SHIITAKE GROWERS HANDBOOK

The Art and Science of Mushroom Cultivation

by
Paul Przybylowicz
John Donoghue

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Shiitake is the second most widely cultivated mushroom in the world and has grown to become a worldwide multi-billion dollar industry.

"The market for shiitake is increasing steadily as more and more people learn about the great taste and other qualities of this excellent, exotic mushroom . . . This book is one of the first items that a person interested in growing shiitake should buy . . . Dr. Paul Przybylowicz, and John Donoghue have brought nearly all the useful knowledge and available practical experience together in this book . . . Even the experienced grower will find a lot of very useful information in this carefully written and well illustrated book."—*Pieter J. D. Vedder, Vice-President Training & Development, Campbell's Mushroom Division.*

This definitive sourcebook clearly describes the underlying scientific principles governing the behavior of shiitake and presents detailed practical mushroom cultivation techniques.

- Understandable descriptions of shiitake biology.
- Traditional and year-round production on logs.
- New methods of shiitake cultivation on sawdust substrates.
- Effective pest and disease controls.
- Informative review of marketing and of health benefits.
- The most comprehensive shiitake bibliography available.

KENDALL/HUNT PUBLISHING COMPANY
Dubuque, Iowa

ISBN 0-8403-4962-9

Shiitake Growers Handbook

The Art and Science of Mushroom Cultivation

Paul Przybylowicz
John Donoghue
Northwest Mycological Consultants, Inc.

KENDALL/HUNT PUBLISHING COMPANY
2460 Kerper Boulevard P.O. Box 539 Dubuque, Iowa 52004-0539

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Illustrations

Lisa Ellingson All illustrations, except figure 2–7.

Library of Congress Catalog Card Number: 88–82081

ISBN 0—8403—4962—9

Printed in the United States of America
10 9 8 7 6 5 4

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Foreword

Shiitake belongs, in my opinion, to a very small group of the overall best and most delicious edible fungi, which also includes the fruiting bodies of *Tuber melanosporum, Amanita caesarea, Cantharellus cibarius, Pholiota nameko* and perhaps a few more. We know from historical documents, literature, and old paintings, that a great number of Asian people have known and cultivated shiitake for centuries.

Researchers at institutes in several countries are working on the development of growing techniques for other well-known and highly appreciated mushrooms. So far, only a few species from the aforementioned group have been successfully cultivated.

Although shiitake has been grown in Asian countries for ages, the big interest there and also in the Western world came after World War II, especially in the last 10 to 15 years. At present, the biggest part of the more than 300,000 metric tons of shiitake produced annually is grown in China and Japan. However, every year a growing number of people in the United States and Europe are trying to make a living by growing this product for a market that is increasing steadily as more and more people learn about the great taste and other qualities of this excellent, exotic mushroom.

There are several similarities between the situation in the shiitake growing business now and in the button mushroom (*Agaricus*) industry of, let's say, 50 years ago. Lured by quite often unprofessional stories about easy money-making and high market prices, a number of people tried to go into this business, completely unprepared, with far too high expectations and, therefore, a very high failure rate. It is amazing how easily so many people can step into such a new business, with hardly any knowledge or experience—and lose their money.

The yearly production of *Agaricus* mushrooms has risen in the last 40 years by more than 10 times: from approximately 100,000 metric tons to the present 1.2 million metric tons. The key for the survivors in the white button mushroom industry is, and always has been, a combination of sufficient knowledge, hard work, and good marketing. I can't see any reason why this should be different for the shiitake business.

It is therefore a very good idea that John Donoghue and my good mushroom friend, Dr. Paul Przybylowicz, have brought nearly all the useful knowledge and available practical experience together in this book.

A book like this is one of the first items that a person interested in growing shiitake should buy. After studying the book intensively, some readers may come to the conclusion that it is not as simple as they had thought and therefore, not the right place for them to put their money. For the group which, after reading, is still convinced that growing shiitake, though perhaps not easy, could be a very fascinating hobby or even a good oppor-

tunity to make a living, this book will be a great help. Even the experienced grower will find a lot of very useful information in this carefully written and well-illustrated book.

Spring, 1988

Pieter J.C. Vedder
Vice-President
Training & Development
Campbell's Mushroom Division

Preface

Shiitake is the second most widely cultivated mushroom in the world. In the past four decades, shiitake cultivation has grown to become a worldwide, multi-billion dollar industry.

For centuries, this mushroom has been grown in Asia using traditional methods. Today, a steadily increasing market for fresh shiitake outside of Asia has created a demand for localized shiitake production in many new areas. By adapting traditional methods and developing new ones, growers around the world are successfully producing shiitake at many different scales, from back-yard hobbyists to part-time farmers to large corporate endeavors.

There are many manuals that describe specific methods for growing shiitake on logs, although the majority of these are not available in English. However, for the most part these publications neglect the underlying biological principles which govern the cultivation process. A thorough understanding of these principles demystifies the process of shiitake cultivation, allowing the grower to successfully adapt and develop cultivation methods as needed.

In this book, we have avoided a "cookbook" approach by first presenting the underlying scientific principles governing the behavior of shiitake, then describing their practical applications to the art of mushroom cultivation. In this context, we have covered the entire range of shiitake cultivation methods currently in use. These include:

- Traditional methods for seasonal production on logs.
- Techniques for year-round mushroom production on logs.
- New methods of cultivation on sawdust substrates.

We have also included an extensive discussion of shiitake pests and diseases, and effective management measures for their control.

Our involvement in the scientific community and in the mushroom industry has afforded us the opportunity to study shiitake from both scientific and applied viewpoints. We have drawn on this experience and have incorporated both approaches in this book; we hope that it will be useful to anyone with an interest in mushrooms. We have attempted to clearly explain and illustrate the underlying principles that are basic to successful shiitake cultivation. In addition, we have drawn extensively from scientific literature and have included a comprehensive bibliography for readers who want additional information.

Because of the wide range of shiitake cultivation techniques and scientific research presented herein, we feel this book will serve as both a handbook and a reference for growers and others interested in the art and science of mushroom cultivation.

We would like to thank Dr. William Denison and Northwest Mycological Consultants, Inc. for providing us the opportunity to pursue our research interests in shiitake. This sponsorship enabled us to visit the major shiitake research and production centers of the world to observe many different systems of shiitake cultivation, including the latest scientific advances.

We would also like to thank our editors, Margo Denison and Maia Fischler, for their unflagging enthusiasm and sharp eyes, Lisa Ellingson for her wonderful illustrations, Al Bob Hollister for his fine photographs, Annette Simonson for assistance in our research endeavors, and the many mushroom growers and researchers who have, throughout the years, so readily shared their experiences, problems and solutions. Finally, we would like to thank Shannon, Ellen and Cas for putting up with us during this project.

Paul Przybylowicz
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702 NW 4th St.
Corvallis, Oregon
1 April, 1988

Dedication

In memory of Jim Roberts (1931 to 1990)

Jim's open, insightful and often humorous nature catalyzed the establishment of the US shiitake industry. He fashioned a global network of people, ideas and resources that continues to be the fabric of this industry. His contributions will provide perspective and inspiration far into the future.

Introduction

"Art is science in the flesh"

—Jean Cocteau

An ancient mushroom is enjoying a recent upsurge in popularity. This mushroom is consumed widely in Asia, where it is prized for its flavor and health-promoting benefits. For centuries, it has been cultivated in China where it is known as "Shiang-gu" or "Hoang-mo." The Japanese name, "Shiitake," is taken from the Shii tree, one of the many tree species that it grows on in nature, and "take," a Japanese word for mushroom. Because Japan is the world leader in production, this mushroom is now widely known as shiitake.

Shiitake's popularity has spread to the West, where it is also known as the "Black Forest Mushroom." Previously available only in its dried form, fresh shiitake has found increasing acceptance with Western consumers due to its distinctive flavor and texture.

This increasing demand for fresh shiitake has resulted in the expansion of cultivation to areas outside its natural habitat. This has created a need to adapt and modify traditional cultivation techniques in order to develop new methods for growing shiitake in new situations. To do this successfully, the grower must be able to distinguish between methods based solely on tradition and those founded on the fundamental relationships between the mushroom and its environment. Thus, the grower must thoroughly understand not only mushroom biology and its response to the environment, but also the various techniques of cultivation. The grower then has a framework within which to adapt—rather than adopt—techniques appropriate for local conditions.

This book explores the biology of shiitake and the underlying concepts basic to all methods of shiitake cultivation. Detailed examples of specific management practices are presented to illustrate applications of these concepts.

The reader should view the examples in the context of these governing principles, and use them as a resource to develop a successful system for shiitake production. The reader is encouraged to use the extensive bibliography as an additional resource.

Section I provides historical and background information on shiitake cultivation, the biology of shiitake, and important environmental factors. Traditionally, shiitake has been grown on logs, and more recently, on sterilized sawdust substrates. Section II covers cultivation on logs, from simple outdoor management to intensive indoor production. Section III presents techniques for shiitake cultivation on sawdust and other particulate subtrates. Each of these sections includes a detailed discussion of diseases and pests.

Finally, Section IV discusses harvesting, storage and marketing; it then details the nutritional and health benefits of shiitake.

Section I
General Information

Successful shiitake cultivation depends upon thoroughly understanding mushroom biology and how the environment influences fungal growth and development. The response of shiitake to the environment is the same, regardless of the cultivation method. The grower applies this information to control the environment in order to provide optimum conditions for each phase of shiitake cultivation.

This first section provides necessary background information about shiitake and its environment. The basic principles of mushroom cultivation are discussed, with emphasis on shiitake cultivation, followed by an in-depth look at the shiitake fungus and at the process of wood decay by which shiitake obtains nutrients. Special terms pertaining to fungi are explained. Finally, important physical and chemical factors which can be manipulated to control the shiitake life cycle are examined.

1

Introduction to Shiitake Cultivation

This chapter explains the basic principles of mushroom cultivation, briefly reviews the history of shiitake cultivation, describes several methods of growing shiitake, and reports on worldwide trends in its production and consumption.

Principles of Mushroom Cultivation

Mushroom cultivation is both a science and an art. Science has investigated basic mushroom biology and determined how environmental factors influence it. It is an art to apply this information to the business of growing a successful mushroom crop.

Shiitake cultivation should not be a haphazard process. Constant attention is required to maintain the competitive edge necessary for economic success. The basic steps in mushroom cultivation are:

1. Creating a selective nutrient base for the mushroom;
2. Introducing the mushroom of choice;
3. Managing the environment to favor mushroom growth and development.

Shiitake grows and produces mushrooms by decaying wood. Materials which serve as both a food supply and habitat are referred to as **substrate**. Shiitake can be commercially cultivated on one of two types of substrate: logs or a **medium** (plural, **media**) consisting mostly of sawdust.

History

The earliest written record of shiitake dates back to 199 A.D. Japanese historical documents recorded that Emperor Chuai praised the shiitake given him by the natives of Kyushu (*182*). Probably, these shiitake were gathered from the wild.

Actual cultivation of shiitake originated in China during the Sung Dynasty (960–1127). Both history and legend credit Wu San Kwung as the originator of shiitake cultivation. Almost every mushroom-growing village in China has a temple in his honor (*22*).

Wu San Kwung lived in Lung-Shyr village in Lung-Chyuan county in southwest Chekiang Province. While collecting wild mushrooms in the high mountains, he found "nice-smelling mushrooms" (shiitake) growing on broken trees which had fallen to the ground. He later discovered that slashing the bark of these logs and beating them vigorously caused more mushrooms to appear.

In 1313, Chinese author Wang Cheng recorded shiitake-growing techniques in his *Book of Agriculture* (*22*). Wang Cheng described how to select a suitable site, choose appropriate trees and cut them down. He outlined basic cultivation methods as follows: cut the bark with a hatchet and cover the logs with soil. After one year, cover the decayed logs with branches, leaves and soil and water frequently. Beat the logs with a wooden club to stimulate mushroom production and mushrooms will appear after a rain. Chinese farmers introduced cultivation techniques into Japan between 1500 and 1600 A.D. (*114, 182*). Since that time, the Japanese have been the leaders in developing techniques for shiitake cultivation on logs.

Mushrooms produce **spores**, minute reproductive structures which are dispersed by air currents. Early cultivation methods depended on spores to transfer the shiitake to new logs. Shiitake growers gathered logs bearing mushrooms and placed them near freshly cut logs, relying on airborne spores to "infect" the new logs (*182*).

Methods of **inoculation**, the process of introducing shiitake to new logs, were improved over the years. Cutting the bark of the new logs increased colonization by the shiitake spores. Another advance was introducing spores directly into the wood, either by inserting spore-covered pieces of paper or by pouring suspensions of spores in water into the cuts. These primitive methods of infecting new logs were unreliable, and when the logs did produce mushrooms, yields were variable.

Shiitake colonizes logs by permeating and penetrating the wood with small, thread-like **hyphae** (singular, **hypha**). Collectively, hyphae are referred to as **mycelium** (plural, **mycelia**) which appears as a white cottony mass.

All mycelia in a shiitake colony contain the same genetic information, but the genes are reshuffled during spore production. Thus, colonies which *start from* spores are different from the parent mushroom(s), whereas colonies resulting from the transfer of mycelium are genetically identical to the parent mycelium.

Early cultivation methods that used spores for propagation were inherently variable. Desired mushroom characteristics, such as higher yields, could not be reliably transferred to new logs. Some growers solved this problem by using pieces of logs which had produced many high quality mushrooms to "infect" fresh logs (*182*).

In the 1920's, K. Kitayama developed pure culture **spawn**, consisting of genetically uniform mycelium growing on a suitable material (*182*). This made it possible to select and propagate shiitake mycelium with improved vigor and higher yields.

In 1943, K. Mori introduced sterilized wooden wedges which were colonized by pure cultures of shiitake (*129*). These wedges were inserted into ax cuts in the logs. Using this method, logs could be rapidly inoculated, and successful colonization of new logs was greatly increased. The higher and more reliable yields that resulted from this innovation allowed the commercial shiitake industry to develop and expand rapidly (*17*). A later development was the use of colonized wooden dowels or plugs which were inserted into holes drilled in the logs. This further decreased inoculation time and increased successful inoculations.

A recent development in log inoculation technology employs thin half-moon shaped wafers of colonized wood which are inserted into deep saw cuts in the log. These wafers distribute the shiitake more evenly in the log, decreasing the time needed to completely colonize the log and producing mushrooms more rapidly (*41*).

Originally, shiitake production was seasonal. **Fruiting** occurred during the spring and fall when rainfall and temperature were conducive. Most mushrooms were dried, for consumption throughout the year. As demand for fresh mushrooms increased, Japanese growers developed methods to achieve year-round mushroom production by inducing shiitake to fruit under controlled conditions. This technique, referred to as **forcing** or **forced fruiting**, is now used in most shiitake-producing areas.

As logs have become scarce in some areas, alternative substrates for shiitake cultivation have been sought. Shiitake produced on heat-treated substrates was first reported in 1933, when it was grown on blocks of wood in glass cylinders (*156*). In 1935, cultivation on sawdust was reported as a means of testing shiitake mycelium for genetic characteristics (*144*).

Commercial cultivation of shiitake on sawdust and other cellulose-containing materials is increasing worldwide. Most sawdust-grown mushrooms are cultivated on sterilized substrates. However, a method developed by Dutch growers produces shiitake on pasteurized substrates on beds in standard button mushroom houses.

As the availability of raw materials fluctuates, and as economic, political and marketing factors shift, shiitake growers develop new techniques to cope with changing conditions. Thus, there is no one ideal method of cultivation, but rather a wide range of methods developed under different constraints. New innovations continually emerge as the balance between these factors shifts and as understanding and technology improve.

Overview of Techniques

Cultivation on Logs

Traditionally, shiitake has been cultivated on freshly cut logs, usually from the oak family. Live trees are cut during the dormant season when their sugar content is high. Within one or two months, the logs are inoculated with

actively growing pure culture spawn. This spawn usually consists of sawdust or wooden plugs which have been permeated by shiitake mycelium.

After inoculation, the logs are placed in stacks and are managed to create favorable conditions for the mycelium to colonize the wood (**spawn run** or **incubation**). The spawn run can occur naturally, in a forest, or under controlled conditions in special structures with irrigation. This process takes from 6 to 18 months depending on the amount of spawn used, the shiitake variety and the conditions during incubation.

After the logs are fully colonized, they are induced to produce mushrooms. Outdoors, seasonal rains will induce mushroom formation. However, to obtain a steady supply of mushrooms, the time of fruiting must be controlled by the grower. Logs can be forced to fruit by soaking or irrigating them with water, either outdoors or in special houses (Fig. 1–1). Depending on their size and the number of fruitings per year, logs can produce mushrooms periodically for two to five years after they have been colonized by shiitake.

Log farms vary widely in size, from households growing a dozen logs as a hobby, to corporate farms with over 500,000 logs.

Much of the dried shiitake produced in Asia is produced by small family farms with 3,000 or fewer logs (*8*). Small-scale, seasonal production needs a relatively low investment and can generate part-time income.

Year-round production requires investment in special fruiting structures and can be a full-time occupation. The long cropping cycle yields a long-term return comparable to growing orchard crops.

Cultivation on Sawdust

Heat-treated substrates for shiitake cultivation usually consist of a mixture of sawdust and/or other cellulose-containing materials supplemented with grain, bran or other sources of carbohydrates and nitrogen. The mixture is placed in heat-resistant containers and sterilized to kill competitors. After cooling, the substrate is inoculated with shiitake and is incubated for 30 to 180 days. During incubation, the shiitake fungus permeates and degrades the substrate; the shiitake mycelium virtually knits the sawdust medium together. Then, fruiting is initiated by moving the substrate to a cooler, humid environment and exposing all or part of its surface. Mushrooms can be produced periodically for three to six months (Fig. 1–2).

In addition to the need for structures, a sawdust farm requires considerable investment in equipment for sterilization, air filtration and environmental control. Sawdust farms are usually large and require a year-round labor force. The entire cropping cycle is much shorter than in log cultivation. This results in a more rapid cash flow. In addition, these operations can react more rapidly to changing market demands.

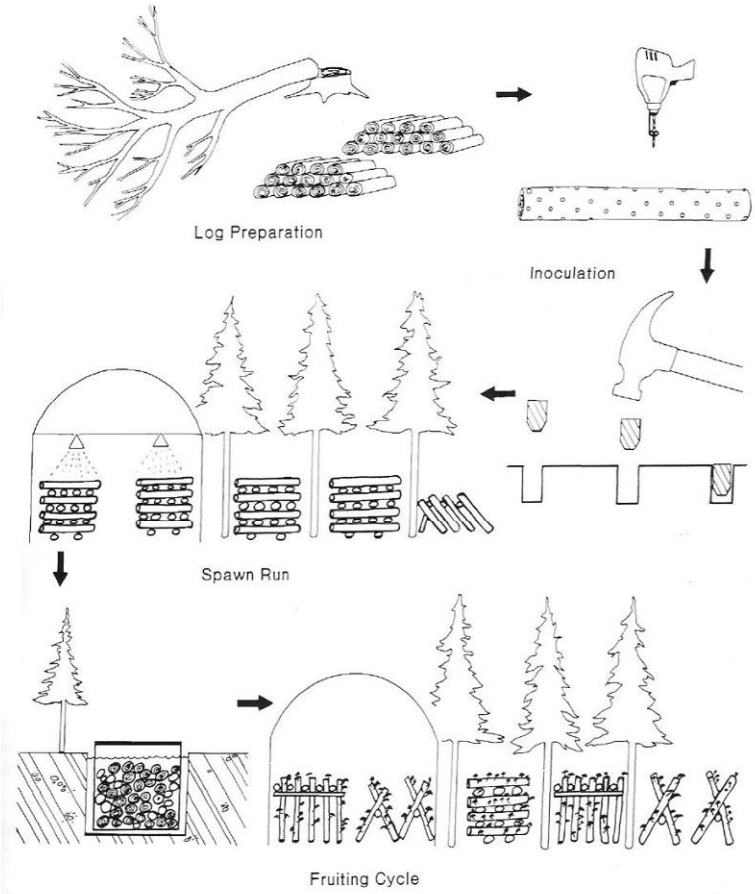


Figure 1–1. Overview of shiitake cultivation on logs.

Trends in Shiitake Production and Consumption

Shiitake ranks second after the button mushroom (*Agaricus*) in total world mushroom production. Worldwide, shiitake production has increased from less than 10,000 metric tons (mt) in 1946 to over 300,000 mt in 1986. During this same period, *Agaricus* production increased from 100,000 mt to 1,226,640 mt (*20, 171*).

Japan is still the leading producer of shiitake, but its share of the world production has dropped from 83% in 1983 (*171*) to 51% in 1986 (*20*)

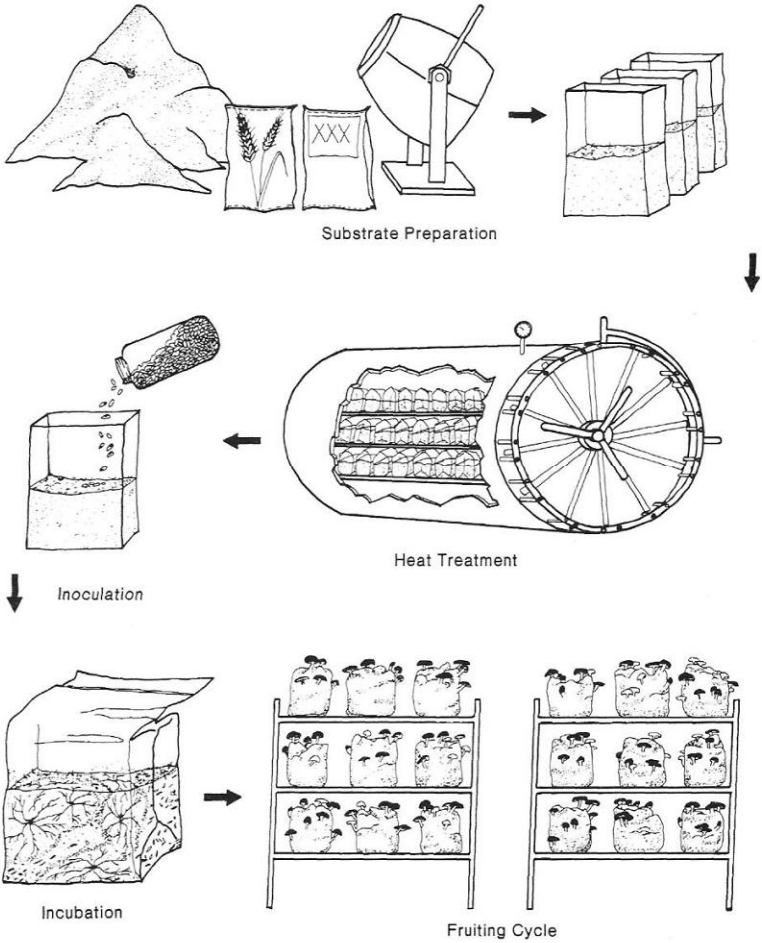


Figure 1–2. Overview of shiitake cultivation on sawdust.

