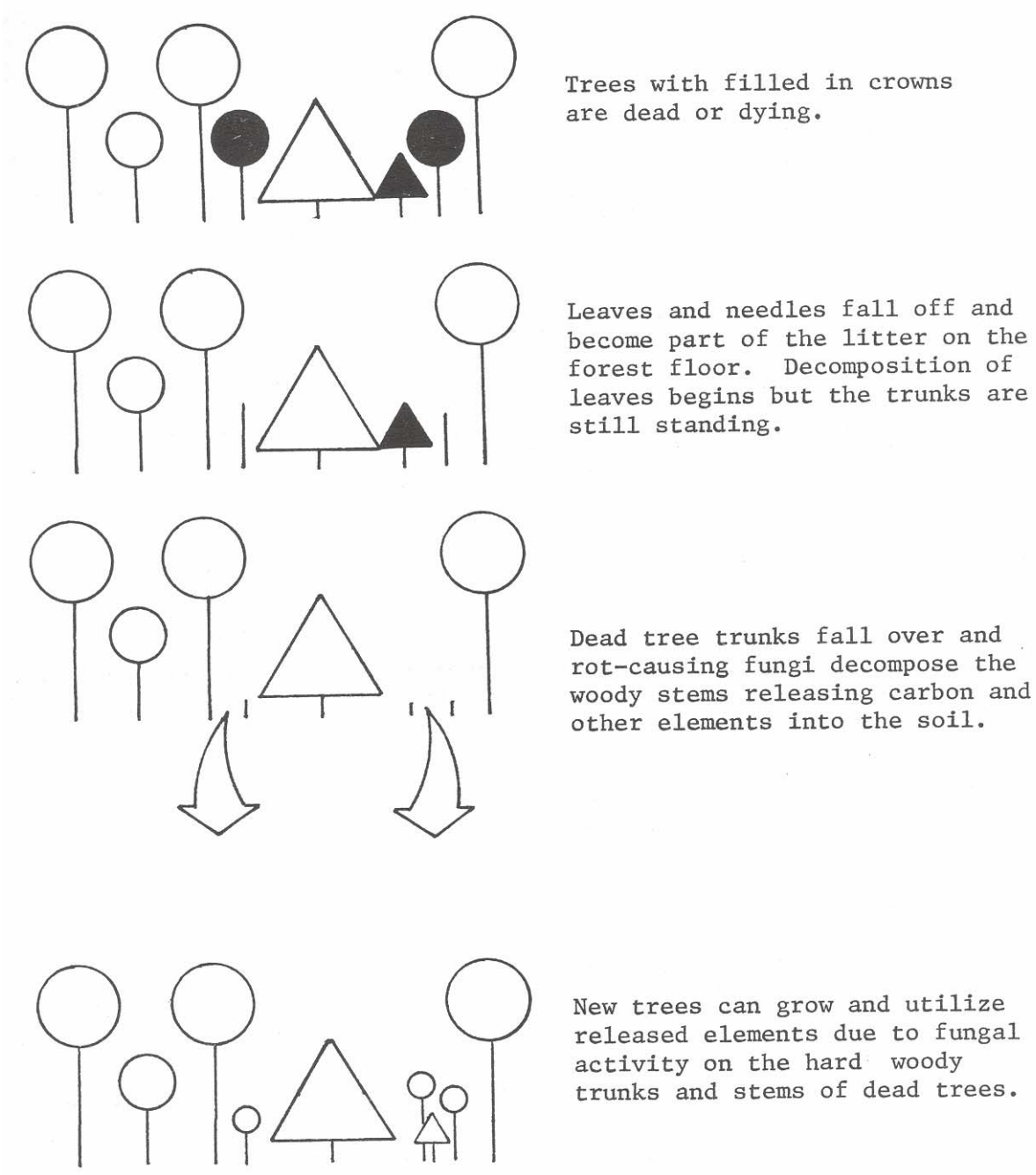

Figure 1.3. The role of rot-causing fungi in the carbon cycle.

Part A: Out Among the Trees

Any area on which a stand of trees exists can be utilized in illustrating the role of fungi in ecology. No matter where the trees grow they are subject to disease and decay as evidenced by shade trees planted in cities and suburbs across North America. For example, the small trees so carefully planted by the city in many municipalities have cankers, heart rot, sap rot, foliage disease, root rot, and scores of other normal "forest" diseases.

One of the most important themes in mycology, especially for fungi found in wood, is that the largest effects are often a result of organisms you cannot see. Large trees may look sound but actually be rotten for many meters up the trunk and into the roots (Boyce, 1961). Have the students stand at the edge of the woods and look into it before you enter. Are there any signs of disease, can you see trees blown over by the wind, is there much leaf litter evident? The answers may change as you actually go into the forest!