(Table 1–1). Approximately 80% of the world production is dried, and 20% is sold fresh (*171*). Most international trade is in dried shiitake, while most fresh production is consumed domestically. Virtually all Japanese production is on logs, but both sawdust and log farms are found in other countries.

TABLE 1–1
Estimated World Production of Shiitake for 1986

Country	Metric Tons	Percentage
Japan	160,000	51.0
Mainland China	120,000	38.3
Taiwan	32,000	10.2
Korea	880	0.3
U.S.A.	200	0.1
Canada	150	–
Singapore	120	–
Holland	75	–
Phillipines	30	–
Finland	30	–
Thailand	15	–
Belgium	1	–
Total	313,501	100.0

Note: Production weights are in fresh weight equivalents.
Source: Chang, 1987 (20).

Production of shiitake for the fresh market has been increasing steadily. For example, Japanese growers produced 38,064 mt of fresh shiitake in 1970; this doubled to 77,517 mt in 1979. Although the Japanese production of shiitake has increased, the number of growers has decreased, reflecting a shift to larger farms. In 1970, there were 251,759 growers: 80% had 3,000 logs or fewer, and only 5% had more than 10,000 logs. By 1979, there were 181,709 growers: 66% had 3,000 logs or fewer, while 13% had 10,000 logs or more (*8*).

Demand for shiitake has kept pace with the rise in production. Long enjoyed in the Orient as an "elixir of life," it is becoming popular in the western world as customers with cosmopolitan palates seek shiitake's unique flavor and reported health benefits.

Recent economic and political factors have encouraged the rapid growth of the shiitake industry in the United States. In the early 1970's, the importation of inexpensive canned button mushrooms from Asia caused an abrupt drop in U.S. button mushroom production. A number of farms went out of business, creating a surplus of unused, specially designed mushroom growing houses. Fresh market mushroom growers seeking to expand their market by offering new mushrooms had a ready supply of inexpensive growing space for cultivation of shiitake on sawdust.

In 1972, the U.S. Department of Agriculture lifted the ban on importation of live shiitake cultures, and the quality of available strains has improved markedly. Since then, the U.S. shiitake industry has expanded rapidly, using

both log and sawdust cultivation techniques. By 1986, sawdust cultivation accounted for more than half of U.S. shiitake production. At least 90% of the shiitake grown in the U.S. is sold in the domestic fresh market.

2

Biology of Shiitake

The shiitake fungus decays wood to obtain the nutrients required to produce mushrooms. Two interdependent systems, the biology of shiitake and the process of wood decay, govern the response of the fungus to environmental changes. The grower needs a basic understanding of these systems in order to solve the problems which may arise during cultivation.

The first part of this chapter deals with academic and scientific aspects of fungi. Fungal classification is outlined, followed by a discussion of various aspects of fungal biology, including mycelial growth, modes of reproduction and life cycles of shiitake and a common disease fungus, *Trichoderma*.

The latter part of the chapter examines the process of wood decay: the components which make up wood, the difference between sapwood and heartwood, predisposing environmental conditions, different types of decay and the succession of decay organisms.

Classification

Currently, the fungi are placed in a kingdom of their own, the kingdom Fungi (*163*). Most biologists now recognize at least five kingdoms:

- **Monera** (bacteria and viruses)
- **Protista** (most algae and single-cell animals, some fungus-like organisms)
- **Animalia** (most multicellular animals)
- **Plantae** (most land plants)
- **Fungi** (most fungi)

The kingdom **Fungi** is further divided into a hierarchy of related groups. In descending order, they are: division, class, order, family, genus, species, and strain.

Another way to classify living organisms is based on the way they get their food (*64*). There are three basic ways: **Primary producers** (algae and green plants) use the energy from the sun, plus water and carbon dioxide to synthesize their own food. **Consumers** (animals) get food by ingestion and digest it internally. **Decomposers** (fungi and bacteria) get their nutrition through the process of decay. The food is usually digested externally, then absorbed.

Fungi are different from either plants or animals, and it is important to understand the differences. Characteristics of the fungi are: 1. inability to syn-

thesize their own food; 2. external digestion and absorption of dissolved materials; 3. generally a filamentous or strand-like growth form with cell walls made of either cellulose or chitin; 4. reproduction by spores.

Ascomycetes and Basidiomycetes

There are many types of fungi; at least 100,000 species are known. Most economically important fungi are in the two major classes: Ascomycetes and Basidiomycetes (Table 2–1). These two classes are collectively referred to as the Higher Fungi.

TABLE 2–1
Greatly Simplified Classification of the Fungi

KINGDOM FUNGI	
Basidiomycetes	**Ascomycetes**
Rusts	Yeasts
Smuts	Lichens
Jelly fungi	Cup fungi
Bracket fungi	Morels
Coriolus	Bulgaria
Stereum	*Peziza*
Mushrooms	Flask fungi
Lentinula (shiitake)	*Hypocrea/ Trichoderma*
Agaricus (button mushroom)	
Puff Balls	Truffles

These two classes are distinguished by their sexual spores (Fig. 2–1). Ascomycetes produce **ascospores** which are formed inside sac-like cells called **asci** (singular, **ascus**). Basidiomycetes produce **basidiospores** which are formed on club-shaped cells called **basidia** (singular, **basidium**). These spores and cells are so small that they can be seen only with a microscope.

On a practical level, Ascomycetes can be distinguished from Basidiomycetes by the size, shape, color and texture of the fruiting body. All "typical" mushrooms are Basidiomycetes. In fact, most fungi that produce fruiting bodies larger than one inch (2.5 cm) are Basidiomycetes. Conversely, most Ascomycetes produce very small fruiting bodies. Exceptions include several well-known Ascomycetes, notably the morels and cup fungi.

The Basidiomycetes are divided into groups based on the structure of the basidium and the spore-bearing surface or **hymenium**. The hymenium may be found on gills, pores, teeth, or folds, depending on the group.

Mushrooms are an order of Basidiomycetes, referred to as the Agaricales, which includes shiitake and the common button mushroom. Typical

mushrooms have gills, a cap and a stem. **Gills** are thin, leaflike plates or flaps of tissue arranged like spokes on a wheel on the underside of the cap. Basidiospores are produced on the gills (Fig. 2–1).

Another order of Basidiomycetes, the Aphyllophorales, also known as the polypores or **bracket fungi**, produce their spores inside pores arranged on the underside of woody or leathery fruiting bodies. Most bracket fungi are wood-decaying fungi. Some members of this group (such as *Stereum* spp. and *Coriolus versicolor*) compete with shiitake for space in logs.

Scientific Names

Although it seems natural to call fungi (and other living things) by their common names, this practice can lead to confusion. The same common name may refer to two or more different organisms. Conversely, two or more common names may refer to the same organism. To avoid this confusion, scientists have adopted an international naming system based upon an organism's position in the classification system.

A scientific name consists of two words. The first word, which is capitalized, is the name of the **genus**; the second word is the name of the **species**. The name of the organism requires both words. A scientific name is either underlined or printed in italic type.

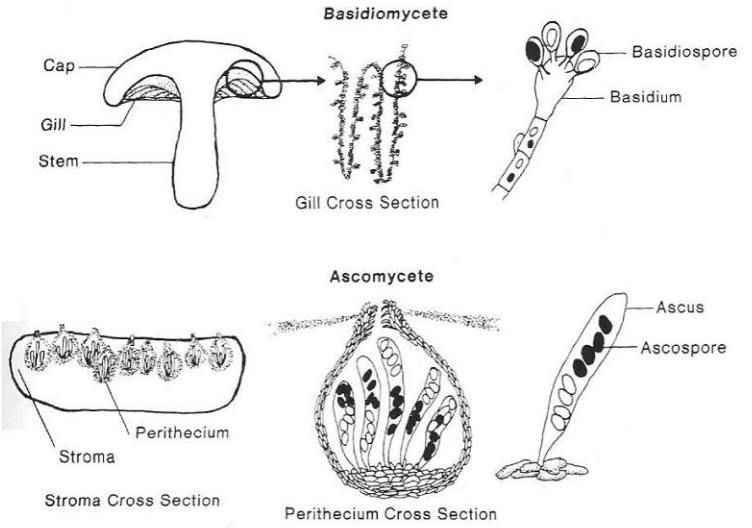


Figure 2–1. Sexual spore production in Basidiomycetes and Ascomycetes.

For example, the scientific names, also called Latin names, of some edible fungi are listed below:

- *Agaricus brunnescens* "grocery store" or button mushroom
- *Boletus edulis* King Bolete, cep, Steinpilz
- *Cantharellus cibarius* Chanterelle, Pfifferling
- *Pleurotus ostreatus* Oyster mushroom, Shimejii

Additional words name the person(s) who described or classified the species. These words may be abbreviated or in parentheses; they are not underlined or italicized. For example, *Lentinula edodes* (Berk.) Pegler is the scientific name for shiitake. The "(Berk.)" means that Berkeley first described the species, but in a different genus. Pegler is the person who placed it in the genus *Lentinula*.

Classifying organisms can be somewhat controversial, and organisms have been classified and reclassified periodically, based on various criteria. Shiitake has been given a number of names over the years (Table 2–2). Currently, the most widely accepted name for shiitake, *Lentinus edodes*, was given by R. Singer in 1941. In 1975, shiitake was placed in the new genus *Lentinula* which resulted in the new name *Lentinula edodes* (Berk.) Pegler (*157*).

TABLE 2–2
Synonyms for *Lentinula edodes* (Shiitake)

Name	Year Assigned
Agaricus edodes	1877
Collybia shiitake	1886
Armillaria edodes	1887
Agaricus russaticeps	1888
Lepiota shiitake	1889
Lentinus tonkinensis	1890
Mastaleucomyces edodes	1891
Pleurotus russaticeps	1891
Cortinellus shiitake	1899
Tricholoma shiitake	1918
Cortinellus berkeleyanus	1925
Lentinus shiitake	1936
Cortinellus edodes	1938
Lentinus edodes	1941
Lentinula edodes	1975

Mycelium and Asexual Reproduction

Mycelium

Fungi spend most of their lives as vegetative mycelium, digesting, absorbing and storing nutrients to prepare for fruiting. The production of a mushroom is analogous to the production of a tomato by the tomato plant. The mushroom is the fruit of the mycelium. Usually, the mycelium is not seen because it is buried in the wood, soil or other substrate it is digesting. Nevertheless, without mycelium, there would be no mushrooms. Healthy mycelium is necessary for good mushroom production.

The cottony white growth, commonly called mold, seen on decaying organic matter is mycelium. Mycelium is very fragile; almost any physical disruption will break or crush it. Mycelium is composed of many interconnected individual strands (hyphae) which are invisible except under a microscope.

Growth occurs only at the tip of each hypha, but here it is almost continuous. Digestion occurs along the length of the hypha. The hypha branches just behind the growing tip, and each branch has its own growing tip. As the mycelium grows, the number of hyphal tips increases until nearly all the substrate is occupied.

Primary and Secondary Mycelium

Fungi have two basic types of mycelium: primary and secondary mycelium. When an ascospore or a basidiospore germinates, it normally grows into primary mycelium. Before an Ascomycete or Basidiomycete can produce ascospores or basidiospores, it must produce secondary mycelium.

Most differences between primary and secondary mycelium can be observed only with a microscope. However, primary mycelium of most Basidiomycetes can easily be differentiated in culture because it grows more slowly than secondary mycelium.

The most important difference is that primary mycelium has only a single nucleus per cell (**monokaryotic**) and is called a **monokaryon**. Secondary mycelium has a pair of nuclei in each cell (**dikaryotic**) and is called a **dikaryon**. Shiitake, and most other mushrooms, can produce mushrooms (**fruitbodies**) only from secondary mycelium.

Secondary mycelium is formed by the mating of primary mycelium. Typically, two genetically distinct and compatible primary mycelia grow together and exchange nuclei to form dikaryotic mycelium. The dikaryon carries genetic information from each parent monokaryon in separate nuclei within each cell.

A distinctive feature of secondary mycelium in many Basidiomycetes, including shiitake, is the presence of **clamp connections**. These peculiar structures form as the nuclei divide to insure that the new cell gets both nuclei of the dikaryon (Fig. 2–2). Clamp connections are easily observed with a microscope.

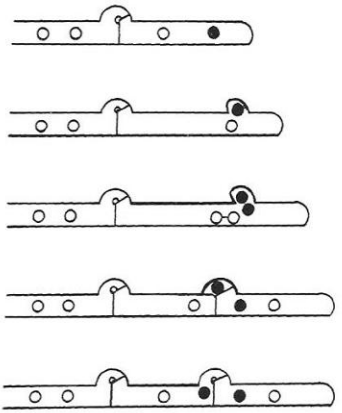


Figure 2–2. Clamp connection formation in Basidiomycetes.

Asexual Reproduction

Ascospores and basidiospores are the result of sexual recombination. During sexual spore production, genetic recombination and mutation help the fungus to adapt to changing conditions.

In addition, most fungi reproduce asexually, without sexual recombination, by fragmentation of the hyphae or by asexual spores produced in specialized structures. Asexual reproduction results in new mycelium of the same type as the parent mycelium.

Mycelial fragments that are placed on new substrates can continue to grow and establish a new colony. Basidiomycetes do not survive fragmentation as well as Ascomycetes. This is one reason why shiitake spawn consists of pieces of solid material which provide protection for the mycelium within them.

Conidia are one type of asexual spore. Some conidia are produced in dusty masses and disseminated by air. Others are sticky and are carried by insects or splashing raindrops. Although individual conidia are too small to be seen with the naked eye, the air contains thousands or millions of these spores. This accounts for the rapid contamination of exposed substrates.

Most Ascomycetes, and many Basidiomycetes, produce conidia. Although shiitake does not produce any asexual spores, its most troublesome disease, the ubiquitous *Trichoderma*, reproduces primarily by this means.

Life Cycles

The life cycle of a fungus describes the developmental stages of the fungus during one generation. Significant differences exist not only between the life cycles of Ascomycetes and Basidiomycetes, but also among species within each class. Two life cycles of importance to shiitake growers are discussed in the following section: shiitake, (*Lentinula edodes*, a Basidiomycete), and *Hypocrea rufa* (an Ascomycete) and its asexual stage, *Trichoderma viride*.

Shiitake Life Cycle

The life cycle of shiitake begins when a mature mushroom sheds basidiospores into the air and they are dispersed by the wind (Fig. 2–3). Basidiospores are thin-walled and perish rapidly when exposed to sunlight

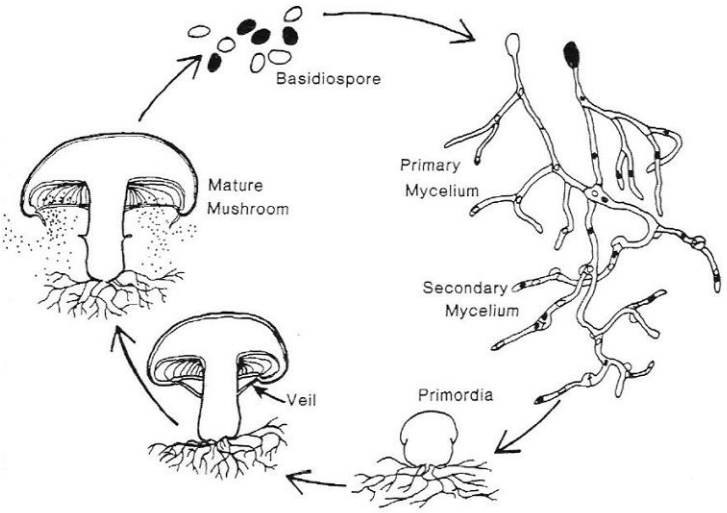


Figure 2–3. Shiitake life cycle.

(*134*). Most of them die; however, those that land on a suitable substrate may, under proper conditions, germinate and establish a new colony.

When the basidiospore germinates, it produces a new hyphal tip which grows into primary mycelium. If the emerging mycelium does not encounter a suitable food supply, it uses up the nutrient reserves in the spore and then dies. However, if the primary mycelium finds a suitable substrate, it could increase in size indefinitely.

In nature, however, the primary mycelial stage usually is brief. Primary mycelium of shiitake can not produce any mushrooms. To develop secondary mycelium, two primary hyphae (monokaryons), containing compatible nuclei, must grow together to form a dikaryon, as discussed earlier. The secondary mycelium resulting from this mating can produce mushrooms and complete the life cycle.

Not all primary mycelia of shiitake are compatible. For all practical purposes, shiitake has four mating types ("sexes") which are compatible only in certain combinations. The ability of two monokaryons to grow together and exchange nuclei is controlled by one or more genes. The **mating system** of *L. edodes* is **heterothallic**, which means that two genetically different spores must mate, and **tetrapolar**, which means that four different genes control the compatibility of the spores (*145, 195*). A detailed discussion of the diverse mating systems of Ascomycetes and Basidiomycetes can be found in fungal genetics texts (*161*).

Shiitake spends most of its life cycle as secondary mycelium. In this vegetative stage, the mycelium colonizes and digests wood, absorbing and storing nutrients in preparation for fruiting. Unless sufficient nutrients have been stored, the fungus can not move on to the fruiting stage.

Mushrooms are produced in response to environmental cues which often stress the mycelium, signaling that it is time to seek new substrates. Mushroom formation begins when small knots of hyphae called **primordia** (singular, **primordium**) or **pins** develop below the surface. These primordia increase in size, creating enough pressure to break through the surface. If the environment is favorable, and water and nutrients are not limiting, they will continue to expand and develop into mature mushrooms.

As a mushroom grows, it gets larger and its stem bends, if necessary, so that the top of the cap is up and the developing gills are down. Initially, the gills are protected by a membrane (called a **veil**), which extends from the stem to the margin of the cap. As the spores mature, further expansion of the cap ruptures this veil, exposing the gills. Veil remnants can be seen as fibers on the edge of the cap and on the stem.

The fertile hymenium on the surface of the gills is covered with basidia and basidiospores. Fusion of the dikaryotic nuclei occurs within the basidia. This is immediately followed by a reduction division (meiosis) which results in four genetically unique monokaryotic basidiospores.

The mature spores fall from the gills onto surrounding areas or are picked up by air currents and carried to new substrates.

Life Cycle of *Hypocrea rufa* (*Trichoderma*)

Hypocrea species are one of the most serious diseases of shiitake. They compete for space and nutrients in the sapwood, and they can attack and consume shiitake mycelium.

Because the sexual stage of *Hypocrea* is rarely seen on logs, it has traditionally been called by the name that had been applied to its rather common asexual (conidial) stage: *Trichoderma viride*. The life cycle begins with ascospores (Fig. 2–4). Germination of the ascospore produces a primary mycelium which is the main feeding stage for this fungus. As this mycelium grows through the sapwood, it degrades both the cell contents and the cell walls of the wood. *Hypocrea* mycelium can also attack and digest the mycelium of other wood-inhabiting fungi.

The primary mycelium can produce asexual spores (conidia). The grower may first notice this pathogen when the spore-bearing hyphae appear as white patches on the surface of the substrate. Then, as the colored conidia mature, the white patches turn green and become powdery with loose conidia.

With a microscope, it is possible to see that the conidia are formed in chains at the tips of bowling-pin shaped cells called **phialides**. A small patch can produce millions of conidia which become airborne, settle on surround-

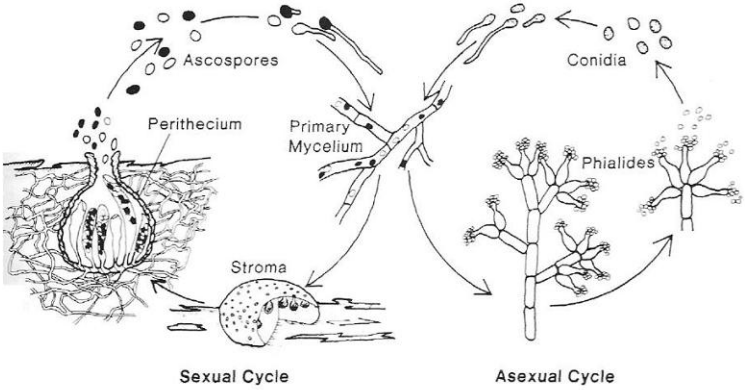


Figure 2–4. Life cycle of *Hypocrea/ Trichoderma.*

ing surfaces, and, in turn, produce new primary mycelium and more conidia. Thus, the conidia serve to rapidly establish *Trichoderma* in a localized area.

The primary mycelium can produce millions of conidia, but it can not produce ascospores until it mates with a compatible primary mycelium. Like shiitake, the mating of two primary mycelia is controlled by a mating system. *Hypocrea rufa* is heterothallic and **bipolar** which means it has two mating types, unlike the four of shiitake.

The developing flask-shaped fruiting bodies (**perithecia**, singular **perithecium**) of *Hypocrea* are enclosed in a cushion-shaped mass of tissue called **stroma**. These structures are formed of primary mycelium. Each stroma contains a dozen or more perithecia.

If mating occurs, sac-like asci are formed within the perithecia. Ascospores are formed following fusion of the parent nuclei, sexual recombination and division. Unlike basidiospore formation, eight monokaryotic spores are produced. The mature asci extend up through the opening of the perithecia and forcibly eject their ascospores. The ascospores of *Hypocrea rufa* are divided into two parts which are commonly ejected as if they were sixteen separate spores.

These spores then begin another generation. Half of the ascospores are of one mating type, and half are the opposite type.

The Process of Wood Decay

Shiitake obtains the nutrients it needs by decaying wood. The hyphae secrete enzymes that break down otherwise insoluble materials (like the cellulose and lignin in wood) and turn them into simple sugars that diffuse back to the fungal hyphae, where they are absorbed as food. As wood decays, it

loses weight and strength, and increases its capacity to absorb moisture (*234*). Decay fungi convert the wood components into fungal tissue and carbon dioxide.

A number of factors influence the rate of wood decay: nutrient content of the wood, moisture level in the wood, temperature, oxygen availability, and the concentration of toxic or inhibitory compounds.

Decay fungi are aerobic: they require oxygen and produce carbon dioxide.

Constituents of Wood

The major components of wood are cellulose, hemicellulose and lignin. These are large, complex molecules, consisting of long chains of smaller molecules. Cellulose is a long chain of glucose molecules, whereas hemicelluloses are composed of other sugars, such as xylose and mannose. Lignin is formed by bonding a number of different phenolic compounds, which are hard for many fungi to degrade.

Wood gets most of its strength from cellulose, which is arranged in bundles in the cell walls of wood fibers. The lignin adds stiffness and is encrusted on the hemicellulose surrounding the cellulose bundles. Additional nutrients in wood include pectins, starch, minerals and simple sugars.

Nitrogen levels in wood are low (0.03% to 0.3%) and nitrogen is often a limiting factor for decay (*123*). Decay fungi need nitrogen to build their cell walls and to produce enzymes. For example, cellulase, the enzyme which breaks down cellulose, is 16% nitrogen.

The rate of wood decay is proportional to the nitrogen concentration in the wood (*123*). Therefore, as shiitake consumes the available nitrogen, its yields decrease (*205*).

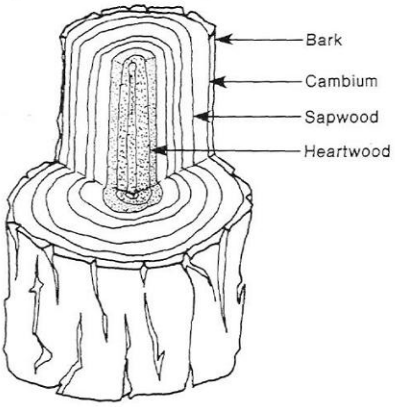


Figure 2–5. Anatomy of a tree trunk.

Sapwood and Heartwood

A tree stem is divided into sapwood and heartwood (Fig. 2–5). The cambium is a thin layer of cells, located just beneath the bark. It is the layer where new sapwood and bark are formed. The sapwood is the active portion of the tree trunk, transporting water and nutrients from the roots up to the leaves and bringing sugars and other compounds from the leaves down to the roots. For this reason, the sapwood contains the most readily available nutrients and has a higher moisture content than the heartwood (*162*). For example, the nitrogen

concentration is highest in the cambium and sapwood, and decreases toward the center of the stem (*123*). This is one reason why shiitake grows best in sapwood.

The heartwood is dead and has a very different chemical composition from sapwood. Heartwood functions as a dump for metabolic wastes generated by the tree. Many of these compounds are inhibitory or toxic to fungi. Usually the heartwood is visible as a darker area in the center of the tree stem.

Water in Wood

The moisture content of wood is an important element in decay. Either too much or too little water inhibits the growth of decay organisms. Wood that is suitable for decay contains enough water to create a film of free water on the surface of the wood fiber cells. Free water first appears in wood at a moisture content of about 23% (fresh wt basis) (*178*). This is called the **fiber saturation point.** Moisture contents above 80% generally inhibit decay by limiting oxygen.

The moisture content of wood eventually equilibrates with the water content of the surrounding atmosphere. Even in very moist air, the wood moisture content at equilibrium will be below the fiber saturation point. This equilibration must be controlled during shiitake cultivation to keep the wood moist enough to support fungal growth.

Water is required for several different steps in the decay process. A water film on the wood cell surface is needed to diffuse fungal enzymes from the hyphae and move dissolved nutrients back to the hyphae. Water swells the wood fibers, allowing degradative enzymes to reach the cellulose bundles. In addition, breaking the chemical bonds of cellulose molecules requires a molecule of water for each bond that is broken.

Types of Wood Decay

Wood-decaying Basidiomycetes cause two different types of decay, brown rot and white rot. Brown-rot fungi degrade mostly cellulose and leave the lignin, which appears brown in the latter stages of decay.

White rots generally degrade lignin and cellulose at about the same rate (*82, 202*). Breakdown of the wood by white-rot fungi is concentrated around the hyphae (Fig. 2–6) and degrades the cell starting from the inside (*234*). In the latter stages of decay, the wood appears white and stringy. Shiitake is a white rot.

Fungi grow fastest along the grain, following the orientation of the wood fiber cells (Fig. 2–7). Hyphae spread more slowly across the grain, for they must penetrate wood cell walls. In sawdust, the wood structure is disrupted and more surface area is exposed. This gives the mycelium better access to the wood, so the fungus can grow and degrade the sawdust more rapidly.

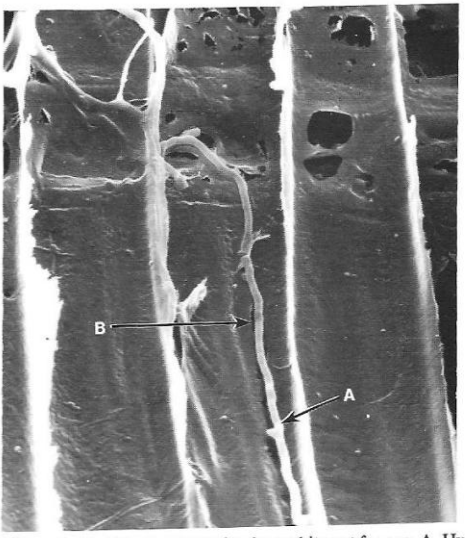


Figure 2–6. Wood cell erosion by a white rot fungus. A. Hypha with clamp connections, B. Localized erosion of cell wall adjacent to hypha.

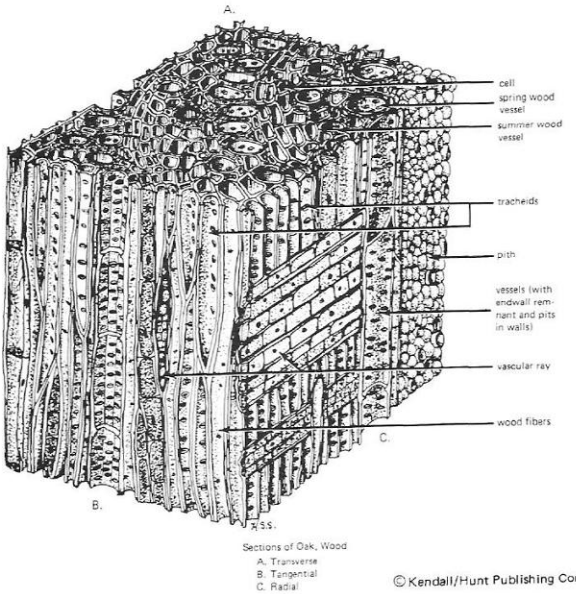


Figure 2–7. Anatomy of oak wood.

Succession of Organisms during Wood Decay

During the natural process of wood decay, a succession of organisms inhabits the wood. Each group of organisms removes part of the wood and leaves residues. This creates a new environment where that group can no longer compete, but which is favorable for the next group of organisms (*14, 159*).

The first organisms to invade wood are bacteria and yeasts. They leave behind growth factors, such as vitamins and nitrogen that become nutrients for the growth of decay fungi which come later in the succession (*14*). There is evidence that some bacteria can raise the nitrogen concentration of decaying wood by converting atmospheric nitrogen into useable forms (*28*).

The next inhabitants are rapid-growing fungi which utilize the easily available sugars in the sapwood. These fungi have a limited capacity to degrade cellulose, and they decline as the sugars are used up.

The first decay fungi to appear can compete for these sugars and can also degrade cellulose. Later decay fungi can completely degrade the wood. In nature, shiitake fits into the succession of decay organisms at this point. Cultivation tries to extend this period by introducing shiitake earlier and managing the logs to secure and prolong its dominant position.

Zone lines are formed by some decay fungi as a protective mechanism, often in response to other fungi, to light or to air (*113*). The decay fungus forms pigmented sheets of swollen hyphae which enclose the main mass of mycelium in the wood (*113*). Generally, this mycelial sheet is dark colored. In cross section, it forms a dark line which defines the boundary of the decay fungus colony. Antifungal compounds are secreted by these pigmented hyphae (*130, 202*) and invasion by other fungi is prevented while available nutrients last (*164, 202*).

Shiitake forms zone lines in response to other fungi, both in logs and in sawdust substrates (*202*). Darkened mycelium also develops in response to light on exposed log ends and substrate surfaces, forming a brown, leathery tissue.

3

Physical and Chemical Factors

Mushroom cultivation attempts to control a natural process by manipulating environmental factors. The science of cultivation involves thoroughly understanding these factors and their effects on mushroom growth. However, sensing what changes are needed and applying this information remains an art.

This chapter considers the key environmental factors which influence the growth of shiitake: temperature, humidity, evaporation potential, substrate moisture content, light, pH and gas concentrations. It also explains how to determine humidity levels and the moisture content of logs.

Temperature

Fahrenheit and Celsius

Everyone is familiar with the use of a thermometer to measure temperature. The two scales commonly used for expressing temperature are named for their creators: Gabriel Daniel Fahrenheit (1686–1736) and Anders Celsius (1701–1744). The Fahrenheit scale designates 32 degrees as the freezing point of water and 212 degrees as the boiling point. The Celsius (centigrade) scale sets the freezing point and boiling points of water at 0 and 100 degrees, respectively. One degree Celsius is equal to 1.8 degrees Fahrenheit (Table 3–1).

TABLE 3–1

Relationship between *Fahrenheit and Celsius* Scales

Fahrenheit	Celsius	Fahrenheit	Celsius	Fahrenheit	Celsius
–22	–30	32	0	86	30
–13	–25	41	5	95	35
– 4	–20	50	10	104	40
5	–15	59	15	113	45
14	–10	68	20	122	50
23	– 5	77	25	131	55

To convert between scales: °F = (°C x 9/5) + 32
°C = 5/9 x (°F–32)

Temperature is one of the most important factors in shiitake cultivation. It affects survival, growth rate, time of fruiting, yield, and even the shape of the mushroom.

Shiitake has been reported to survive in logs from –22° to 113°F (–30° to 45°C) (*100*). The survival of fungi at high temperatures is determined by both temperature and exposure time: the detrimental effects of short exposures at high temperatures are equivalent to longer times at somewhat lower temperatures. For example, shiitake is killed at temperatures around 113°F (45°C) (*208*), but prolonged exposure at temperatures above 95°F (35°C) also can kill the mycelium.

Temperature controls the growth rate of the fungi by changing the rate of chemical processes associated with mycelial growth. Growth increases as temperature rises to an optimum, then declines with higher temperatures. Shiitake mycelium will grow between 40° and 95°F (4°–35°C), but its optimum temperature is from 75° to 82°F (24°–28°C) (*210*), usually at 77°F (25°C) (*144*).

Temperature also plays a very important role in fruiting, affecting both mushroom initiation and development. Mushroom initiation (**pinning**) is induced by a sudden shift in temperature or by a period of fluctuating temperatures (*85*). The temperature during pinning is critical for maximum yields. Optimum pinning temperatures are strain-dependent and range from 50° to 77°F (10–25°C), usually from 50° to 60°F (10°–16°C) (*150, 151, 210*).

Temperature affects not only yields, but mushroom shape as well (*150*). High temperatures cause long stems and thin caps, whereas mushrooms grown under cool conditions have short stems and thick caps (*208*). Developing mushrooms will tolerate freezing temperatures for short periods, but can be damaged or killed by prolonged exposure.

Humidity

Effects of Humidity on Shiitake

Evaporation of water is important during shiitake cultivation. Mushrooms consist of 85% to 95% water and are constantly losing water to the air. The substrate is also losing water. If too much water is lost, both the quantity and quality of the crop suffer.

Water loss is controlled by regulating humidity levels. For this reason, the grower must understand the concept of humidity and the tools and techniques which monitor it.

Absolute and Relative Humidity

Humidity measures the amount of water vapor in the air, usually expressed as absolute or relative humidity. **Absolute humidity** is the actual amount of water vapor (pounds, grams or grains) in a unit of air (cubic feet, cubic meters, pounds). The **saturation point** is the maximum amount of water

a volume of air can hold; this increases with temperature. When the absolute humidity equals the saturation point, water begins to condense; this is referred to as the **dew-point.**

Relative humidity (RH) indicates how dry or damp the air is. A practical definition of relative humidity is: the ratio of the amount of water in a unit of air (absolute humidity) to the maximum water-holding capacity of that unit of air at a given temperature (saturation point). Air at the same relative humidity will contain different amounts of water at different temperatures.

For example, a cubic foot (cu ft) of air at 80°F (27°C) contains 5.8 grains (gr) of water (7,000 grains = 1 lb). The saturation point of air at 80°F is 11.04 gr per cu ft, resulting in a relative humidity of 5.8/11.04 which equals 53% RH. If this air is cooled to 60°F (16°C) without losing any water vapor, the relative humidity would increase to 100% RH (Fig. 3–1). Thus, the dewpoint would be 60°F (16°C). A one or two degree drop in temperature at high relative humidities can saturate the air, creating condensation problems on developing mushrooms.

Relative humidity is measured in several ways using an apparatus called a hygrometer. Some hygrometers use a humidity-sensitive element, often a human hair, which changes length with humidity. These hygrometers are inexpensive, work well and are accurate within 5% RH at humidities below 80%.

A more accurate type of hygrometer is the pyschrometer. This tool is often used to calibrate other kinds of hygrometers. A psychrometer consists of two identical thermometers, a water reservoir, and a wick around one thermometer bulb. Evaporation of water from the wick lowers the temperature of the wet bulb thermometer. The difference in temperature between the dry bulb and wet bulb is used to calculate the relative humidity. Some pyschrometers can be mounted on a wall where the air circulation is sufficient, while others (sling-type) are whirled in a circle to get an accurate reading.

Other methods of measuring relative humidity are based on cooling the air until it reaches the dew-point, then measuring changes in the electrical conductivity of humidity-sensitive materials.

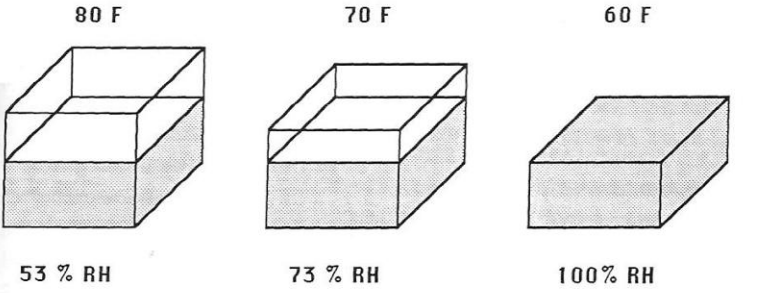


Figure 3–1. Water holding capacity of air at different temperatures.

Evaporation

As water evaporates from a surface, that surface is cooled. This phenomenon, known as **evaporative cooling**, is due to the latent heat of vaporization. Each molecule of water requires 540 calories to vaporize, and it obtains this energy (heat) from the surrounding environment. Thus, as the water vapor escapes, it carries a great deal of heat with it.

The rate of evaporation, **evaporation potential**, is determined by temperature, relative humidity and air movement. Temperature and relative humidity determine how much additional water the air can hold, whereas air movement influences the rate at which the water is removed.

For example, assume that some mushrooms are in contact with just one cubic ft of air at 55 °F (13°C), 90% RH. The saturation point of air at 55°F is 4.9 gr per cu ft. Therefore, at 90% RH, the absolute humidity is 4.4 gr per cu ft. If there is no air movement or condensation, the maximum amount of water that could be lost from the mushrooms is the difference between the saturation point and the absolute humidity (i.e., 4.9 gr minus 4.4 gr = 0.5 gr).

However, if 20 cubic feet per minute (cfm) of this air were moving past the mushrooms, the evaporation potential would increase to .086 lb of water per hour (20 x 0.5 x 60 min/hr = 600 gr/hr divided by 7,000 gr/lb = .086). This translates into a loss of 2 lb of water per day into the air.

As has been shown, evaporation potential and humidity are very important in mushroom growing. Relative humidity and air movement must be carefully controlled to avoid rapid drying, yet provide enough ventilation for good mushroom growth. The final moisture content of a mushroom depends, in part, on the humidity during maturation. Mushrooms grown at high humidities contain more water than those grown at lower humidities, given the same temperature, air flow, substrate moisture content and strain.

Moisture Content

Proper moisture content in the substrate is crucial for a good spawn run. **Moisture content** is the amount of water in an object expressed as a percentage of the total weight. It is calculated by comparing the initial weight of a sample with its weight after drying in an oven.

Calculating Moisture Content

The moisture content is calculated by determining the weight of the water in a sample and expressing this weight as a percentage. Substrate moisture content can be determined as follows: Remove a fresh sample of substrate (sawdust mix or a thin log slice) and wrap it in plastic to keep it from drying before it is weighed. Then weigh the sample and record its fresh weight. Place the sample in an oven at 200°F (93°C) for 6 to 12 hours until it stops losing weight. Weigh the dry sample and record its oven-dry weight.

The weight of the water is the difference between the fresh and oven-dry weights.

For example, a freshly cut log section weighs 5.5 oz (156 g) initially and weighs 2.2 oz (62.4 g) after 12 hours in the oven. Subtracting the dry weight from the fresh weight gives a difference of 3.3 oz (93.5 g) as the water weight.

The moisture content can now be calculated. It can be expressed in two ways: as oven-dry moisture content (ODMC) or as fresh-weight moisture content (FWMC).

To find the **oven-dry moisture content (ODMC)**, divide the weight of the water by the oven-dry weight. Using figures from the example above, the ODMC equals 3.3 / 2.2, or 150%. Note that this method can result in a total moisture content of over 100%. This method is widely used in the lumber and forest products industry.

Electronic pin-type moisture meters manufactured for the lumber industry also register moisture content on an oven-dry basis. These meters use electrical resistance of the wood to estimate moisture content and are accurate only if there is no free water in the wood. Since this is below the range required for growth of shiitake, these meters are of little value for shiitake cultivation.

To calculate the **Fresh Weight Moisture Content**, divide the weight of the water by the fresh weight. Using the figures from the same example above, FWMC equals 3.3 / 5.5 or 60%. This method is widely used in the mushroom and produce industries, and is the method used in this book.

The relationship between these two ways of expressing moisture content is:

$$FWMC = ODMC / (1 + ODMC) \text{ or } ODMC = FWMC / (1\text{-}FWMC)$$

Effects of Moisture Content on Shiitake

The moisture content of the substrate is a critical factor in the success of the spawn run. Optimum substrate moisture levels for growth of shiitake mycelium are from 55% to 68% moisture content (MC) in sawdust culture (*125*), and from 35% to 75% log moisture content (LMC) in logs (*2*). Above or below this range, growth will be inhibited. Primordia (pin) formation on logs occurs from 35% to 65% LMC, with the optimum range from 55% to 65% LMC (*98, 211*). If the substrate dries to the fiber saturation point (23% MC), the fungus will be killed.

Light

Shiitake requires light during both its vegetative and fruiting stages.

Exposure to light increases the rate of decay by certain decay fungi (*178*), and light exposure during its period of vegetative growth is a prerequisite for the fruiting of shiitake (*65*). However, the duration needed is not well defined and a brief exposure (20 min/day) may be enough.

Light is characterized in terms of its wavelength and intensity. Wavelength determines the color of light and is expressed in nanometers. Visible light is from 380 nm (near-UV) to 750 (infra-red). The wavelength of light can be measured with a spectrometer. Shiitake responds to wavelengths in the near-UV to blue, 370 to 420 nm (*5, 65*). If artificial lighting is used, these wavelengths can be obtained by using fluorescent lamps.

Intensity is most easily expressed in lux. Light intensity can be estimated with a light meter. A light meter on a camera can be used as follows: set the film speed to ASA 400 and the shutter speed to 1/60 second. When focused on a gray card, the meter should read f4 (*53*). A general rule of thumb for the amount of light required during fruiting is: there is enough light if a newspaper can be read at arm's length.

The spawn run requires from 180 to 940 lux, with 550 lux as an optimum. Research with other Basidiomycetes confirms these findings. It also suggests that the mycelium becomes sensitive to light only after available nutrients decrease (*115, 222*).

Light also plays a role in fruit body development, especially of the gills and spores (*86*). The color of the mushroom is affected by light during maturation. Mushrooms grown in the dark are light-colored and often misshapen (*144, 160*).

Chemical Factors

pH

The degree of acidity or alkalinity of a solution is measured by determining the concentration of hydrogen ions in solution; this is expressed as pH units. pH ranges from 0 to 14, with pH 7 (distilled water) being neutral. Numbers above pH 7 indicate increasing alkalinity; numbers below pH 7 indicate increasing acidity.

Commonly, pH is measured with an electronic pH meter or with colored, pH-sensitive chemicals. These chemicals turn specific colors at known pH values and are available as pH paper or in soil test kits.

Fungal growth is influenced by pH in several ways. pH directly affects the reaction between degradative enzymes and the wood. Each enzyme has an optimum pH; as the pH gets further above or below this optimum, the enzyme works more slowly until it is completely inhibited. In addition, pH affects the solubility of compounds, which, in turn, determines their availability to the fungus.

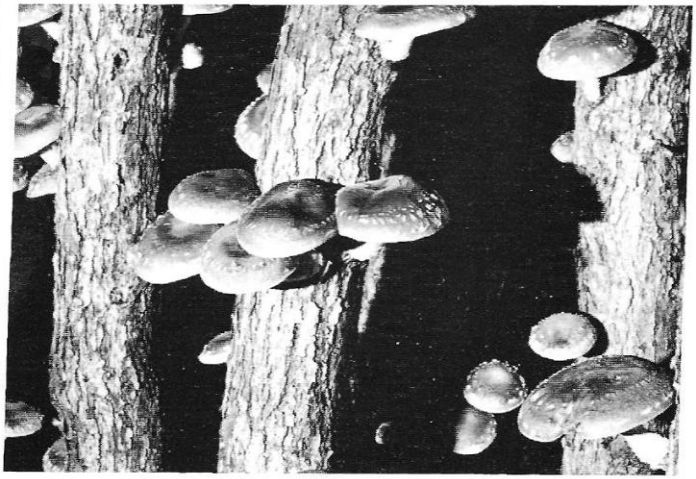
The optimum pH for wood decay fungi is from 4.5 to 5.5; growth is halted at pH 2 (*178*). Reported pH optima for shiitake mycelial growth are 3.5 (*65*) and 4.3 (*208*). The pH of wood is from pH 4.5 to 5 and becomes increasingly acidic as shiitake decays the wood (*178, 211*). The optimum pH for fruiting is from 3.5 to 4.5 in laboratory culture on artificial media (*207*) and pH 5 in sawdust culture (*52*).

Gas Concentration

Mushrooms are aerobic, using oxygen and respiring carbon dioxide. The composition of the atmosphere, especially the concentration of carbon dioxide and other volatiles, such as ethylene, influences mushroom growth and development (*138, 227*). Gas concentrations are generally expressed as percentages or as parts per million (ppm, 100 ppm = .01%). Oxygen makes up about 21% of the atmosphere, carbon dioxide is about 0.03% and nitrogen is about 78%.

Studies of the common button mushroom have shown that increasing the carbon dioxide level up to 0.6% (6000 ppm) enhances mycelial growth. However, at levels from 0.4% to 0.6%, primordia formation is completely inhibited. Between CO_2 concentrations of 0.2% and 0.4%, the primordia are deformed and the mushrooms have long stems and small caps. Carbon dioxide levels below 0.2% are optimum for fruiting (*227*). Shiitake appears to react similarly to carbon dioxide levels (*168*).

Section II
Shiitake Cultivation on Logs



Shiitake has traditionally been cultivated on hardwood logs. This method of cultivation is still widely used throughout the world. Cultivation involves a series of steps, and since each step can be accomplished in several ways, the grower has many options to consider.

Shiitake grows on logs, but it does not grow equally well on all logs. The tree species selected, where it has grown, and when it is cut all make a difference. Shiitake has specific temperature and moisture requirements, but they are not constant over its life cycle; they differ from stage to stage. The shiitake grower must learn to recognize these stages, monitor conditions within the logs, and manipulate their environment to achieve the most vigorous, productive growth.

This section explores shiitake cultivation on logs: determining a production area, selecting trees and obtaining logs, choosing shiitake spawn and strains, inoculating, incubating, fruiting, and controlling diseases and pests. Discussions include basic concepts of shiitake growth, summaries of pertinent research, and management practices.