Try and see if there are differences in a single stand or two different stands situated close to one another. Conifer stands consisting of pines, spruce, or fir trees have slow decomposing needle litter while deciduous broadleaf stands will have rapidly decomposing material (Cooke, 1979). Even the soils are different so don't hesitate to dig a foot or so down and compare them.

Look for individual trees that have lost their needles or leaves prematurely as these are signs of rot and decay. Branches on the ground are also probably heavily colonized by rot fungi; stumps of trees cut in the past are also good sources. Some stumps are soft and will disintegrate if touched with your foot while others are hard and dry and seem indestructible. Such stumps may exist in the same area or even beside each other. If the trees were killed by a fungus, the organisms responsible may be a white soft or brown hard rot. This will be reflected in the condition of the stumps. The other possibility is that the trees were cut when healthy and the stumps were invaded by different fungi. The result is that either the cellulose or the lignin has been removed. These fungi in some respects do not compete directly since they are in search of different carbon sources (Cooke, 1980). The reality is that when one fungus gets a solid foothold a different fungus may be unable to colonize the stump or trunk. In these cases the wood may not be colonized by other fungi until the pioneer rot has finished utilizing those nutrients necessary for its growth.

Monocultures in forestry, as in agriculture, can sometimes result in situations when disease-causing organisms such as fungi which cause rot and decay can take advantage of a uniform and reliable food source. In an even-aged all-spruce stand you will notice the results of wind-throw and physical damage. Often this is caused by white rots which weaken the roots and stems causing them to be susceptible to wind. Take samples from any broken tree and feel how soft the wood has become due to the action of the fungi. One fungus, *Fomes betulinus*, causes a brown rot in birch and is a common site in hardwood stands. Birch trees are also susceptible to white rot caused by *Trametes versicolor* (turkey-tail). In each case a different fungus colonized first and has dominated. Investigate how light in weight the rotted wood is. Even though it is difficult to see the fungus it has effectively destroyed the strength and integrity of the wood. Regardless of where you sampled from the piece of wood you should be able to isolate the rot-causing fungus.

These fungi, along with others, can be found in a variety of places in any stand. Look in woodpecker holes in the base of the trees. Sample from the stumps of freshly cut trees and from stems and trunks previously cut. Often all you can see are the fruiting bodies of the fungi. If you can see a fruiting body (conk or cap) you may be assured that the fungus hyphae have worked their way throughout the substrate (the tree trunk).

Questions and Answers

1. Q.: Are fungi which cause rot and decay easy to see in a forest?
   A.: No. In general the majority of fungal biomass is small indiscrete hyphae. These hyphae are in the trees or in the soil.

2. Q.: What are indications of rot?
   A.: Fungal fruiting bodies such as conks, trees which have fallen down, trees with no leaves, or decomposing leaf litter on the forest floor.

3. Q.: Does the amount of rot and decay in a forest vary within forest type and site conditions?
   A.: Yes, tropical forests have a higher turnover of material, deciduous forests have a moderate rate of turnover, and conifer forests have a slow rate of turnover.

4. Q.: Is there much overlap between different fungi which cause rot and decay?
   A.: No. After a fungus has become established it can dominate an area and exclude other fungi.

5. Q.: What is the source of the difference in wood condition between white soft rot and brown cubical rot?
   A.: White soft rot-causing fungi use enzymes that digest the hard brown lignin while brown cubical fungi digest the cellulose and leave the lignin behind.

Part B: Laboratory Demonstrations

To help illustrate how pervasive, variable, and powerful the rot-causing fungi are, there are several demonstrations that are easy to arrange.

Fruiting Bodies

Display several different samples or photographs of fruiting bodies associated with both white and brown rots. Many will be familiar some may not be. The fruiting body is a relatively small part of the fungus itself. Often characteristic of the fungus, the fruiting body is used for identification.

Materials and Methods

Collect conks or pieces of wood with fruiting bodies on them whenever you are in wooded areas. If possible ask permission and take only what is needed. If possible get your class to collect with you or produce a disease collection of fungi. Have them look for five or 10 common fruiting bodies found on wood and associated with rot and decay. There are several texts (Agrios, 1978; Boyce, 1961) and guides (Lincoff, 1981; McKnight and McKnight, 1987; Miller, 1977) available in libraries and book stores which make this task relatively easy and fun.

Questions and Answers

1. Q.: Where do you find the fruiting bodies?
   A.: The fruiting bodies or conks can be found on living trees, dead standing trees, stumps, and fallen branches.

2. Q.: Can you tell by the conk what fungus it is?
   A.: The fruiting body and the host material are often used to identify the fungus.

3. Q.: What do the fruiting bodies do?
   A.: The fruiting bodies of the rots are the basidiomycete structures in which the spores are produced.

4. Q.: How do the spores get into trees and other woody material?
   A.: The spores are dispersed by wind or water, or both. Insects and some animals also disperse rot- and decay-causing fungi from one place to another.