4

Selection and Preparation of Logs

The obvious first requirement for cultivating shiitake on logs is a source of logs. These logs must be carefully handled to maintain their selective nature in order to favor growth of shiitake, and not its rivals.

This chapter will discuss climatic factors that make shiitake cultivation feasible in a particular region. It will also show why some kinds of logs and some tree species are more likely to produce a successful shiitake crop. It will then tell how to select trees in the forest and suggest ways to cut, store and handle logs. Finally, it will show how to determine and monitor log moisture content.

Suitable Regions

Natural Distribution and Habitat

Lentinula edodes occurs naturally throughout Asia. It is reported from China, Korea, Thailand, Burma, Nepal, North Borneo, the Philippines, Japan and Papua, New Guinea (Fig. 4–1) (*127*). A similar species, *L. boryana*, occurs in sub-tropical America, throughout Florida, Mississippi, Louisiana, the Caribbean Islands, Cuba, Venezuela, Brazil (*157*) and Costa Rica (*111*).

Shiitake's natural habitat is dead hardwood logs in a warm, moist climate. This combination of warm temperature and plentiful moisture prevents excessive drying of the wood and promotes rapid fungal growth. Mushrooms are produced whenever the weather permits, but primarily in the spring and fall (*182*).

Shiitake grows on a number of broad-leaved trees (hardwoods), mostly in the oak family (Fagaceae). These include: oak (*Quercus*), beech (*Fagus*), chestnut (*Castenea*), hornbeam (*Carpinus*), and chinkapin (*Castanopsis*) in the Fagaceae as well as trees from many other families (*68, 182*).

Areas Suitable for Cultivation

Improved cultivation techniques that developed in the mid 1900's rapidly expanded the shiitake industry. New methods were developed to create favorable environments around the logs, thus making shiitake cultivation possible in areas unsuited to outdoor natural cultivation.

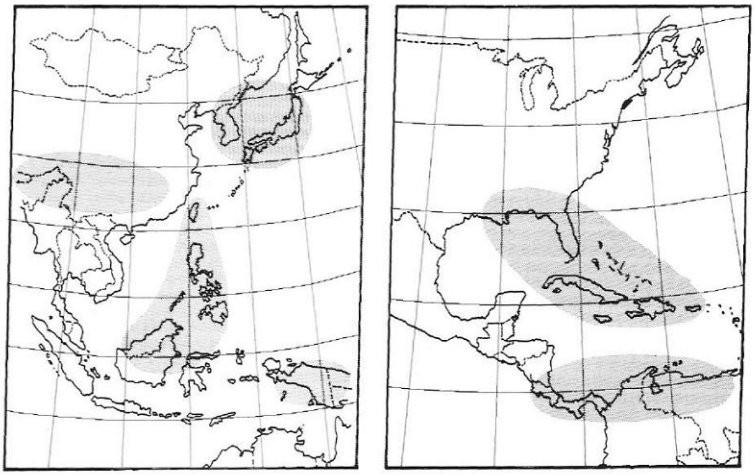


Figure 4–1. Natural distribution of *Lentinula edodes* and *L. boryana.*

Shiitake can be grown successfully almost anywhere suitable trees are found. Factors affecting the success of a commercial shiitake operation include: a supply of trees, availability of labor, access to markets, market price and demand, a source of quality spawn, cultivation tools and equipment, and, above all, the management skills to put it all together.

Although an unfavorable climate does not necessarily preclude shiitake production, it does greatly affect the economics of a commercial operation. As climate becomes less conducive, greater capital investment is needed to artificially create a favorable log environment. Investments include not only buildings, but also systems for heating, cooling, humidification and ventilation. Managing these systems requires a high level of skill. To justify these expenditures, a profitable market must exist.

Important Properties of Logs

Logs are a selective substrate. The low nutrient levels in wood limit the number of organisms able to survive on it. Uncut trees are relatively sterile. The living tree has built-in defenses against fungal attack: its bark and its ability to produce anti-fungal compounds. The log is protected by its bark, which decreases water loss and provides a physical barrier to invading fungi. Since the bark contains few nutrients, fungal colonization and penetration are

discouraged. This selectivity should be maintained by avoiding damage to the bark.

For successful shiitake cultivation, logs must contain sufficient nutrients and water. However, wood density, moisture content and nutrient concentration may vary considerably within the same tree, between trees from different sites and between tree species.

Sapwood/Heartwood Ratio

Sapwood, the part of the tree between the central heartwood and the bark, is metabolically active and contains most of the available sugars. Shiitake colonizes and decays sapwood rapidly. Heartwood is not readily colonized by shiitake. Generally, it has a lower moisture content and often contains compounds which inhibit fungal growth (*141*).

Ideal shiitake logs have little or no heartwood (Fig. 4–2). The ratio of sapwood to heartwood varies considerably between tree species and also among trees of the same species. Generally, the proportion of heartwood increases with age and diameter. It also depends on the tree's growth rate. Trees in poor sites usually grow slowly and have a higher percentage of heartwood than rapidly growing trees.

Wood Density

Other factors being equal, the productive life and total yield of a shiitake log rise with increasing wood density. Wood density is expressed as **specific gravity**: the ratio of the weight of wood compared to the weight of an equal volume of water.

Labor costs are a significant portion of the cost of producing an inoculated log, and this initial cost is the same regardless of the log's ultimate yield.

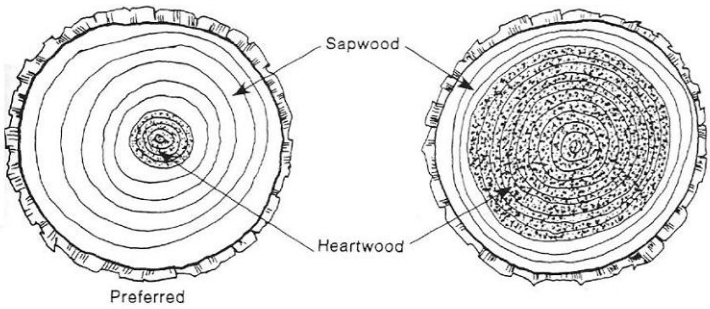


Figure 4–2. Sapwood/heartwood ratio in shiitake logs.

One reason oaks are widely used for shiitake cultivation is that they are one of the more dense woods available (Table 4–1).

TABLE 4–1
Specific Gravity and Moisture Content of Some Tree Species Suitable for Shiitake Cultivation

Species	Specific Gravity	Moisture Content of Freshly Cut Wood (1)
Alder	.37	49
Beech	.56	39
Birch	.57	42
Chestnut	.40	55
Chinkapin	.42	57
Hickory	.65	39
Hop hornbeam	.63	34
Magnolia	.46	48
Maple, soft	.44	61
hard	.56	44
Oak, white	.60	41
red	.54	43
black	.51	43
Tanoak	.56	47
Tupelo, black	.46	50
Willow, black	.34	58

1. Moisture content was calculated from an average of heartwood and sapwood, using fresh weight as the base.
References: 162, 37

Log Moisture Content

Maintaining a proper moisture content in the logs prior to inoculation is critical for successful shiitake cultivation. Shiitake grows best at log moisture contents between 35% and 55%. Initial log moisture content differs among tree species (Table 4–1). It also varies between the heartwood and sapwood, between different points along the length of the trunk (*58*) and between trees from different sites.

Bark Characteristics

The bark provides a protective layer on the outside of the log. It also limits moisture loss and decreases contamination by competing fungi.

Removal of the bark from freshly cut logs results in rapid colonization of the exposed sapwood by numerous pest fungi (*159*). Shiitake logs require a bark covering in order to produce mushrooms; areas of logs that have lost their bark are no longer productive.

Thick bark resists physical damage and water loss better than thin bark. Thin-barked logs must be carefully handled to prevent bark damage, and they require more watering to maintain appropriate moisture levels. Many trees have thin bark when young and develop thicker bark during maturation.

Handling Characteristics

Profitable mushroom cultivation depends on efficient movement of materials. Therefore, the maximum size of shiitake logs depends in part on whether they will be moved by hand or by machine. Although uniform log length facilitates handling for commercial operations, there is no magic length. Most shiitake growers find that three- to four-foot logs (0.9–1.2 m) can be lifted easily without back strain.

The maximum log diameter is limited by log weight and the proportion of sapwood to heartwood. Logs greater than 6 to 7 inches (15–18 cm) in diameter are difficult to lift by hand and often have a high proportion of decay-resistant heartwood. Some shiitake farms use logs with diameters 20 inches (51 cm) or greater which are never moved after they are inoculated.

Most shiitake growers use logs from 3 to 8 inches (8–20 cm) in diameter. Smaller logs are less desirable because they produce lower yields for the same handling costs. Also, they dry rapidly because of their high surface-to-volume ratio.

Tree Species

The choice of tree species determines many management practices during cultivation. Each wood has unique characteristics which affect shiitake growth. Not only do nutrient contents differ, but wood densities and bark characteristics are also unique. Methods that work for a dense, thick-barked species will not work for a lighter, thin-barked species.

Shiitake cultivation has been attempted on many tree species. While the list in Table 4–2 is by no means complete, it illustrates the range of species tried. Mushrooms have been produced on most of the listed species, with the exception of apple. Properly managed, some of the less suitable species might produce satisfactory shiitake crops.

The oak family (Fagaceae) contains most of the tree species used for commercial shiitake cultivation. The true oaks (*Quercus*) are preferred, where available, because of their high yields and long life. However, other members of the oak family, such as tanoak and chinkapin, are widely used.

TABLE 4–2
Tree Species Tested for Suitability for Shiitake Production

Common Name	Family	Genus	Species
High Suitability			
Oak	Fagaceae	Quercus	acutissima, alba, brandisiana, crispula, dentata, garryanna, kelloggii, kerii kingiana, mongolica, muehlenbergii, prunis, rubra, semiserrata, serrata, variabilis
Chinkapin	Fagaceae	Castanopsis	accuminatissima, argentea, chrysophylla, cuspidata ,indica
Tanoak	Fagaceae	Lithocarpus	auriculatus, densiflorus, lanceafolia, lindleyanus, polystachyus
Hornbeams	Fagaceae	Carpinus	betula, caroliniana, japonica, laxiflora, tschonoski
Medium Suitability			
Alder	Betulaceae	Alnus	glutinosa, japonica, rubra, serrulata, tinctoria,
Aspen, Popular Cottonwood	Betulaceae	Populus	balsamifera, deltoides, grandidentata, nigra, trichocarpa
Beech	Fagaceae	Fagus	species.
Birch	Betulaceae	Betula	nigra, pendula
Chestnut	Fagaceae	Castanea	crenata
		Cyclobalanopsis	acuta, glauca, salicina, myrsinfolia
Hickory	Juglandaceae	Carya	species
Maple	Aceraceae	Acer	rubrum, macrophyllum
Sweetgum	Hamamelidaceae	Liquidambar	styraciflua
Tupelo	Nyssaceae	Nyssa	silvatica
Willow	Salicaceae	Salix	nigra
Low Suitability			
Cucumbertree	Magnoliaceae	Magnolia	accuminata
Tulip-popular	Magnoliaceae	Liriodendron	tulipifera
Dogwood	Cornaceae	Cornus	florida
Apple	Rosaceae	Malus	sylvestris
Sycamore	Platanaceae	Platanus	occidentalis
Virginia pine	Pinaceae	Pinus	virginiana

Note: Trees considered highly suitable are widely used, while medium suitability trees require careful management. Trees with low suitability are not recommended for commercial shiitake production.
References: *18, 68, 83, 146, 160, 173, 182*

Log Preparation

Log preparation includes all the steps needed to convert a standing tree into shiitake logs.

Selecting Trees

The best shiitake logs come from fertile sites where the trees are grown close together (Fig. 4–3). Crowding produces straight trees with few branches, so many logs can be cut from the same tree. Only live, healthy trees that are relatively straight and contain a high percentage of sapwood should be selected. Trees with numerous decayed branch stubs, discolored wood or other signs of internal decay should be avoided.

Moss, lichens or other organic matter on the bark are rarely a problem, but should be removed prior to inoculation. In moist sites, bark-decaying fungi may thrive underneath this material. If trees are densely covered with moss, the underlying bark should be examined for fungal mycelium. If mycelium is noticed, these logs should be watched for contaminants during the spawn run. Usually these bark-inhabiting fungi die if the bark is allowed to dry.

Season to Cut Trees

Trees for shiitake cultivation are cut during the dormant season. In the autumn just prior to dormancy, the leaves fall off and the sap flows down to the tree roots (Fig. 4–4). During winter dormancy, sap does not flow. In the spring, the sap rises through the stem to the leaf buds.

Figure 4–3. Typical stand of trees for shiitake logs (USA).

Shiitake logs should be cut before the buds break in the spring (*180*). During dormancy, the sapwood contains high sugar levels and the bark adheres tightly. If trees are cut after the sap starts to rise, the bark slips off easily. Shiitake will grow on logs cut at other times, but yields may be lower and losses to contaminants greater.

Cutting Logs

Trees are generally cut into logs immediately after felling. However, if they will not be inoculated within 2 to 3 weeks, the limbs should be trimmed and the tree trunks left in longer lengths. This will minimize drying and contamination by decreasing the exposed end grain. Because of the orientation of wood fiber cells, moisture loss through the end grain is much greater than across the grain (Fig. 2–7). The logs should be handled carefully to avoid damage to the bark and to keep them free from mud. Soil contains mycelium and spores of many wood- inhabiting fungi which could infect the logs.

Storing Logs before Inoculation

If cut logs must be stored for a long period before inoculation, it is important to avoid either excessive drying or wetting. They should also be protected from direct sunlight.

During warm, dry weather, water loss from the logs should be minimized. Proper stacking helps to control the moisture level. Stacking logs in a bulk pile decreases air movement around them (Fig. 4–5). Plastic tarps, shade cloth or similar materials can further retard drying. Bulk stacks of logs should not be watered because the bark will remain wet and be colonized by mold fungi.

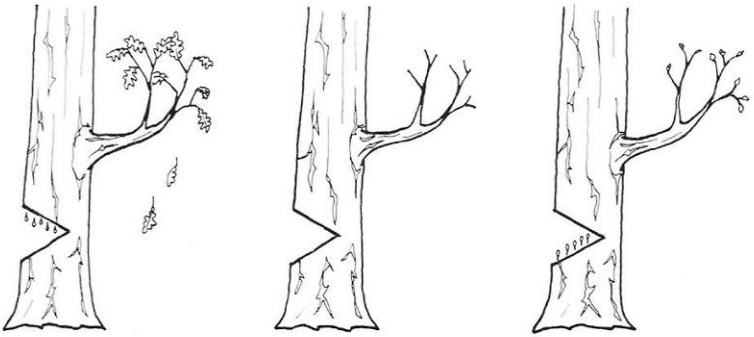


Figure 4–4. Sap flow during periods of the year.

Conversely, if the weather is cold and rainy, the logs should be stacked in low crib stacks to promote good air circulation around the bark (Fig. 4–5). This arrangement lets water drain off the logs. In areas with high rainfall, plastic, fiber glass, tin or other water-proof materials should be placed on top of the crib stacks to shed the rain.

Reference Logs

The difference between obtaining a good shiitake crop or a poor one depends on maintaining a favorable moisture content in the logs during cultivation. The log moisture content (LMC) is monitored through the use of reference logs.

Reference logs are marked logs with known dry weights. They are given exactly the same care as the rest of the logs. By placing these reference logs in several representative locations and periodically weighing them, changes in LMC in all the logs can be estimated. This is the most accurate and reliable way to monitor changes in log moisture content during the spawn run.

Reference logs should be selected from each source of logs and be representative of the logs from that site. Select and weigh reference logs (2 to 4 logs per 100) when the trees are cut into logs. A screw-eye placed in one end of each reference log will allow easy weighing with a hand-held spring scale. Number and mark these logs with flagging or paint so they can be spotted easily.

Determining Log Moisture Content

At this same time, select a *different* set of logs for moisture content determination. The moisture content of samples cut from these logs will be used to estimate the initial moisture content of all the logs, including the reference logs. This estimated initial moisture content will then be used to calculate the dry weight of the reference logs.

At least five different trees should be used. One or more samples should be cut from each log as follows: Cut thin slices, less than 1 inch (2.5 cm) thick, across the grain to form a round "cooky." If the trees are already

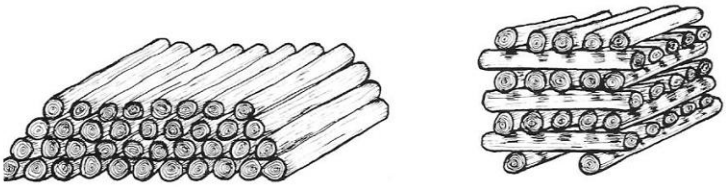


Figure 4–5. Stacking methods for shiitake logs before inoculation.

cut into logs, cut the "cookies" at least 6 inches (15 cm) from the log ends. To determine the wet (or fresh) weight, weigh the "cookies" immediately, or prevent moisture loss by wrapping in plastic bags until they are weighed.

After determining the fresh weight, the samples should be dried in an oven at 200°F (93°C) for 6 to 12 hours or until they stop losing weight. This weight is the dry weight.

The moisture content of the "cookies" equals the weight of the water in the "cookie" (wet weight minus the dry weight) divided by the initial wet weight (see Chapter 3 for more details). The moisture contents from all samples should be averaged to estimate the log moisture content (LMC) of all logs from that source. The LMC is stated as a percent of total weight.

The average LMC can now be used to calculate the dry weight for each reference log. Calculated dry weight equals the weight of log x (1 minus the average LMC). Record the log number and calculated dry weight in a notebook and also on a permanent tag (soft aluminum, plastic etc.) which should be attached to the log.

Although logs lose weight as shiitake decays the wood, the calculated dry weight can be used during the spawn run to estimate log moisture content. As weight is lost, the actual LMC will be higher than calculated.

Changes in the LMC of reference logs can be computed using the calculated dry weight as follows: Weigh the reference log and subtract the calculated dry weight to get the water weight. Then divide the water weight by the log weight to find the log moisture content.

For example, a 25 lb. log (11.3 kg) initially at 45% moisture content has a calculated dry weight of 25 x (1-0.45) or 13.75 lb. (6.2 kg). This weight is written on the log tag. Suppose this log weighs 20 lb. (9 kg) at a later date. Using the same calculated dry weight, the LMC is (20–13.75) / 20 = 31%. This simple method accurately tracks the log moisture content, eliminating the need to cut new samples.

It should be noted that dry weight does decrease markedly during the latter stages of cultivation as the log is decayed. Thus, the reference logs are most useful during the spawn run.

5

Strains and Spawn Suitable for Logs

The importance of strain selection is often overlooked, but it is critical to successful cultivation. Throughout cultivation, management must be tailored to the unique characteristics of the strain being grown. Although the choice of spawn type is not as important as strain selection, it also affects management practices.

This chapter examines differences between types of shiitake strains and presents a guide to strain selection. It also considers the advantages and disadvantages of kinds of commercial spawn. Finally, it suggests ways to inspect and store spawn.

Strain Characteristics

In nature, shiitake is propagated by spores. Due to sexual recombination during spore formation, mycelium produced from spores is genetically different from the parent mycelium. While this genetic variability helps shiitake adapt to changing conditions in nature, it is not desirable for commercial production.

To obtain high yields predictably and reliably, one must use genetically uniform mycelium with desired characteristics. This mycelium is called a pure **strain.** Strains are carefully maintained and propagated to avoid changing their genetic makeup. Just as there are different varieties of tomatoes, there are numerous strains of shiitake. Successful shiitake cultivation depends on understanding the behavior of each strain used and managing environmental factors to obtain maximum yields.

Although the mycelia of all strains grow best at comparable temperatures (72°–77°F, 22°–25°C), they differ in other important respects: growth rate, resistance to competing fungi, propensity to fruit, optimum fruiting temperature, and mushroom size and shape. These traits are discussed below.

Temperature Range

Shiitake strains may be classified by their optimum fruiting temperatures into three basic types: warm weather, cold weather, and wide-range strains (Fig. 5–1).

Warm weather strains produce mushrooms from 50° to 80°F (10°–27°C), whereas cold weather strains produce best from 45° to 60°F (7°–16°C). Cold weather strains can fruit at lower temperatures, albeit more slowly.

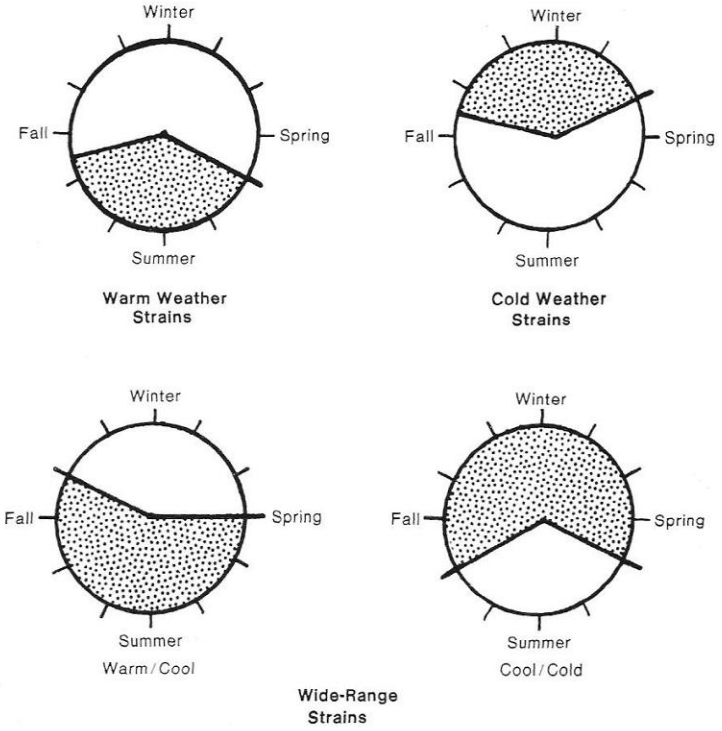


Figure 5–1. Fruiting seasons of different shiitake strains (Fruiting season is shaded).

Wide-range strains are either cold/cool (fall, winter, spring) or cool/warm (spring, summer, fall). These strains are widely used for forced fruiting (see Appendix).

Mushroom Quality

Shiitake quality is based on mushroom size, shape and thickness of the cap and stem, flavor intensity, flesh density, and moisture content. Many of these features are strain-dependent, but are also influenced by environmental conditions during fruiting.

Temperatures during fruiting affect mushroom quality. Thick- fleshed mushrooms are produced at cool or cold temperatures, mostly by cold-weather strains. Thinner-fleshed mushrooms are produced at warm temperatures by wide-range and warm weather strains.

Growth Rate

The growth rate of shiitake strains varies in two distinct ways: in the time needed to colonize wood and in the total time from inoculation to fruiting. Aggressive strains which colonize the logs rapidly are desirable because they discourage contamination. However, the speed of colonization is not directly related to the time until fruiting. Rapid-fruiting strains tend to be aggressive colonizers, but some aggressive colonizers take a long time to fruit (*160*).

Response to Fruiting Stimuli

Shiitake produces mushrooms in response to changes in nutrient availability, temperature and log moisture content. Each strain responds differently to these cues and requires a certain combination of stimuli for maximum yields. Some strains require very specific conditions, while others fruit well under a wide range of conditions.

For example, wide-range strains such as CS–15 and CS–41 respond well to drying, followed by soaking, within a wide temperature range. Cold weather strains, like CS–16, require a cold period followed by a warming trend, whereas warm weather strains respond best to warm temperatures, followed by a cold shock.

Strain Selection

Strain selection is an important management decision. Strain characteristics determine subsequent management strategy and total yields.

The prevailing climatic conditions during cultivation, and to a lesser extent, the tree species, dictate the most suitable strains. Each shiitake farm has a different micro-climate; therefore strains that work well for one grower may not be best for another. The choice of mushroom production strategy, that is, whether to fruit naturally outdoors or to force fruit inside, must also be considered.

A combination of strains can produce mushrooms throughout the year. Artificial temperature control, heating in the winter and cooling in the summer are usually necessary. The cost of these temperature manipulations can be decreased by choosing strains with fruiting temperatures that match the local climate.

To test the performance of a strain under local conditions, samples should include about 100 logs. Usually, just a few strains should be grown because each additional strain complicates management and production schedules. However, one should avoid growing only one strain because this places "all the eggs in one basket."

Inexperienced growers or growers starting in a new area should begin with rapid fruiting, wide-range strains. These strains fruit easily and may produce mushrooms within a year. After production systems have been

developed, growers can identify which strain characteristics are best adapted to the conditions on their farms. Because cold weather strains are often more difficult to manage, they should be added as the growers' experience increases.

Spawn and Its Production

Spawn

Shiitake growers use spawn to introduce the shiitake fungus into the logs on which the crop will be produced. Spawn consists of a growth medium which has been permeated by mycelium. The process of introducing spawn into the substrate is called inoculation or spawning. This is analogous to transplanting vegetable starts into soil in order to grow vegetables.

Most commercially available spawn for shiitake production consists of a pure strain grown on sterilized wooden plugs or sawdust. These strains have been selected for desirable traits and are then propagated by mycelial transfers.

It is very important for shiitake growers to use high-quality spawn. Many failures can be traced to poor-quality spawn or weak strains which were unable to compete in the log. Unfortunately, by the time the grower notices these problems, a considerable investment of time and materials has been wasted. Spawn should be purchased only from reputable spawn producers who are knowledgeable and can demonstrate the quality of their product.

Spawn Production

Commercial mushroom growers need large volumes of spawn. Spawn is produced by taking a pure strain of mycelium from a culture tube and multiplying it (Fig. 5–2). It is an exponential process: each successive stage increases the mycelium by a factor of ten or more. To assure high quality spawn, sterile conditions are required throughout the process. Stringent quality control is essential or undetected contaminating organisms will be multiplied along with the shiitake.

Fungus cultures are usually stored on a semi-synthetic agar medium which contains sugars, a nitrogen source and trace nutrients. As the rapidly dividing cells consume the available nutrients, cultures are maintained by repeated transfers to new media.

Sometimes, during this rapid cell division, a genetic mutation that is particularly well-adapted to the agar medium is produced. A mutation that helps the mycelium grow faster on sugar can eventually dominate the culture. Although this altered mycelium grows well on high-sugar media, it may have lost the ability to grow or fruit well on wood. Modern spawn laboratories prevent this problem by storing cultures in "suspended animation," frozen in

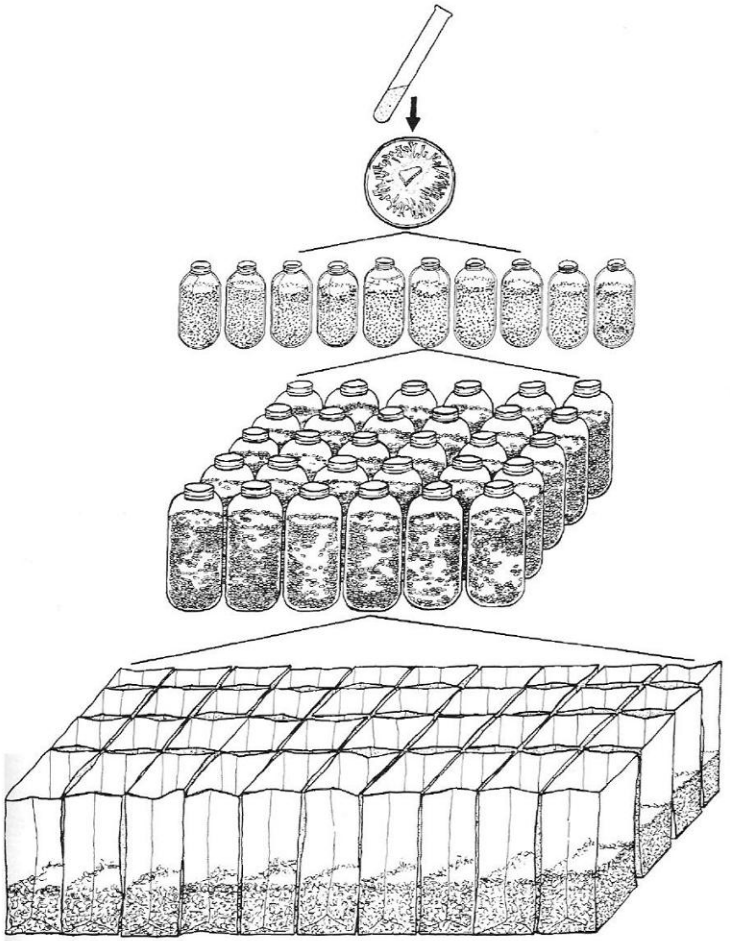


Figure 5–2. Spawn production.

liquid nitrogen. These cultures can be stored for many years with no genetic changes (*35*).

Spawn production begins when small bits of agar with growing hyphae are transferred from a test tube onto agar medium in a thin flat dish (petri

plate) (Fig. 5–2). The mycelium grows over the surface of the medium, usually within two weeks.

The medium in the petri plate is then divided and transferred under sterile conditions to a number of jars or bottles containing sterilized grain. Grain is used because it is high in nutrients. The inoculated grain is incubated for 2 to 3 weeks. During this time it is shaken several times to separate the grain. This evenly distributes the growing hyphae throughout the substrate and encourages rapid growth. The tough seed coat of each grain protects the mycelium within.

These jars, known as "grain masters," are inspected closely to assure purity. Grain masters may be used either to create more grain spawn or to inoculate sterilized sawdust or wooden plugs for inoculating the logs.

Sawdust and plug spawn are lower in nutrients than the agar and grain media used earlier. This is because high-nutrient spawn is more susceptible to colonization by competing or disease fungi during inoculation.

Types of Shiitake Spawn

Commercial shiitake spawn for logs comes in two forms: plugs and sawdust. Each has its advantages and disadvantages, and both are extensively used. Other types of spawn for logs are still experimental or are not widely available. The choice of spawn depends on several factors: cost, availability, climate, number of logs and the inoculation system.

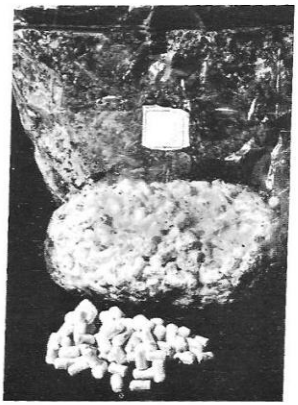


Figure 5–3. Shiitake plug spawn (USA). Note microporous filter on bag.

Plug Spawn

Plug spawn consists of wooden dowels thoroughly permeated by shiitake (Fig. 5–3). Generally, they are 8.5 mm in diameter (5/16 in) and about 16 mm long (5/8 in). Often, one end is tapered or chamfered to facilitate insertion into the log.

Plugs have several benefits. They are easier to use than sawdust spawn and do not require specialized tools. Also, plugs resist drying better because they have less exposed surface (*180*). Under moist conditions, sealing plug inoculation holes to prevent water loss is not needed. This considerably decreases the time and labor needed for inoculation.

Sawdust Spawn

Sawdust spawn is a mixture of sawdust and bran (4:1), which has been colonized by shiitake mycelium (Fig. 5–4). It has higher nutrient and moisture levels than plug spawn and is usually inoculated into larger holes in the logs. Therefore, the mycelium begins colonizing the wood more rapidly.

A disadvantage is that sawdust spawn has a higher surface-to-volume ratio because of its particulate nature. This makes it more susceptible to drying out during the spawn run (*160, 180*). Therefore, sawdust-filled inoculation holes must be sealed.

Other Types of Spawn

Other methods of inoculating shiitake logs have been developed and tested. Liquid spawn, consisting of mycelium suspended in a liquid slurry, has been injected into logs, but with poor results (*176*).

Different methods of inoculating saw kerfs (cuts) in logs have been tried. These methods generally speed log colonization because they increase contact between the spawn and the wood end grain. *"Comb spawn," available* in Japan, consists of thin slices of colonized wood which are inserted into saw cuts in the log (*41*). A similar approach using fiberboard instead of wood wafers has been successfully tested in Europe and the United States (*108, 160*). Chainsaw kerfs filled with sawdust spawn (*29*) and spawn disks produced from colonized grain (*175*) have also been successfully used in small trials.

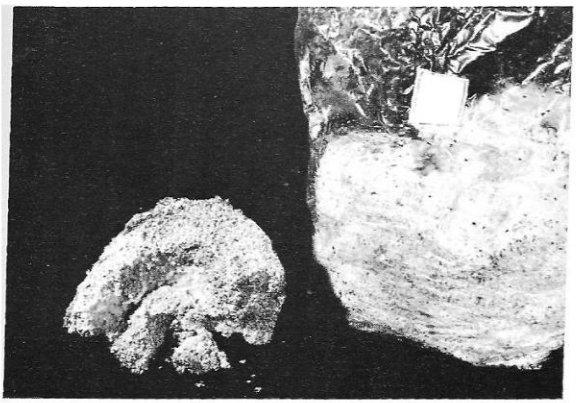


Figure 5–4. Shiitake sawdust spawn (USA).

Judging Spawn Quality

Shiitake spawn is commonly packaged in plastic bags or bottles. Healthy spawn is bound together with white mycelium and may have brown, leathery or crusty patches on the surface. The brown color is shiitake's natural response to aging and light exposure. Some strains also produce lumps of tissue on the spawn surface as they mature.

Colors other than white and brown indicate the presence of other fungi. Common molds produce patches of colored spores: green, dusty olive-green, grey or buff. Any spawn which is contaminated upon arrival should be returned to the supplier. If the spawn is stored, tiny punctures may occur in the container, and small patches of mold may grow near these openings. Contaminated spawn should not used.

Healthy shiitake spawn has a characteristic smell, similar to the fresh mushroom. Often, mold contamination will cause a musty smell. Bacterial contamination is hard to see, but often gives the spawn a sweet or sour smell similar to apple vinegar. Prolonged spawn storage under anaerobic conditions may also cause a sour odor.

If there is any doubt about the spawn quality, it can be tested as follows. Pour boiling water over a paper towel and place the wet paper inside a small, unused polyethylene bag. Then, use rubbing alcohol or a 10% bleach solution to sterilize a spoon, your hands, and the outside of the spawn container. Carefully open the spawn container only enough to remove some spawn with the spoon; then reseal it promptly. Place the spawn inside the plastic bag with the wet paper and close the bag with a tie, leaving some air space. Then, through the bag, crumble sawdust spawn or rub the surface of plug spawn. This disturbance will make the white mycelium "disappear," just as it does when placed in the log. Shake the spawn into a corner of the bag and place it in a dark, warm spot (68°–80°F, 20°–27°C) with a little air movement (above the water heater or refrigerator).

After several days, the mycelium should begin to recover and the spawn surface should appear white and fuzzy. If the spawn does not recover within two weeks (longer at lower temperatures), it should not be used.

Storing Spawn

The mycelium in the spawn is actively digesting the medium and respiring carbon dioxide. Spawn that has been stored too long loses its vigor as lack of nutrients limit further growth. Oxygen will decrease and carbon dioxide and other volatiles will build up during storage if there is inadequate air exchange in the spawn container. In totally sealed containers, this can weaken or kill the mycelium.

The temperature affects how long spawn can safely be stored. Spawn will last for a month or two at cool room temperatures. At temperatures from 36° to 42°F (2°–6°C), spawn can be stored up to eight months. Sawdust spawn will not keep as long as plug spawn because the readily available nutrients in the sawdust are depleted sooner.

Preparing Spawn for Inoculation

Spawn that has been stored at cold temperatures is inactive. Prior to inoculation, it should be warmed for several days at room temperature to initiate mycelial growth and encourage rapid recovery after inoculation. Spawn should be closely examined for contamination at this time.

6

Inoculation of Logs

Proper inoculation establishes the competitive edge necessary for successful cultivation. The shiitake mycelium grows from the inoculation sites out into the wood. It is important to introduce the shiitake when the wood is fresh to give the shiitake a head start over other competing fungi.

This chapter considers materials and techniques for successful inoculation of logs: attaining desirable log conditions; selecting a drilling pattern; drilling tools and techniques; inoculating techniques; sealing methods and materials; and designing inoculation areas and log handling systems.

Preparing the Logs

Wood Conditions

Research has given conflicting recommendations on how much time should elapse between cutting and inoculating logs. Some research indicates that shiitake grows rapidly only after the tree tissues die (*68, 97*), and advises waiting four to eight weeks before inoculation. However, other research recommends inoculating logs soon after cutting (*2, 160, 180*). The uses of different tree species for these studies may account for their different results.

If a log has the proper moisture content, the fungus can recover quickly from the stresses of inoculation and begin growing out into the log. The ideal log moisture content at inoculation is from 35% to 55% (*180*). Usually, this suggests inoculating as soon as possible after felling the trees (*2, 180*).

Logs that are too dry can be soaked or sprinkled to raise the moisture content. However, little water moves through bark; mostly it enters logs through the exposed end grain (*2*). Because uncolonized logs do not absorb water readily, it is important to prevent moisture loss between felling and inoculation (*2, 160*).

Surface Conditions

The bark surface should be intact, clean and dry. Protruding branches are a hindrance and should be trimmed off. Young branches which may sprout during incubation should also be removed as they withdraw nutrients from the wood.

Organic matter, such as moss or lichens, can provide a moist area for competing fungi to flourish during the spawn run. Therefore, such organic

matter should be removed from the bark before inoculation. In geographic areas where heavy moss covers the logs, mechanical brushes or pressure washers can remove it. Logs should be kept clean because mud and dirt serve as a source of inoculum for competing fungi.

The bark should dry before inoculation. Wet bark encourages molds which may inadvertently get into the spawning holes. Moreover, sealing wax does not adhere well to wet bark. Covering the tops of log stacks and letting air circulate around the logs will usually dry the bark.

Drilling the Logs

Colonization Patterns

Figure 6–1 shows the colonization pattern of mycelial growth on the end of a log during the spawn run. The dark brown V-shaped areas outlined with white mycelium are colonized by shiitake. Mycelial browning is shiitake's natural protective reaction to light and air. This growth pattern reflects the spacing, width and depth of the inoculation holes and demonstrates the limited growth across the grain.

Shiitake grows much faster along the grain than across it due to the orientation of cells in the wood (Fig. 2–7). In fact, after nine months incubation, growth with the grain is six to ten times greater (Fig. 6–2) (*180*). The grower should select an inoculation pattern that uses this differential growth to achieve rapid, even colonization.

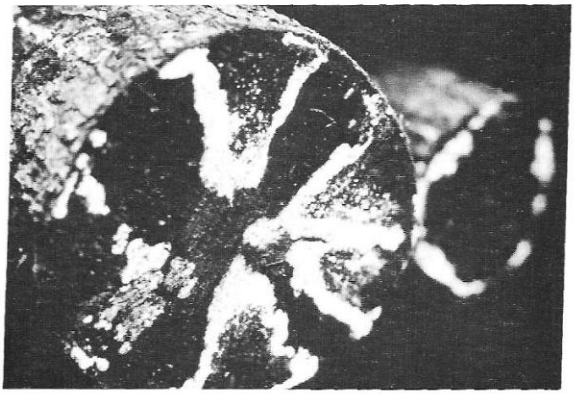


Figure 6–1. Log end showing shiitake colonization pattern (USA).

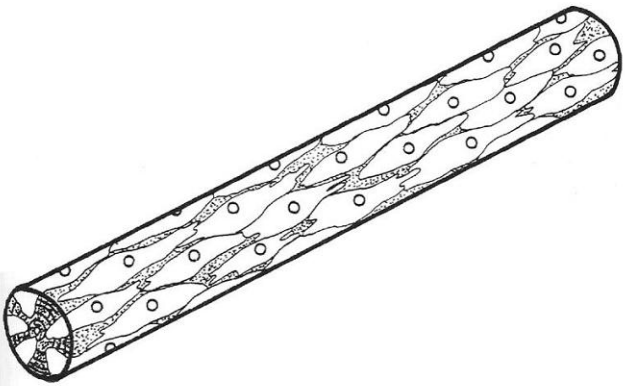


Figure 6–2. Growth pattern of shiitake in a log.

Spacing the Holes

Inoculation holes are spaced at intervals over the log surface to help the growing mycelium to permeate the sapwood and rapidly colonize the log.

The time from inoculation to fruiting is influenced by the inoculation pattern, tree species, log moisture content, strain and temperature. Higher spawn rates speed colonization and produce mushrooms sooner, but also increase spawn costs. There is no standard number of holes per log, and many different inoculation patterns are used.

Spawn rates can be increased in several ways. The number of rows per log can be increased, by evenly spacing the holes over the log surface. A modified diamond pattern places several holes next to each other, across the grain (Fig. 6–3). This is faster to drill and inoculate than an evenly spaced pattern and gives comparable results.

A general rule of thumb for estimating the number of holes per log is: no less than one row of holes for every inch of log diameter, with holes spaced every 6 inches along the row. Additional holes near log ends, branch stubs and wounds quickly establish shiitake near the most likely sites of invasion by other fungi (Fig. 6–4).

Although a ring pattern can be used with log species that are rapidly colonized by shiitake, the diamond pattern is the most common pattern (Fig. 6–3, 6–4). Rows are spaced every 2 to 3 inches (5–8 cm) around the circumference, and holes are drilled every 6 to 10 inches (15–25 cm) along the row. Holes in adjacent rows are offset to form a diamond pattern.

The drilling pattern must be applied to the logs in a rapid, uniform manner. Figure 6–5 shows an easy way to space holes evenly in a diamond pattern. A pattern stick can be made from a piece of lath, the same length as the logs, as follows: Mark the desired spacing along the stick using two different colors. Starting several inches from the end of the stick, use the first

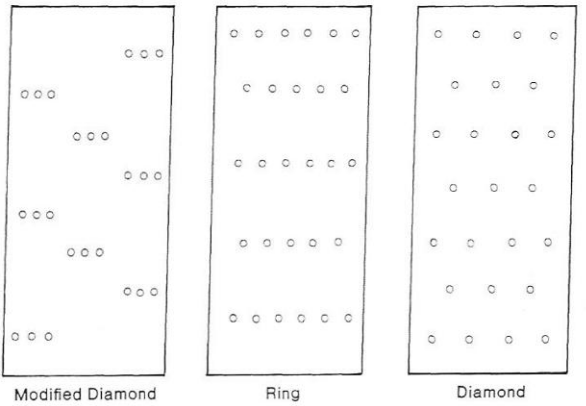


Figure 6–3. Log inoculation patterns.

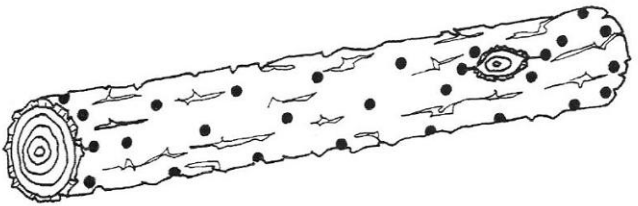


Figure 6–4. Diamond pattern for inoculation; note extra holes around branch stub and near ends.

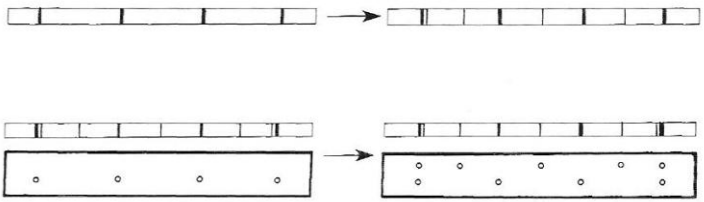


Figure 6–5. Making and using a pattern stick.

color to draw lines every 6 to 10 inches (15–25 cm). Next, use the second color to draw another set of lines halfway between the first ones, and add an extra line several inches in from each end.

Use this pattern stick as a drilling guide. Drill the first row of holes to match the first color. Then, after rotating the log, use the second color as a guide for the next row. This results in even, staggered spacing.

Depth of Holes

The ideal depth of the spawn hole is a function of the amount of sapwood, the log diameter and the type of spawn. Deep inoculation results in faster total colonization and may increase early production. However, it may not reduce the time until fruiting; this is influenced more by hole spacing. Holes should not be drilled into the heartwood because it is not initially utilized by shiitake.

Holes for plug spawn are usually drilled 1.5 to 2 times the length of the plugs. If the plugs are pounded in flush with the surface, this creates an empty space under the plug. This space is rapidly colonized by mycelium. Results are comparable to using a longer plug.

Holes for sawdust spawn are drilled to match the capacity of the spawn transfer tool. These tools are made in several depths for various log conditions (Fig. 6–6).

Tools Required

A drill and the proper bit are needed to make inoculation holes. There are several options, depending on the type of spawn and availability of sup-

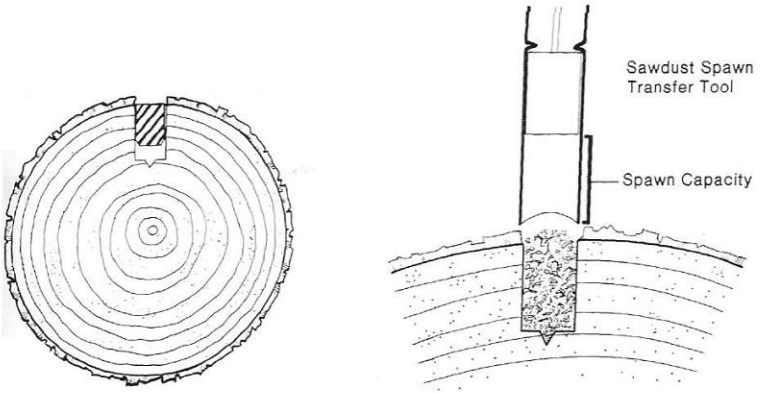


Figure 6–6. Proper spawn hole depth.

plies. Specialized shiitake bits are available, both with and without depth stops. These bits are designed for 10,000 rpm and have a lead screw and a single cutting edge to reduce binding in green wood. The lead screw pulls the bit into the wood, considerably reducing labor (Fig. 6–7).