5. Q.: If you cannot see a fruiting body does that mean no rot is taking place?
   A.: No. The fruiting body is produced only when there is a need to reproduce. Such needs may result from environmental stress or depletion of a food source.

Different Sources

Small amounts of rotted wood can be collected from stumps, cut logs, woodpecker holes, and litter and placed on nutrient media. Essentially invisible until placed on the media, fungi will grow within 24 hours. Stress how quickly the fungus can grow. White rot-causing fungi are the best to use as they grow very quickly.

Materials and Methods

1. Make up 2% malt extract agar, a good general isolation media which allows for relatively clear viewing of the fungus (see next section).

2. Collect a few grams of woody material from fallen branches, cut stumps, holes created by insects or birds, rotten logs, or fence posts and place in separate bags. This can often be accomplished as a field trip but can also be done in advance.

3. Store material in a refrigerator until ready to place on media (to prevent competing fungi, bacteria, and other organisms from overtaking the fungus of interest).

4. Using forceps dipped in alcohol and passed through a flame, place small amounts of each of the samples on the plates containing the nutrient agar.

5. Seal plates with parafilm or tape, place in the dark at room temperature, and the fungi grow for 1 or 2 days.

6. Fungal colonies should be evident very quickly and are often the hyphae of the rot-causing fungus associated with the sampled source.

Questions and Answers

1. Q.: Do you expect the same fungus will be found in similar sources of rotten or decayed wood?
   A.: Not necessarily. Often the fungus that causes the rot or decay was simply the first fungus to colonize the wood.

2. Q.: What advantage is there to growing fast as noted on the plates?
   A.: Fast growers can establish themselves on a nutrient source such as a fallen log and dominate.

3. Q.: Would you expect more than one fungus from a single sample?
   A.: This can happen, but in wood interference competition can cause the dominant fungus to prevent anything else from growing.

Different Media

Some of these fungi are very adaptable and sensitive to the available nutrients. By placing them on different media they will look different and even grow differently.

Materials and Methods

The use of different nutrient media often helps with the identification of some fungi. In the case of basidiomycetes it is unlikely that fungal hyphae will form a fruiting body in a petri dish. This should not discourage you from using two or three different types of media and seeing if the fungus in question grows differently on the various nutrient sources, will grow radially quicker, or change color on different media?

2% malt extract agar
- 20 g malt extract/liter
- 16 g agar/liter
- distilled water

Oatmeal agar
- 40 g Quaker oatmeal/liter
- 16 g agar/liter
- distilled water

V8 juice agar
- 20 ml V8 juice/liter
- 40 g Quaker oatmeal/liter
- 20 g Quaker Cream of Wheat/liter
- 16 g agar/liter
- distilled water

Cornmeal agar
0.5 g cornmeal/liter
16 g agar/liter
distilled water

Preparation

1. Dissolve agar in hot distilled water while mixing.
2. Add the malt extract/oatmeal/V8 juice et al./cornmeal and continue to stir.
3. Autoclave to sterilize.
4. Pour agar mixture into plates and allow to harden.

Questions and Answers

1. Q.: Are all the colonies of the same color?
   A.: This will vary depending on the species isolated.

2. Q.: If color change did occur, what might this mean?
   A.: It may mean that the metabolic pathways or the efficiency of the pathways has changed resulting in production of a pigment.

3. Q.: Do some fungi grow faster on a certain media?
   A.: This will depend on the species isolated.

4. Q.: Why would the radial growth be different on different media?
   A.: The fungus may have to change strategy in order to maximize the nutrient source. On rich sources it may not have to grow radially as quickly since the immediate area is nutrient rich. If nutrient poor it may have to utilize energy in growing out further in reach of food.

Patterns of Growth

Fungi grown on dialysis membrane (cellulose tubing or sheets, available from most scientific supply companies or hospitals) placed over nutrient agar can be lifted off and viewed under the microscope. Note the pattern of growth. If you can find a reference point and watch carefully for 3 to 5 minutes, you should be able to actually see the hyphae growing! Branches form and elongate and sometimes join other hyphae. This is one of the few times one can watch an organism grow.

Materials and Methods

Dialysis material allows the fungus to absorb from the nutrient media without becoming immersed in it. This causes the fungus to "sit up" higher and is more easily viewed under a dissecting or compound microscope. It also enables you to remove the dialysis membrane with the fungus in it for other manipulations.

1. To prepare the dialysis material, cut it into squares or circles small enough to fit into a glass petri dish, place a filter paper between each layer of dialysis membrane (makes them easier to handle), add water, and autoclave.

2. After autoclaving, allow the dialysis material to cool, place on the nutrient media with a small amount of sterile water, and then inoculate with a white rot-causing fungus and incubate for 1 to 3 days.