The choice of bit diameter depends on the type of spawn used. Plug spawn usually needs a 5/16-inch (8.5 mm) hole, but sawdust spawn needs larger holes, usually 12 mm, to match the sawdust spawn transfer tools.

Other commonly available drill bits such as spade bits, twist bits and auger bits can be used. However, if many logs must be drilled, specialized bits save time and effort.

Special shiitake drill bits work best with a high speed drill (6,000–10,000 rpm) and greatly accelerate the drilling process. The ideal drill is high speed, lightweight, quiet and has a built-in adjustable stop to limit the depth of the hole.

A regular electric hand drill will work for small numbers of logs, but low speeds (550–1,800 rpm) are impractical for large quantities of logs. Several types of high-speed drills (6,000–10,000 rpm) are made especially for drilling shiitake logs. Lightweight, hand-held models are used in Japan but are not widely available outside of Asia (Fig. 6–8).

Several common shop tools can be adapted for drilling shiitake logs. Angle grinders with speeds from 6,000 to 12,500 rpm work well if the arbor is replaced with a chuck and a depth stop is added. Routers are also used; however, their excessively high speeds (22,000 to 25,000 rpm) may not only damage the drill bits but also endanger the operator. Devising an overhead suspension system for hand-held drills helps to speed drilling and avoid strain on the operator.

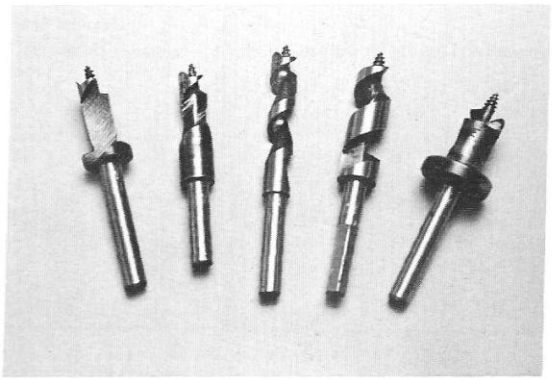


Figure 6–7. Drill bits for shiitake inoculation (USA).

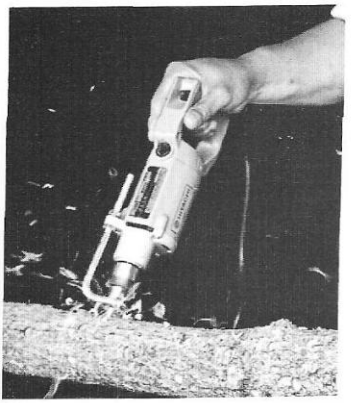


Figure 6–8. High-speed shiitake drill (USA).

Flexible-shaft drilling machines are widely used on large farms because of their durability. They use a motor to drive a drill chuck by means of a flexible shaft (Fig. 6–9). These machines can be built to desired rpms and are quieter than drills.

Machines that simultaneously drill multiple holes greatly speed drilling (Fig. 6–10). These machines are expensive and are efficient only with straight logs.

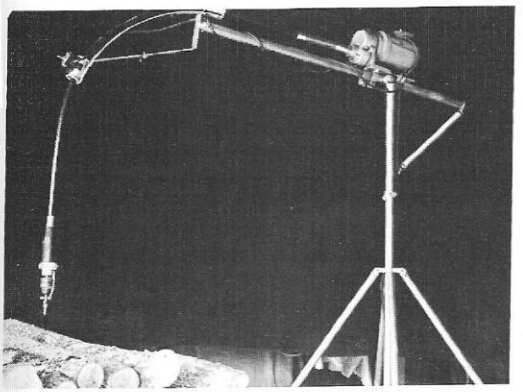


Figure 6–9. Flexible-shaft drilling machine (USA).

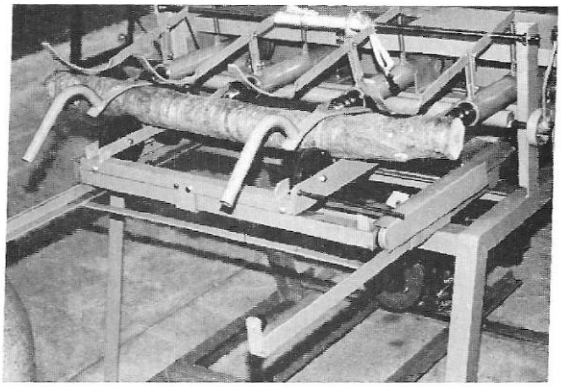


Figure 6–10. Mechanical gang drill (Japan).

Inoculating the Logs

Inoculation is the act of putting spawn into the log. Inoculating promptly after drilling the holes avoids two problems. First, if holes are left exposed, spores of other fungi can get into them. Secondly, if holes dry out, it takes longer for the spawn to recover and grow into the wood.

Plug Spawn

Plug spawn is placed, tapered end down, into the holes and pounded down flush with a hammer. If the plugs fit well, one or two blows will place the top of the plug just below the surface of the bark. Two people can do this job efficiently: the first person inserts the plugs, and the second pounds them in and rotates the log. To avoid contamination, the person inserting the plugs should have clean hands and avoid handling the logs.

Sawdust Spawn

Sawdust spawn can be transferred by hand or with a specialized transfer tool. In either case, periodically check the amount of spawn in the hole by pressing with a finger or nail. Holes should be firmly packed, but not too full. Holes may appear full, but really have just a little spawn near the top.

During spawning, the spawn breaks up entirely and the white surface mycelium "disappears." Once in the log, however, new mycelial growth begins and the spawn will become white again.

Tools for transferring sawdust spawn include spawn plungers and mechanical spawn guns. Spawn plungers are simple devices consisting of a tube and piston. They are widely available and work well (Fig. 6–11). To fill

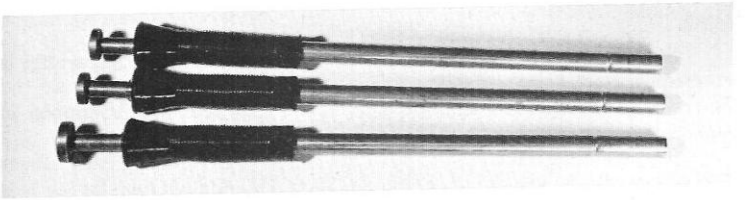


Figure 6–11. Sawdust spawn plungers (USA).

the plunger, press it several times into the spawn. Then place it on top of an empty hole and push down the top of the piston to expel the spawn and pack it into the log (Fig. 6–12).

Mechanical spawn guns have a canister filled with spawn. Pulling back on the handle loads a chamber with spawn, and pushing the handle inserts the spawn into the holes.

Completely automated systems that combine drilling, spawning and sealing are available in Japan.

Sealing the Holes

Sawdust spawn must be sealed, and many growers also seal plug spawn, especially in drier areas. Many sealing materials have been tried, but hot wax and foam plugs remain the most effective and commonly used materials. Each has advantages and disadvantages; the choice depends on the grower.

Figure 6–12. Log inoculation with sawdust spawn plunger (USA).

Wax

Several kinds of wax can be used to seal inoculation sites. Paraffin is commonly used; some growers add up to 20% mineral oil to improve the flexibility of the seal. Cheese wax, a softer, more pliable wax, is popular in some areas with cold winters.

The temperature of melted paraffin during application greatly affects the quality of the seal. Melted paraffin gives an effective long-lasting seal when applied at about 260°F (127°C). At this temperature, the wax forms a thin, tough, translucent coating over the spawn. Although the hot wax kills the spawn on the surface of the inoculation hole, most of the spawn is unaffected because the wax cools so rapidly. Paraffin applied at lower temperatures is white and opaque after hardening and is easily knocked off when the logs are handled.

When hot wax is applied to the spawn hole, log sap and water from the spawn vaporize and boil out. The wax cools and hardens to produce a good seal. However, if the wax is not hot enough during application, the seal hardens as the water is boiling, leaving small pin holes in the seal. These pin holes create avenues for water loss and for the entry of contaminants. Conversely, if wax is too hot during application, it takes longer to cool, and wax may run off when the log is turned to seal the next row of holes. Small electric deep-fat fryers work well for heating wax as they are thermostatically controlled within the desired temperature range.

Wax Applicators

The molten wax must be applied neatly and rapidly to the filled holes. A variety of applicators are used, from paint brushes to glass wax droppers. The ideal applicator holds enough wax to cover at least one row of spawn holes and keeps the wax hot. A simple device widely used in Asia is the "drumstick" applicator, which consists of a wad of foam or cloth on the end of a stick. The "drumstick" is plunged into hot wax and then padded onto each hole. Disadvantages are that the "drumstick" does not hold much wax and it cools rapidly.

Glass bulb-syringe wax applicators hold enough wax for a row of holes and keep it hot. The suction bulb pulls wax into a glass tube, then squirts it onto the spawn hole through a wire mesh screen (Fig. 6–13).

An effective, inexpensive wax applicator can be adapted from a common turkey baster. Most turkey basters are made of polypropylene, which can withstand high temperatures. However, to work well, the flow of wax must be reduced.

A valve can be constructed in the tapered end of the baster: Cut a 16 penny nail in half, remove the rubber bulb on the end of the baster and drop the nail (head up) into the baster. The cut-off end should protrude through the hole while the head of the nail remains inside the baster, creating a valve (Fig. 6–14). Pushing the nail up lets the wax flow in or out. When the spawn hole is lightly touched with the nail and the bulb is squeezed gently, a small

Figure 6–13. Glass bulb-syringe wax applicator (USA).

amount of wax flows out and neatly seals the hole. When the baster is lifted from the hole, the pressure of the hot wax closes the valve and stops the flow.

Foam Plugs

Foam plugs are small discs of plastic foam, sized to fit the inoculation hole. Foam plugs do not seal holes as well as properly applied hot wax. Therefore, it is important to maintain proper log moisture content immediately after inoculation. Unlike wax, foam plugs can easily be removed temporarily to inspect the growth of sawdust spawn in the hole.

Foam plugs are pressed flush into the filled inoculation holes, compressing the spawn. Usually, this is done by hand, but mechanized inoculation systems also work well. Some automatic inoculation systems insert spawn and foam plugs in one step.

Foam plugs are available commercially, or they can easily be made. One way is to punch them from flat sheets of foam with a circular punch like those

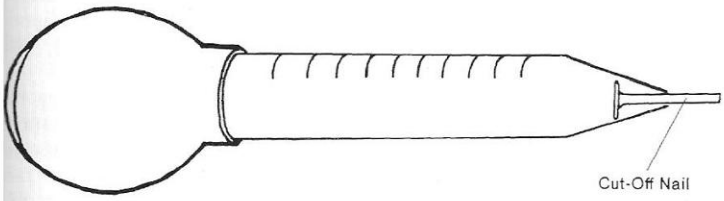


Figure 6–14. Turkey-baster wax applicator.

used for leather. Another way is to use "backing cord" or "backer rod," which is designed for filling cracks in concrete slabs. This rope-like foam can be sliced into thin (1/4", 5 mm) discs with a sharp razor. Larger quantities of plugs can be cut "en masse" from bundles taped together, using a bread or meat slicer.

Other Sealing Materials

A number of other materials can be used to seal inoculation holes. Small discs of cork or bark are used. Special punches are available to punch discs from fresh bark. Various grafting seals and tree paints have been used with varying results. Some of these compounds contain fungitoxic ingredients which may weaken or kill the spawn by contact or vapors.

Regardless of the material and method used for sealing, it is important to achieve a tough, effective seal. Logs should be handled carefully to avoid dislodging the hole coverings.

Inoculation Area

The design of the inoculation area should provide for a favorable inoculation environment. It should also plan for efficient materials handling, including the ease of transporting logs into the area, the efficient flow of logs during the stages of inoculation, and proximity to the spawn run area.

The inoculation area should be protected from the sun, wind and rain. Heat from direct sunlight, as well as drying caused by both sunlight and wind, can reduce the vigor of the spawn. Also, because wind carries many spores, inoculation on dry, windy days can increase contamination (*180*). Rain is another source of contamination. In addition to encouraging molds by wetting the bark, rain can carry spores into the spawn holes. Therefore, spawning should take place under trees, in buildings or under some other protective structure.

Materials Handling

Efficient materials handling will significantly decrease inoculation costs. The distance that logs must be moved and the number of times they are handled should be minimized. Logs can be moved in many ways: by hand, with motorized and non-motorized carts, tractors, forklifts, etc.

Log inoculation involves several stages: cleaning the log, drilling the holes, inserting the spawn, and sealing the inoculation holes. Work stations should be at a comfortable working height. The flow of logs through the inoculation process can be expedited considerably if logs can be rolled on rails from one station to the next (Fig. 6–15). At each station, notches are cut into the rails to hold the log. Sufficient space should be allowed between work stations to hold several logs awaiting the next step. With such a system, the logs

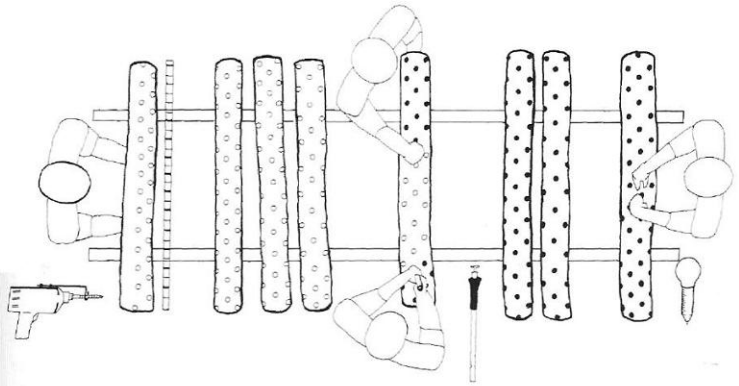


Figure 6–15. Four-station inoculation system (top view).

are lifted only twice: onto the rails for drilling and off after sealing, thus avoiding the bark damage caused by repeated lifting and stacking.

Although rates vary with log size, inoculation method and type of spawn, a four-person crew using such a system can drill, inoculate and wax from 40 to 80 logs per hour. Thus, 300 to 600 logs can be inoculated during an average eight-hour work day.

7

Incubation of Logs

During incubation or spawn run, shiitake mycelium permeates and decays wood. The goal of incubation is to achieve rapid colonization of the wood by shiitake and minimize contamination by other fungi. Most failures in shiitake cultivation can be traced to poor management of conditions during the spawn run (*146, 180*). Thus, it is very important to understand both the optimum conditions favoring mycelial growth and the management techniques used to maintain them.

This chapter discusses factors that influence the location of the spawn run area and considerations in managing both natural and artificial spawn runs. It suggests designs, equipment and practices to control temperature, humidity and ventilation to provide a favorable environment for the logs. It then details management considerations for the critical spawn recovery period and the spawn run areas. Finally, it considers ways of monitoring shiitake growth and recognizing that the fungus is ready to fruit.

Management Principles

A key principle of mushroom cultivation is that conditions should be created that favor the desired fungus, while discouraging its competitors. At no time is this more critical than during the spawn run; it is then that shiitake must establish the necessary "competitive edge." Once shiitake has colonized the logs, the spawn run is complete and the fruiting cycle can begin.

Warm temperatures and free water in the wood are required to promote rapid growth of shiitake mycelium through the log. The optimum temperature for shiitake incubation is from 72° to 77°F (22°–25°C); lower temperatures result in a longer spawn run. Shiitake grows best in wood which is well above the fiber saturation point (23%). Log moisture contents between 35% and 55% are ideal.

During the spawn run, logs must be either in a natural environment that favors mycelial growth, or in a controlled environment where favorable conditions can be created. The physical layout of the spawn run area, the equipment used, and the care given to the logs are all designed to provide this ideal environment.

The environmental factors of temperature, humidity, and air flow are interrelated. Temperature, log moisture content, log stacking method and air flow are affected by the location and design of the spawn run area. In addition, log moisture content and temperature can be regulated through irrigation and ventilation.

The degree of active management needed depends on climate, design of the spawn run area, tree species used, stacking method, and number of logs. Generally, mushroom quality and yield per log increase with additional management. All the aforementioned factors, combined with efficient log handling, contribute to the economic viability of a shiitake farm.

Location and Design of Spawn Run Areas

Location

The location of the spawn run area depends on the climate, availability of suitable sites, and number of logs to be incubated. Although the spawn run area does not have to be close to the fruiting area, proximity is desirable because it simplifies log handling.

Aspect, elevation, air drainage, access, surrounding landforms and vegetation also influence the suitability of a particular site. These factors, combined with the climate, determine the microenvironment at the site. An ideal site should be warm and shaded; it should have easy access, good air movement and a source of water if rains are not dependable.

The choice of the spawn run site determines how much active management, such as irrigation and the use of windscreens or log coverings, will be required (*47*). A range of sites can be used, depending on the climate. For example, in warm moist climates, logs can be incubated in the forest with very little attention or under a shade structure with little watering. In a drier climate, it may be necessary to decrease water loss by increasing watering and by building structures to shade the logs. In colder climates, it may be necessary to use greenhouses or incubation methods that increase log temperature.

Natural Spawn Run Areas

In warm, moist climates, a full or partial forest canopy can provide a good, inexpensive area for incubation (Fig. 7–1). The buffered forest environment avoids the fluctuations in temperature and humidity found in exposed sites. The surrounding vegetation raises the humidity and provides cooler temperatures. Temperature is also influenced by the aspect and elevation; northern aspects and higher elevations are cooler.

The type of overstory tree affects both the temperature and the disease potential of the area. Evergreen forests provide shade year-round, whereas deciduous trees admit sunlight to warm the logs during the winter. The build-up of leaves from deciduous trees may promote undesirable mold growth on the bark. Another factor to consider is whether the forest is harboring shiitake diseases and pests. Many of the fungi found in coniferous forests are not problems during shiitake cultivation; however, most of shiitake's major competitors are present in hardwood forests.

Figure 7 – 1A. Natural spawn run areas. Log incubation in deciduous forest (USA).

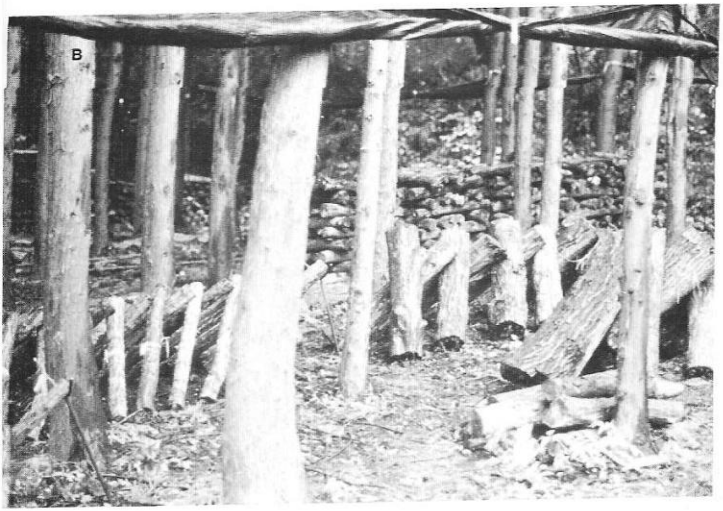


Figure 7 – 1B. Log incubation in evergreen forest (Japan). Note adjustable wind screen.

A site should have good air movement. Sites in depressions where air movement is restricted and cool air collects should be avoided. Air movement can be increased by pruning and brush removal. Conversely, sites with excessive air movement should also be avoided, except in very wet climates. Water loss can be decreased by using brush, shade cloth or similar fabrics either as windbreaks or as a covering for the logs. If rainfall does not provide enough water to maintain the log moisture content at suitable levels, a water source is necessary. Good access is critical; however, level ground is not required for many of the log stacking patterns.

Generally, logs incubated outdoors require a longer spawn run than logs in artificial spawn run areas because forest temperatures are cooler. However, the longer spawn run may be offset by lower costs.

Logs incubated in a natural spawn run area can be transported elsewhere for fruiting. For example, in Japan most growers incubate their logs in the forest where climate is favorable for growth. Then, after the spawn run is complete, the logs are trucked to a fruiting site closer to the market.

Artificial Spawn Run Areas

Artificial spawn run areas can provide good incubation conditions in regions otherwise unsuited to shiitake cultivation. Favorable conditions for shiitake growth are created by using shade houses, greenhouses or other structures adapted to local climatic conditions.

Artificial spawn run areas have both advantages and disadvantages. Because the temperature and humidity around the logs can be regulated, shiitake grows faster and the spawn run is shorter. This results in a more efficient use of space. In drier areas, the higher humidity in a greenhouse slows drying and reduces water use. On the negative side, artificial spawn run areas cost more and require more management. One problem is that uncontrolled heat buildup within the structure can cause the humidity to drop rapidly.

An artificial spawn run area should have good access. If tractors or similar machines will be used to move stacks of logs, the ground should be nearly level. In cool areas, the site should have full solar exposure to warm the logs, especially in the winter. A water source is needed and good drainage is desirable. The area must be able to provide 60% to 85% shade and a means to vary the air flow through the structure.

Structures. A number of different structures are used; two of the most common are shadehouses and greenhouses (Fig. 7–2). Shadehouses consist of lath or shade cloth stretched over a simple frame or suspended from cables strung between poles. These structures are inexpensive and allow very good access, but are cooler than greenhouses. The evaporation rate is relatively high because of air movement. Shadehouses do not shed rain; this can be a problem in areas with heavy rainfall. In regions with little rainfall, irrigation will be required.

Figure 7–2A. Artificial spawn run areas (USA). Log incubation in shade house.

Greenhouses covered with glass, fiberglass or plastic are also used to in cubate logs in cool areas. Because greenhouses are warmer than shadehouses the spawn run is shorter.

Figure 7–2B. Log incubation in greenhouse.

Greenhouses exclude some environmental influences, such as rainfall, and increase others, such as solar heating. Therefore, the grower must design the structure and plan management strategies that provide for the temperature, humidity, and air flow needs of the incubating logs.

Greenhouses for shiitake cultivation must allow complete control of air movement. For this reason, the roof covering ends at least two feet (60 cm) from the ground to create side vents, and the vent covering can be opened or closed as needed. Large access doors at the ends of the house are covered during the winter and left open or partially covered during the summer.

Because clear greenhouse coverings offer no protection from the sun, shade cloth or whitewash is needed in the summer to lower temperatures and prevent excessive drying. Shade cloth comes in several densities; 60% to 85% shade is generally needed for adequate protection. It can be removed in the winter when solar heating is desired.

Other buildings have been used for the spawn run with good results. Steel buildings often have good air circulation and some solar heating. Buildings that remain cool can slow the growth of shiitake. Wooden buildings are generally unsuitable because irrigation and high humidity may cause them to deteriorate.

Irrigation Systems

More often than not, some type of irrigation system is required, especially in artificial spawn run areas. The type of irrigation system selected depends on the design of the spawn run area, the volume of water available at a constant demand and the number of logs. The ideal system can cover the logs evenly and apply enough water to saturate the bark and keep water dripping off the log. The misting systems commonly used for plants are not suitable because the water volume is too low.

A wide variety of sprinkler heads are used. Sprinkler heads with adjustable, even coverage are best. A common type has two arms that spin around a central pivot. Most sprinkler heads with no moving parts (spray-type) do not provide even water distribution.

Irrigation systems can be fixed or moveable. Fixed systems are best suited to greenhouses and other structures with an overhead frame. Overhead mounting facilitates access. Moveable systems, such as soaker hoses, work well under shade structures and in natural spawn run areas.

Automatic irrigation control allows greater flexibility in water management. Some water timers can be programmed to irrigate on a 7- or 14-day cycle. Other systems have additional settings which allow short waterings during the day to cool the logs or to water the top courses of logs.

Stacking Methods

The method of stacking the logs during incubation is an important management decision because it greatly influences the microenvironment of

the log; this, in turn, determines the growth rate of the mycelium. Stacking methods differ depending on the climate, space available, method of moving logs and design of the spawn run area. Many different stacking methods are used; common ones include crib, lean-to, A-frame and bulk stacking. The choice of an appropriate stacking method reduces the management required to maintain optimum conditions.

Crib Stacks

Crib stacks, also known as "criss-cross," "square" or "cabin- style" stacks, are built of horizontal layers of logs. The logs in each layer run at right angles to the logs in the layer below. Depending on the size of the logs, a layer may contain from four to eight logs. Stacks are from 2 to 4 feet (60–120 cm) high (Fig. 7–3).

Crib stacks are space-efficient and allow good air movement around the logs. Because entire stacks can be moved with a fork lift, this method is widely used in forced-fruiting operations which require efficient log handling (Fig. 7–4). Crib stacking allows logs to be stacked off the ground. This is an advantage where drainage is poor or termites are a problem. Crib-stacked logs effectively shade out vegetation which keeps the air space below the stack open.

The disadvantages of crib stacks are several: near-level ground is required, there is little solar warming of the logs, and differences between conditions at the top and bottom of the stacks are pronounced. Although the logs at the top often dry while the bottom logs remain damp, mycelial growth within the same log is relatively uniform. These disadvantages can be minimized through careful watering and stacking.

Air movement through a crib stack can be controlled by the spacing of the logs. After the logs have been watered, evaporation cools the air, which then sinks. The stacks must be designed to allow this cool, moist air to drain, or the bark will remain wet too long. For this reason, logs in the lower part of

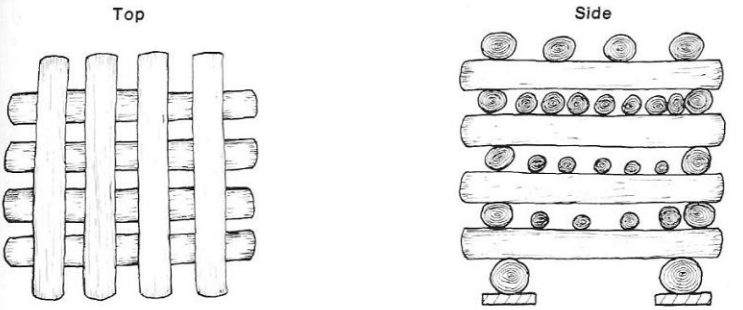


Figure 7–3. Crib stack for incubating shiitake logs.

Figure 7–4. Moving shiitake logs in crib stacks with forklift (USA).

the stack should be more widely spaced than those nearer the top. An air space of eight to twelve inches (20–30 cm) at the bottom of the stacks is also necessary. This is often created by stacking the bottom two courses with only two logs each.

Differential drying between logs at the top and bottom of the stack can be controlled by placing smaller logs, which dry more rapidly, toward the bottom and inside of the stack, while placing larger logs near the top. Other ways to minimize differences between the top and bottom include watering, using a log covering and reversing the stack after several months.

Lean-to Stacks

Lean-to stacking, also known as "centipede stacking," is widely used, both in artificial and natural spawn run areas. This stacking method works well on sloping or uneven terrain. Lean-to stacks are built against a triangle stack, a pair of forked logs, or some other stable support. A row of slanting to near-vertical logs are leaned against a single horizontal log near their top ends. The horizontal log, which provides spacing between the slanting rows of logs, is held in place by the weight of the logs leaning against it (Fig. 7–5).

Because the logs are close to the ground, they stay moist and dry slowly. Contact with the ground lets the mycelium penetrate the soil, which then acts as a moisture reservoir.

Lean-to stacks require more space than crib stacks and must be handled several times. The elevated end of the log is in a different microclimate from the end in contact with the ground. Consequently, to achieve even growth, the logs should be flipped end-for-end midway through incubation. Surrounding vegetation can grow up through the stacks; this increases the

humidity around the logs, which is an advantage in dry areas, but a problem in damp ones.

Lean-to stacking is a very flexible method that offers the grower many options for passively controlling conditions around the logs (*47*). Elements that can be varied include the stacking angle, orientation of the stack relative to the sun and the spacing of the logs.

A low stacking angle decreases drying and increases the exposure to sun and rain. As the logs approach an upright position, air circulation increases and more rain drains off.

The solar orientation of the stack controls the amount of solar heat that the logs receive. In addition, if logs are lined up parallel to logs in adjacent rows, each row shades the next and reduces its exposure to sun and rain. If, on the other hand, log position is alternated from one row to the next, this staggered placement provides maximum exposure (Fig. 7–5).

The number of logs per row influences air movement and the drying rate. Placing more logs in each row by spacing them more closely will decrease drying, while spreading the logs out will increase air movement and drying.

For example, a grower in a dry, cool climate might create low lean-to stacks with the north end of the logs elevated. This would expose the logs to the maximum amount of solar heat. The logs in alternate rows would be staggered for maximum exposure to the sun and rain. Logs would be closely spaced in the row to decrease drying.

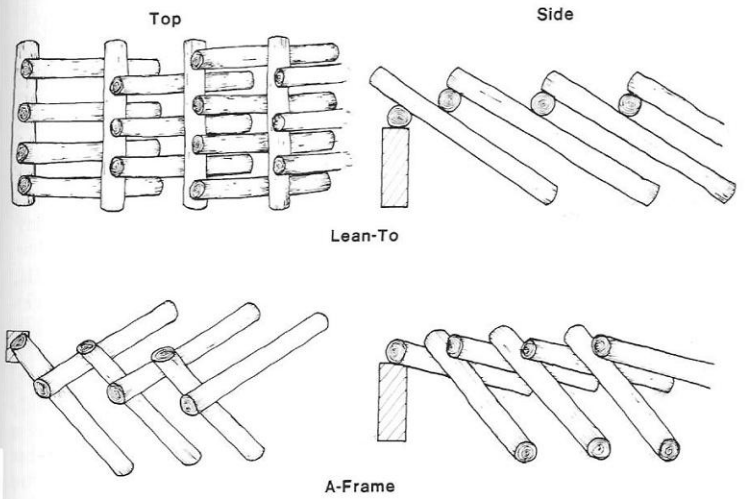


Figure 7–5. Lean-to and A-frame stacks for incubating shiitake logs. Note staggered arrangement of logs in lean-to stack.

Conversely, a grower in a warm, rainy climate would stack the logs nearly upright, with fewer logs per row, and place each row of logs parallel to the preceding one. This would limit the absorption of rain and increase air circulation for drying. In addition, the ends of the logs toward the sun could be elevated to decrease solar heating.

A-frame Stacks

A-frame stacks are similar to lean-to stacks. A-frames rest the lower end of the log on the ground and the upper end on a log. The first log leans on a support; subsequent logs are placed alternately at an angle to the preceding log (Fig. 7–5). A-frame stacks are managed like lean-to stacks. This method is well-suited for wet areas. In addition, logs in A-frame stacks can be fruited without restacking.

Bulk Stacks

Bulk stacks consist of logs piled parallel to each other, either horizontally (similar to firewood) or vertically. This method buffers changes in temperature and humidity by utilizing the thermal mass of the logs and the metabolic heat produced by the growing mycelium. Air movement is limited, decreasing water loss. Bulk stacks are the most space-efficient stacking system, having the most logs per unit area.

The disadvantages of bulk stacking are generally related to restricted air movement. This can lead to increased mold on the bark; this is hard to monitor because of the stacking density. Increased log contact also encourages diseases to spread rapidly. Another problem is that rodents like to make nests in the stacks, often removing significant amounts of bark in the process. Many of these disadvantages of bulk stacking are less serious when the logs are stacked vertically rather than horizontally.

Horizontal bulk stacking is often used during the spawn recovery period when the spawn is especially susceptible to drying (Fig. 4–5). A disadvantage of this method is that watering from above soaks the bark and may create mold problems due to limited air movement. Logs stacked in horizontal bulk stacks should be protected from rain and watered only on the ends, where water loss occurs most rapidly. Generally, logs are left in these stacks for a maximum of several months; longer periods can result in the mycelium binding the logs together. If this happens, the bark often rips off when the logs are separated.

A horizontal bulk stacking method that eliminates many of these disadvantages involves placing the logs in a container and surrounding them with a dry cellulosic material (*53*). Layers of logs are packed into boxes or similar containers and covered with dry sawdust, rice hulls or similar materials. The logs must be dry on the surface to prevent contamination. The boxes are then sealed, usually without vents. The packing material slows water loss and insu-

lates the logs, eliminating the need for watering. This method allows a year-round spawn run without extensive management.

The disadvantages of this box method of incubation are the labor and materials involved and the difficulty in assessing the progress of the logs. Another problem is that the mycelium tends to grow out of the spawn holes and log ends and into the packing material. This binds the material to the log, and the bark may become permeated with mycelium. Because these logs usually do not receive any light, fruiting is delayed after the spawn run until the light requirement has been met. Although careful monitoring can limit some of these problems, this method is most suitable for hobbyists and for testing strains on a small scale.

Vertical bulk stacks share some of the problems associated with horizontal bulk stacks, but eliminate others. Usually the logs are placed on top of twigs or other materials, such as coarse conifer bark chips, to allow for air movement. Some air also circulates vertically between the logs. Logs can be watered freely since water drains off the bark. The water loss is minimized, decreasing the need for watering. The logs are in contact with the ground, which can act as a water source for the mycelium, resulting in faster growth. Disadvantages include the difficulty of monitoring contamination and non-uniform growth in the logs.

Vertical stacks are created in two different ways: either around a pole or supported between stacks of logs or other restraining devices.

To create a circular stack, a pole is driven firmly into the ground, which has been covered with twigs and needles from coniferous trees. A small circle of logs is arranged and tied to the pole. Logs are then leaned against this core, creating a pile 4 ft to 6 ft (1.2–1.8 m) in diameter (Fig. 7–6).

Evergreen twigs and needles are placed on the top and sides of the stack to create a "breathing space" around the logs. This prevents wet spots on the bark where molds would grow.

After this step, the procedures for warm or cold areas differ. In warm areas, the pile is covered with plastic by wrapping a sheet around the lower

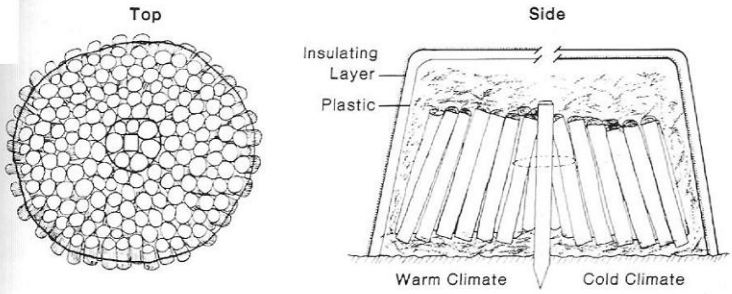


Figure 7–6. Vertical bulk stack for incubating shiitake logs.

portion first, then around the upper portion. The plastic is then covered with light-colored insulating materials (such as straw mats or humidity blanket) to decrease solar heating. In colder areas, the insulating layer is placed under the plastic to increase solar heating (*2, 180*).

The plastic covering should be opened every two to three weeks to check the ends of the logs; the logs can be watered lightly or ventilated as needed. Because humidity is high in these stacks, water loss is slight.

This method allows rapid spawn growth in the absence of a spawn run structure, although log piles can also be placed in greenhouses. This method works well in drier areas and for thin-barked tree species that lose water rapidly.

Logs can also be stacked vertically between supporting structures, creating one large vertical stack. This method is generally used in greenhouses where the spawn run will be rapid and there will be no rain on the logs. The application of water must be carefully controlled to successfully use any type of vertical stacking method.

Management Practices

Spawn Recovery Period

The recovery period is critical for successful shiitake cultivation. The shiitake mycelium has been disrupted and damaged during inoculation and, as yet, occupies a very small area within the log. Optimum conditions enable the hyphae to recover rapidly and resume their growth. Delays in recovery may let competing fungi become established.

During the spawn recovery period, shiitake mycelium is especially sensitive to drying. Stresses which would not affect established mycelium can greatly reduce the recovery of the spawn. The greatest danger is drying of the spawn.

Spawn moisture content is generally higher than that of the surrounding wood. This creates a moisture gradient which causes water to move from the spawn into the log. Sawdust spawn is particularly susceptible to drying during this period.

Moisture loss during the spawn recovery period can be controlled in several ways. An effective seal over the spawn will limit water loss in this critical area. Also, because bulk stacking retards water loss, this stacking method is often used for the first month or two. An alternative is to stack the logs in the pattern selected for the spawn run; in this case, they must be monitored carefully and watered more frequently during recovery.

Several signs show that the mycelium has recovered and is colonizing the log. White mycelium on the ends of plug spawn or under the wax seal on sawdust spawn indicate resumed growth. If foam plugs are briefly removed to inspect growth, the spawn should appear knitted together by white mycelium (which may turn brown with age and exposure).

Spawn Run

During the spawn run, the environment around the logs is managed by balancing water application and air movement. This balance regulates temperature, log moisture content (LMC) and incidence of disease. Under natural incubation, periodic rains may provide sufficient water. However, irrigation can supplement or replace rainfall to create more uniform conditions during incubation.

Irrigation water can come from a variety of sources. Surface water is satisfactory provided it is relatively free of suspended solids and organic matter. Chlorinated water from community water systems is also suitable. The concentration of chlorine is not high enough to damage the shiitake mycelium.

Water is added during the spawn run to replenish water lost from the logs, to raise the humidity and to cool the logs. Air movement can control evaporation rates and lower temperatures. Knowing when and how much to water and ventilate borders on art, as well as science.

Maintaining log moisture content. The management of log moisture content during the spawn run is where many growers fail. Replacing water lost from the logs through evaporation requires careful management. Log moisture must be kept high enough to maintain shiitake's "competitive edge," but the bark must be kept relatively dry. Establishing an irrigation schedule is a complex task, requiring considerable monitoring and experimentation.

A maximum-minimum thermometer and a hygrometer are useful tools for monitoring conditions during the spawn run. They can be moved about the area to estimate the drying rate in different spots. The reference logs described in Chapter 4 are also very useful for monitoring water loss. Until the conditions in the spawn run area are well understood and an irrigation schedule is established, reference logs, placed throughout the stacks, should be weighed once each week.

Other signs, such as cracking of the log ends and changes in the feel of the bark, tell the experienced grower when watering is needed. On the other hand, colonies of *Trichoderma* will appear on logs that remain too wet, usually near ground level.

Logs should be watered to maintain a moisture content between 35% and 55%. The time between waterings varies with the rate of water loss, but is usually between 7 and 30 days. Each watering may take from six to twelve hours, depending on the volume of water applied, the temperature and the log moisture content. After a day or two, the moisture content of the reference logs should be checked and the schedule adjusted, based on these results.

After the logs have been thoroughly watered, the bark surface must dry in order to prevent invasion by *Trichoderma* and other molds. *Trichoderma* spores usually require a film of water to germinate and colonize shiitake logs (*88, 91*). Ventilation is increased to speed drying. If the bark remains wet for

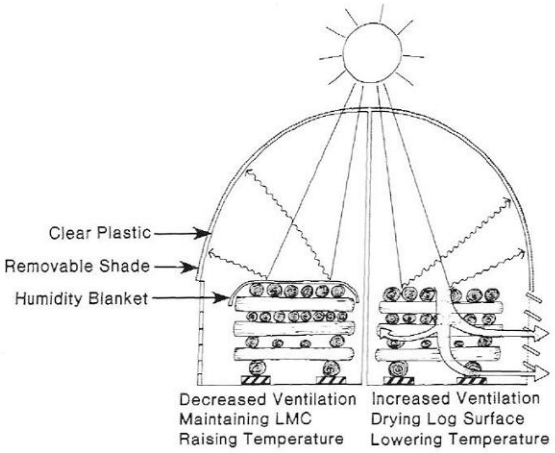


Figure 7–7. Managing spawn run conditions in a greenhouse.

more than a few days after watering, the air circulation is inadequate and should be improved. Once the bark has dried, ventilation can be reduced to lower the evaporation potential, thereby slowing water loss.

For example, after irrigating logs in a greenhouse, evaporation of water from the logs cools the surrounding air, which then sinks. Opening side vents allows this cool, moist air to escape, drying the bark (Fig. 7–7). Once the bark has dried, ventilation is decreased by closing the side vents and covering the logs with humidity blanket to reduce the downward movement of air through the stacks.

Optimum humidities during spawn run are between 50% and 70% RH. The humidity can be raised with irrigation. The water need not be applied directly to the logs, but can be sprayed on the ground, the walls or an absorbent log covering such as humidity blanket.

Controlling temperature. Providing optimum temperatures for shiitake during the spawn run will increase the speed of mycelial growth. Temperature is managed by irrigation and by controlling the amount of sunlight reaching the logs through shading.

Log temperature is usually raised by solar heating, either directly on the logs or by placing the logs in a greenhouse. The percent shade, orientation and angle of the logs all influence the degree of heating. In addition, the logs can be covered by a light-colored material (such as humidity blanket) to prevent direct solar heating.

Irrigation can effectively cool the logs. Briefly watering the logs during the hottest periods of the day will lower log temperature by evaporative cooling. However, the water does not have to be applied directly to the logs. It can be applied to the roof or walls of the building or, most commonly, to an absorbent log covering such as humidity blanket.

Although watering affects both the temperature and the water content of the logs, its influence varies with the time of day. Watering in the early morning when the logs are cool has little effect on log temperature, whereas watering during the hottest hours of the day reduces log temperature. Also, because high midday temperatures raise the evaporation rate, less water will be absorbed at that time. Water applied during the evening and at night evaporates the least, allowing a higher percentage to be absorbed.

Monitoring mycelial growth through the logs. A number of signs indicate the progress of the fungus through the log. Under moist conditions, mycelium often appears on the log ends. This mycelium is white initially, but turns brown with exposure and forms a pattern traceable to the inoculation holes close to the ends (Fig. 7–8). However, under drier incubation conditions, there may be no mycelium on the ends.

There are more subtle signs as well. The characteristic odor of shiitake becomes noticeable. Also, the wood softens, especially around the inoculation holes. This is easiest to detect a day or two after watering; immediately after irrigation, the contrast is hard to feel because the bark is saturated and spongy.

Features of Mature Logs

Shiitake logs are ready to fruit when the shiitake has colonized the outer cylinder of available sapwood and has utilized the easily obtainable carbohydrates. At this point, the mycelium has stored enough nutrients to form mushrooms. Generally, this requires between 6 and 24 months.

A number of signs indicate that logs are nearing the end of the spawn run. The most obvious is the appearance of primordia and mushrooms on the logs. This usually occurs on logs near the tops of the stacks since these have

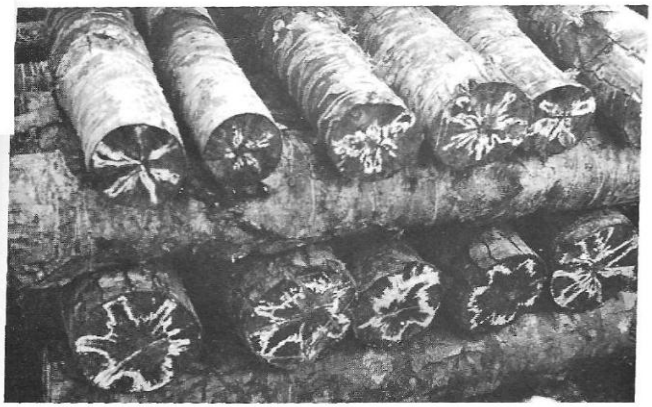


Figure 7–8. Shiitake mycelium on ends of fully colonized logs (USA).

experienced greater stresses from wetting and drying. Other signs also indicate readiness for fruiting: the logs become increasingly absorbent; most of the bark becomes elastic and spongy; and the logs no longer "ring" when struck.

If logs are induced to fruit before they are fully colonized, yields will be poor, mushroom quality may be low and the risk of disease may be high. If the mycelium does not have sufficient reserves for fruiting, mushrooms may have small or missing caps and thick stems. Another sign of incomplete colonization is a yield of only a few mushrooms per log, with fruiting occurring only from the inoculation sites.

In logs fruited prematurely, the shiitake mycelium will be weakened by allocating its limited reserves toward mushroom production. This weakness, combined with the fact that competing fungi can easily become established in uncolonized areas, can result in high disease levels. It is better to incubate logs a little longer than to risk losing the crop to disease.

8

Shiitake Fruiting Cycle

Once the logs have been completely colonized, the fruiting cycle can begin. Mushrooms will be produced periodically for two to six years.

During the fruiting cycle, the mycelium undergoes a series of unique changes, the rate and degree of which are controlled by the environment. Consequently, the ability to understand and manage the environment during each stage of the fruiting cycle will result in maximum yields.

This chapter covers the four stages of the shiitake fruiting cycle: induction, pinning, fruiting and resting. It also discusses the effect of the environment on each stage. Chapter 9 will explore management techniques for manipulating the environment during the fruiting cycle.

Overview of the Fruiting Cycle

The four stages of the fruiting cycle follow one another to form a continuous cycle of vegetative growth alternating with periodic mushroom production (Fig. 8–1). Initially, logs fruit after completing the spawn run.

Induction is the first stage of the fruiting cycle. During induction, environmental stresses (including changes in temperature, moisture, and nutrients) signal that the environment is no longer favorable for vegetative growth, and that the time has come for reproduction by spore dispersal. The mycelium responds by shifting from its vegetative phase to the production of fruiting bodies.

Pinning. After the mycelium has been induced to fruit, the hyphae aggregate to form knots at the wood/bark interface. This signals the onset of pinning. These hyphal knots then differentiate into specialized mushroom tissues. At this point, they are called pins or primordia, the earliest stage of the mushroom (Fig. 8–2).

Fruiting or maturation begins after the primordia have emerged and a cap and stem are discernible. During this period, the mushrooms develop and expand (Fig. 8–3). Fruiting ends when the shiitake are harvested or, in nature, when spores are produced.

Resting. The post-fruiting period has traditionally been called the resting period. However, although the fungus is resting from mushroom production, it is by no means dormant. The mycelium is actively growing, decaying wood, and storing nutrients in preparation for further fruiting. Following resting, logs can again be induced to fruit, beginning another round of the fruiting cycle.