3. Place the hyphae plate under a dissecting microscope. Observe the patterns of growth, note any regular, repeating formations. Remove the dialysis membrane (with hyphae in it) and place under a compound microscope. Locate a reference point and watch for 3 to 5 minutes. You should actually see the hyphae growing, branching, and sometimes joining branches.

Questions and Answers

1. Q.: Do the hyphae arrange themselves in obvious patterns? Do the branches ever join?
   A.: Often a branching pattern occurs that does include joining branches.

2. Q.: Can you explain any observed patterns?
   A.: The fungus is taking up nutrients in the most efficient pattern possible so that nutrients may be absorbed and sent around the hyphal mat.

3. Q.: Can you see hyphae growing or joining?
   A.: Hopefully, yes.

4. Q.: If you can actually see the growth of the hyphae what does this mean in terms of resource exploitation?
   A.: Root fungi are fast and powerful in a competitive situation. The result is carbon released and other compounds and elements made available to the fungus and to the ecosystem.

Part C: Student Activities

As mentioned previously there are a number of things the students can do, making use of the "hands on" approach, to enhance their understanding that fungi play an important role in forest ecology. Students can make general notes at the wooded sites and record observations about the trees and forest floor. They can compare these notes with notes taken after they have entered the stand of trees. Collection from one or two sources of woody material for macroscopic and microscopic investigation in the laboratory is also possible. These samples can be placed on media at a later date. Students can be encouraged to count the number of different places fungi were noted in the woods. Modest collections of fruiting bodies of rot- and decay-causing fungi can be done.

In the laboratory, students can observe the demonstrations you have provided and be asked to comment on them or answer relevant questions pertaining to them. Have students put woody material suspected of being colonized by fungi on prepared plates one week and make observations the following week.

Have different groups of students collect from different sources (e.g., stumps, leaves, standing trees, fallen logs). Discuss any differences in sources of material and what may have grown out from them.

Inoculating Plates

Carefully wipe down the area of the laboratory bench and transfer hood where you will be working using the 20% Javex solution provided (please be careful as the Javex will bleach clothing). *Do not take the top off the petri dish until you are ready as this will cause the media to be contaminated by other fungi and bacteria.* When you are ready, pick up the sample with forceps and carefully and quickly lift one edge of the top of the petri dish up enough to allow you to place the wood sample on the media. Immediately put the cover back on and label the top with your name and laboratory session. Place the plate in the area indicated. Plates will be incubated for 1 week and returned in the following laboratory session.

Making a Transfer Hood from a Cardboard Box

Fume hoods are not appropriate places to pour media into plates or place material onto plates because they move air from the room across your sample and up the duct work. Laminar flow hoods blow air into the room or from the top to bottom through a microfilter and are the best places to work aseptically, but they are very expensive and always in short supply. A compromise is a transfer hood or cabinet. Commercial cabinets are usually plastic with both incandescent and ultraviolet lights. Transfer cabinets are effective because they prevent material from falling down onto the plates; by providing an oasis of relatively still air, reasonable control can be maintained over possible contaminates.

Transfer hoods or cabinets made of painted wood, panelling, and a number of other materials may be used. In a pinch a home-made transfer hood may be made using a large cardboard box, tape, and clear plastic that is adhesive on one side. A box measuring 60 cm × 60 cm × 90 cm is a good size as long as the box is not too big to allow air movement or too small to work in. Turn the box on its side, open the flaps. Suspend the top flap out from the box for extra protection. Remove the bottom flap and cut the side flaps on an angle to support the top flap. Line the inside of the box with clear adhesive plastic (which usually comes in a roll). The plastic assists in keeping the box clean.

Acknowledgements

I greatly appreciate the time and effort spent by Audrey Miller in typing this manuscript.

Literature Cited

Agrios, G. N. 1978. Plant pathology. Second edition. Academic Press, Toronto, 703 pages.
Boyce, J. S. 1961. Forest pathology. Third edition. McGraw-Hill, New York, 550 pages.
Cooke, W. B. 1979. The ecology of fungi. CRC Press, Boca Raton, Florida, 275 pages.
Cooke, R. C. 1980. Fungi, man and his environment. Longman Press, London, 144 pages.
Hudson, H. J. 1980. Fungal saprophytium. Second edition. Institute of Biology, Studies in Biology no. 32, Edward Arnold, London, 120 pages.

Lincoff, G. H. 1981. The Audubon Society field guide to North American mushrooms. Alfred A. Knopf, New York, 926 pages.

McKnight, K. H., and V. B. McKnight. 1987. A field guide to mushrooms of North America. Houghton Mifflin, Boston, 429 pages.

Miller, O. K. 1977. Mushrooms of North America. E. P. Dutton, New York, 368 pages.

Odum, E. P. 1975. Ecology. Second edition. Holt, Rinehart and Winston, New York, 180 pages.