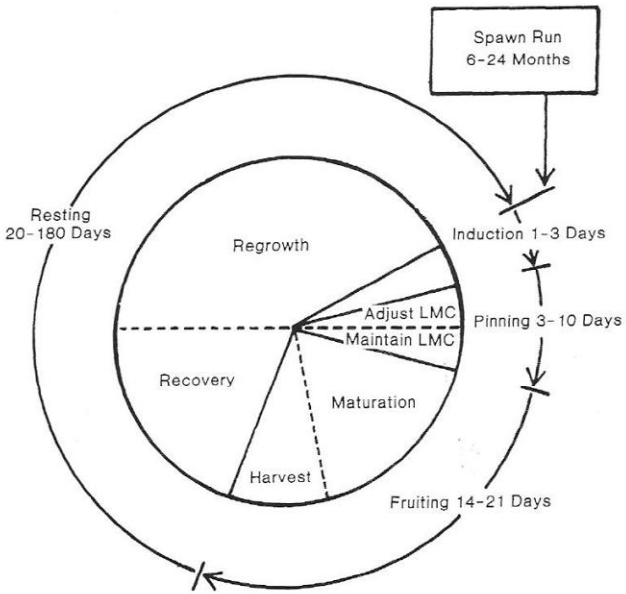


Figure 8–1. Shiitake fruiting cycle on logs.

Environmental Influences

Induction

Induction causes the fungus to reallocate its energy and resources from vegetative growth to the production of mushrooms. Although research with other fungi has given insights into the environmental stresses that induce this change, the mechanisms are not well understood. However, several interrelated stimuli which cause induction have been identified.

As discussed in chapter 3, the ability of shiitake to enzymatically decay wood is directly affected by temperature and log moisture content. Changes in these environmental factors cause shifts in metabolic activity which can trigger induction.

Light. Prior to induction, shiitake mycelium must have been exposed to light. The requirement is slight and is usually fulfilled during the spawn run.

Nutrient depletion. As long as readily available nutrients are present, shiitake continues its vegetative growth. However, when the readily available sugars in the sapwood become depleted, the mycelium begins to accumulate nutrient reserves for fruiting (*105*). Studies of other fungi have shown that fruiting is favored by low levels of readily available sugars (*57*).

Temperature. Sudden drops or shifts in temperature often cause induction. However, the temperature differential required depends on the shiitake strain. Some strains respond best to being warm and then experiencing a rapid drop; others respond to warmer temperatures following a prolonged cold period.

Low temperatures may indirectly induce fruiting by slowing metabolic activity, thus lowering nutrient availability. As is the case with other fungi, temperature may also have a direct effect by favoring specific metabolic processes which trigger induction (*57*).

Moisture. Extremes in log moisture content (LMC) can also induce fruiting. This may be because moisture acts to influence both nutrient levels and temperature. Low LMC can temporarily reduce nutrient availability by limiting the free water needed for enzyme activity and diffusion of nutrients. Conversely, when LMC is high and wood cells are saturated, the nutrient concentration around the mycelium is lowered by dilution.

Figure 8–2. Shiitake primordia on logs (Japan).

In its natural habitat, shiitake fruiting is induced by spring and fall rains which thoroughly wet the logs at a time of changing temperatures. During cultivation, the grower can control temperature and LMC to synchronize fruiting.

Shiitake logs are commonly irrigated or submerged in water to induce fruiting. Because drying creates additional stress in the mycelium, drying the logs before soaking often increases the strength of the fruiting stimulus. During soaking, logs quickly adjust to the water temperature; thus, log temperature can be controlled by regulating water temperature.

The ability of shiitake mycelium to respond to induction is determined by the nutrient and water reserves it has accumulated. Research has shown that adding nutrients to the soak water will increase yields over non-supplemented logs (*206*). A solution of ammonium sulphate (0.1%) and glucose (0.5%) resulted in a 71% increase in yield. However, supplementation during soaking may also increase contamination during subsequent flushes, resulting in a net loss.

Pinning

After induction, hyphal knots differentiate into specialized mushroom tissue called "primordia" or "pins." If the mycelium does not have sufficient nutrient and water reserves, the primordia either abort or form small, deformed mushrooms. Since mushrooms are from 85% to 95% water, they require a considerable water reserve in the substrate to mature. Thoroughly saturated logs provide a large water reservoir for mushroom development.

Of all stages in the fruiting cycle, pinning is the most sensitive to environmental influences. Log moisture content, temperature and relative humidity are all crucial. Extreme values of any of these three factors can inhibit or halt the pinning process, aborting mushroom production.

Pinning occurs as the logs dry, at LMCs from 35% to 65%, with the optimum LMC range from 55% to 65% (*98, 211*). Pinning will not occur if logs are either saturated or too dry (*98*).

The length of time that the logs remain within the optimum LMC range for pinning affects the crop in several ways. If logs dry too rapidly, the limited water will reduce the number of primordia that emerge through the bark. In extreme cases, no pins will appear.

Air temperature during pinning has a major influence on yield (*150, 151*). Each strain pins best within a specific temperature range. The fruiting seasons for various strains are determined by the temperature required for pinning. In general, most strains pin best from 55° to 68°F (13°–20°C) and require from three to five days for primordia formation.

Bark thickness has been shown to influence pinning. Thin bark allows more primordia to emerge, but also holds less water and dries faster. For this reason, thin-barked logs require more care to maintain adequate water levels during pinning and fruiting.

Fruiting

Prior to the onset of fruiting, shiitake mycelium has been storing nutrients. These nutrients and water are transported through the mycelium and into the developing fruiting body (*206*).

Temperature. Once the primordia have emerged and have begun to expand, temperature has more influence on mushroom quality than on yield. Higher temperatures speed maturation, but produce lower quality mushrooms with thinner caps. Mushrooms grown at lower temperatures are more dense and have a more intense flavor.

Humidity. Although mushroom moisture content is partly strain related, it is largely determined by the relative humidity during fruiting. Humidities between 60% and 85% RH are optimal. Higher humidities result in dark soggy mushrooms with shorter storage life and less flavor. Lower humidities result in light-colored mushrooms with cracked caps. Extremely low humidity can stop mushroom growth entirely by drying the log.

Humidity during fruiting can also influence the occurrence of disease fungi such as *Trichoderma*. High humidities favor spore germination and growth of these fungi. Limiting the amount of time the logs remain under fruiting conditions will lower the incidence of disease.

Light. Light is required during fruiting for proper mushroom shape and color. If light levels are too low, the mushrooms will be light-colored. With adequate light, the cap will be a dark brown. More light is needed at higher temperatures because the mushrooms are growing faster. Conversely, less light is required at lower temperatures to achieve the same cap color.

Harvesting. The point at which shiitake is harvested depends on the demands of the market. Mushrooms are usually picked within a day or two after the veil covering the gills ruptures. Generally, all the mushrooms on a group of logs subjected to the same induction conditions mature within several days. This crop is referred to as a **flush**. The details of harvesting and post-harvest handling are discussed in Chapter 14.

Resting Period

After the mushrooms are harvested, the mycelium must again accumulate nutrients in order to produce another flush of mushrooms. Fruiting is a very "expensive" process for the mycelium. Large amounts of carbohydrates, nitrogen and water have left the mycelium, leaving it weak and susceptible to invasion by other organisms (*206*). These nutrients must be replenished. To allow the mycelium to recover, the logs must be incubated under conditions

that promote rapid mycelial growth. The resting period has two stages: recovery and regrowth.

The mycelium is weak during the recovery period and the logs must be treated accordingly. Log moisture content should be maintained from 30% to 40% to promote mycelial growth while inhibiting pinning. Warm temperatures (59°–77°F, 15°–25°C) speed recovery. The end of the recovery period is indicated by a browning of the exposed shiitake mycelium.

During the following period of regrowth, the mycelium replenishes its depleted nutrients. The length of the resting period is determined by the cropping pattern and the strain. It can be as short as three to five weeks.

Cropping Patterns

There are numerous possible schedules for flushing a group of logs. These are called cropping patterns. Under natural conditions, logs usually produce just two crops per year with an almost six-month resting period between crops. Using forced fruiting, however, three to five fruitings can be evenly spaced throughout the year. Some strains respond well to forcing two or three flushes in rapid succession. Although these logs have only brief resting periods between flushes, they are eventually given a long resting period during the remainder of the year.

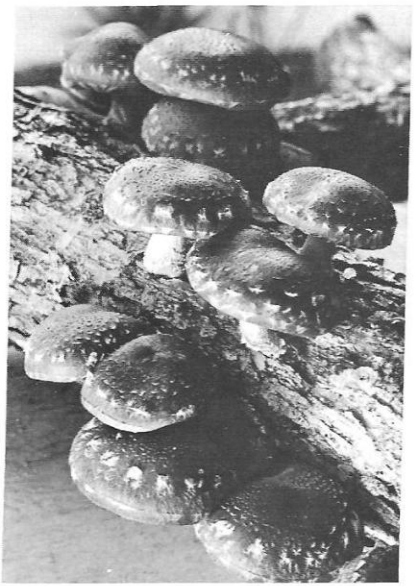


Figure 8–3. Mature shiitake on logs (USA).

Generally, logs that are fruited more than four times per year produce lower yields per flush because the mycelium has not had enough time to accumulate and store nutrients. However, yield can be increased by providing optimum conditions during the resting stage. Intensive forced fruiting leads to increased decay and higher levels of contamination, which decrease the productive life of the log.

Fully colonized logs follow a cycle of fruiting and resting over a period of two to five years. During this cycle, shiitake continues to decay the wood. This results in a gradual drop in log pH

from 4.5–5 to about pH 4 over a period of two to three years (*211*). The pH optimum for fruiting is from 3.5 to 4.5. However, these figures probably reflect the stage of decay rather than an actual influence of pH upon growth. After five years, about 57% of the cellulose and 53% of the lignin in the wood have been removed by the shiitake (*205*).

Yields

The total number of fruiting cycles depends on the log size, tree species and cropping management. Generally, yields are highest during the second and third years of production. Under natural production, 75% of the total yield is harvested during this period. Yields decline in the third year, paralleled by decreases in nitrogen and potassium levels in the wood (*205*).

Yield can be expressed either as a percentage of the initial log weight, or on a per log basis. Expressing yields as a percent of log weight is useful for comparing yields from different areas or from logs of different sizes.

Biological efficiency (BE) is used in the mushroom industry to express yield. BE is a ratio of the fresh weight of the mushrooms to the initial dry weight of the substrate, expressed as a percentage. The maximum biological efficiency for shiitake production on logs has been reported as about 33% (*171*).

For example, assume that a 25 lb (11.3 kg) log is initially at 45% moisture content. The dry weight is 13.75 lb (6.2 kg). Using 33% BE to estimate the maximum total yield gives 4.5 lb (2 kg) as the maximum yield over the entire cropping period. Actual yields would probably be lower.

Most growers refer to yields on a per log basis and assume an average size log. Yields on a per log basis are from 0.25 to 0.33 lb (100–150 g) per log per fruiting, assuming eight to twelve fruitings for each log.

Thin-barked logs usually give higher yields at early fruitings because primordia are able to break through the bark easily. However, thick-barked logs yield more overall because their tough bark protects them and enables them to produce for a longer period (*146*). Mushroom production ceases in any area where the bark is lost, and the productive life of a log ends when it has lost most of its bark. Fruiting also ceases when logs are so decayed that the mycelium is weakened and overrun by other fungi.

9

Management of the Fruiting Cycle on Logs

A number of different techniques and strategies can be used to produce shiitake from colonized logs. After logs have completed the spawn run, the fruiting cycle can begin. Management of the log environment during this period determines the timing, quality and quantity of the mushrooms. The management strategy is dictated by market demand.

This chapter examines different strategies for fruiting shiitake logs. It shows how marketing, capital and labor factors influence the choice between natural production or forced fruiting and explores these two basic fruiting strategies in detail. A hypothetical shiitake farm is used to illustrate each strategy.

Choosing a Fruiting Strategy

The grower must consider both marketing demands and management practices when selecting a method for fruiting shiitake on logs.

Market Considerations

One of the most important decisions is whether the mushrooms will be marketed fresh or dried. Once the market has been defined, decisions can be made concerning the fruiting strategy and the amount of capital and labor that can be profitably invested.

Dried shiitake has a long storage life and can endure long shipping times without losing quality. This means that the fruiting area can be remote from the market. Dried shiitake can be produced seasonally in large quantities and then sold throughout the year. Generally, dried shiitake brings a lower price than an equivalent weight of fresh mushrooms sold on the fresh market.

Fresh-market shiitake must be grown where it can be rapidly delivered to the market. Because, even under ideal conditions, fresh shiitake has a shelf life of about two weeks, long shipping times can result in mushroom deterioration and loss of revenue. The higher price paid for fresh shiitake compensates the grower for the higher facility and labor costs needed to produce, market, package, store and transport a perishable product.

Management Considerations

There are two basic approaches to the management of logs during fruiting: natural fruiting and forced fruiting.

Natural fruiting is possible in areas with warm, moist climates. Logs can go through the fruiting cycle outdoors with little management. Mushroom production is seasonal, but may be extended with simple management techniques. Generally, shiitake produced under natural outdoor conditions are sold dried.

Naturally fruited shiitake can also be sold fresh, but the seasonal grower is at an economic disadvantage in the fresh market. Natural crops peak during the spring and fall, causing a seasonal abundance of mushrooms which lowers the market price. The seasonal grower may receive a lower price for fresh shiitake than does the year-round grower who has been steadily supplying a market.

Forced fruiting is the process of managing each step of the fruiting cycle to control mushroom production. By controlling induction, forced fruiting can schedule mushroom crops to be sold fresh throughout the year. Forcing usually occurs in greenhouses or other structures.

Forced fruiting has several economic advantages that offset its increased costs. Controlled conditions can produce higher quality mushrooms which command a higher price. Overall yields per log are higher than in natural fruiting and each fruiting cycle is shorter. This allows logs to be cycled through the fruiting area faster, producing more mushrooms in a given time period.

Comparisons between natural and forced fruiting. Figure 9–1 compares log conditions during natural and forced fruiting under warm temperatures. Under natural conditions, logs are induced to fruit by a drop in log temperature accompanied by periodic rainfall. The logs do not experience rapid, extreme temperature and moisture changes during induction. Weak induction stimuli, combined with limited periods in the optimum pinning range, result in fewer primordia. Because the log microclimate fluctuates, pinning is sporadic. This results in a longer fruiting period with lower yields.

During forced fruiting, on the other hand, the logs are soaked to induce fruiting. The changes in log temperature and moisture content are both faster and greater. This single, strong induction results in synchronous mushroom development and enables the grower to manage the log environment to provide optimum conditions for each stage of development.

Because the temperature and rate of drying are controlled to maintain optimum pinning conditions for a longer period, more primordia are produced. Once the pins have emerged, the temperature is raised and the relative humidity is lowered to stop further pinning and to produce high quality mushrooms. Such management gives higher yields in a shorter time.

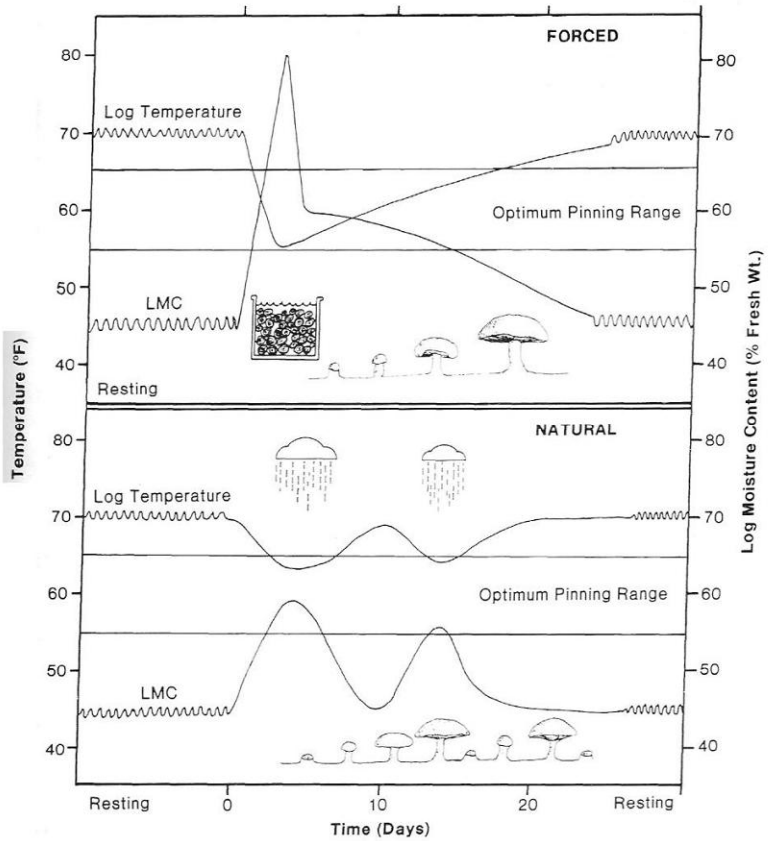


Figure 9–1. Log conditions during forced and natural fruiting of shiitake. Wide-range strain CS–41, ambient conditions 70°F, 60% RH.

Seasonal Outdoor Fruiting

Outdoor fruiting is characterized by seasonal production. Mushrooms produced outdoors are often dried, but can also be sold fresh. The production season can be extended by water management techniques and by using strains with different temperature requirements for fruiting. Lengthening the fruiting season is especially desirable when growing shiitake for the fresh market.

Suitable Areas

Requirements for the fruiting area are similar to those for the spawn run area, except that the fruiting yard must be cooler (50°–68°F, 10°–20°C) and more humid. Japanese growers express this concept as "6 dry, 4 wet" for spawn run and "4 dry, 6 wet" for fruiting (*182*). The area needs good air movement and 60% to 85% shade. Good access is essential because mushrooms are harvested daily. A water source is desirable to increase log moisture content for forcing and to maintain humidity during dry spells.

Stacking Methods for Outdoor Fruiting

Before fruiting, logs are usually restacked to provide the increased space needed for mushroom expansion and harvesting. Restacking the logs also allows the grower to change the microclimate around the logs and to cull contaminated logs.

Alternatively, logs may be kept in the same stacks for both the spawn run and fruiting, thus saving labor. In this case, the logs should be loosely stacked during the spawn run. Because the increased air circulation around the logs causes more drying, this method is limited to areas with sufficient water, either irrigation or rainfall. Both horizontal and vertical stacking methods are used.

Horizontal stacking. Some horizontal stacks work well for both spawn run and fruiting. Loose crib stacks and triangle stacks are space-efficient for fruiting (Fig. 9–2, 9–3).

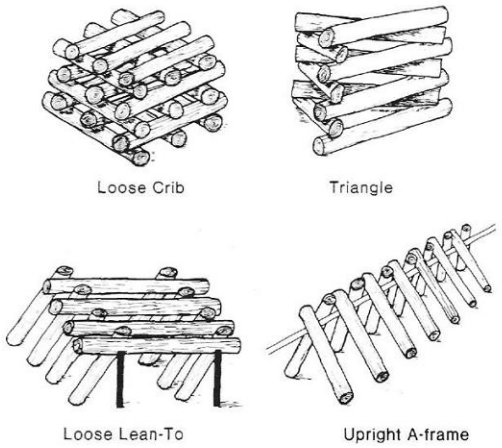


Figure 9–2. Log stacking methods for fruiting shiitake.

Horizontal stacking has several disadvantages. Picking takes longer because some of the mushrooms are difficult to reach, especially those in loose crib stacks. Even under the same conditions, horizontal logs often produce a lower quality mushroom than vertically stacked logs do. The mushroom caps are dirtier because dirt and debris fall onto them from the logs above. In addition, a higher percentage of mushrooms are misshapen because they press against adjacent logs as they expand.

Vertical stacking. Vertical stacking has several advantages over horizontal stacking. Water rapidly drains off vertical logs; this is desirable under wet conditions. Access for harvesting is easier, and mushroom quality is higher. Also, it is easier to remove contaminated logs.

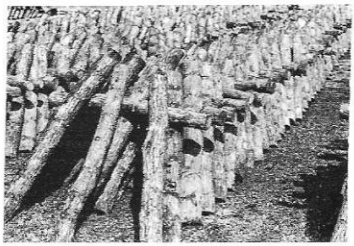
The most commonly used vertical stacking methods for outdoor fruiting are the upright A-frame stack and a loose lean-to stack with the middle logs removed from each row (Fig. 9–2, 9–3). The upright A-frame is usually stacked against a supporting rail or wire. The logs can also be stacked in an A-frame similar to that used during the spawn run (Fig. 7–5).

Managing Logs During Outdoor Fruiting

Logs can be fruited outdoors with little active management, or management may be intensive. If the fruiting yard and stacking method are chosen

Upright A-Frame

Triangle Stack



Loose Lean-to Stack

Figure 9–3. Shiitake logs stacked for seasonal outdoor fruiting (USA).

carefully, little active management will be needed. However, managing to create better conditions during specific stages of production will result in improved mushroom quality, increased yields and an extended fruiting period. Several active management practices are presented below, beginning with very simple steps and progressing to more intensive ones.

Improving mushroom quality. Once the logs have been thoroughly wetted, protecting them from rainfall will markedly improve their quality. Plastic tarps, sheets of rigid roofing or other materials placed over the logs will shed rain, while not decreasing air movement around the logs. The amount of water reaching the logs can then be controlled as needed, allowing the mushrooms to mature without getting soaked by an untimely rain.

Increasing shiitake yields. Managing conditions during pinning can increase shiitake yields. Logs that have been induced by rain can be covered with humidity blanket or plastic tarps to control the drying rate. Humidity blanket is a porous, white, synthetic, felted material which holds water, allows air movement and provides some insulation. The log moisture content can be controlled by watering the humidity blanket or by raising the tarp as needed. In this manner, the logs can be held between 55% and 65% LMC for three to five days. This increases the number of primordia which develop into mushrooms. Once the primordia have emerged, watering should be decreased or the tarps should be rolled up to expose more of each log. This will lower the humidity around the logs and produce dry, firm mushrooms.

Extending the fruiting period. The fruiting period can be extended by several methods, used alone or in combination. These include using strains with different fruiting temperature ranges, irrigating the logs and preventing induction by rain.

Logs can also be force fruited by soaking as described below. Soaking requires additional handling, but the increased yields may offset the additional costs. Sometimes soaking can be combined with moving the logs to a fruiting site or with restacking them for fruiting.

Figure 9–4 shows management options for extending the fruiting period in a temperate climate. Favorable weather for unmanaged outdoor fruiting occurs during the spring (April and May) and in the fall (September and October). If all the logs were inoculated with a warm weather strain, fruiting would be concentrated in a few weeks in May and September (Fig. 9–4 A) .

If half of the logs were inoculated with a cool weather strain, they would fruit in April and October, thus extending each fruiting season by one month (Fig. 9–4 B).

Steady production could be achieved by covering all the logs in March to prevent induction, then inducing a few logs each week. Assuming 2,000 logs total, 250 logs of the cool weather strain could be uncovered to the rain each week in April. Beginning in May, logs containing the warm weather strain

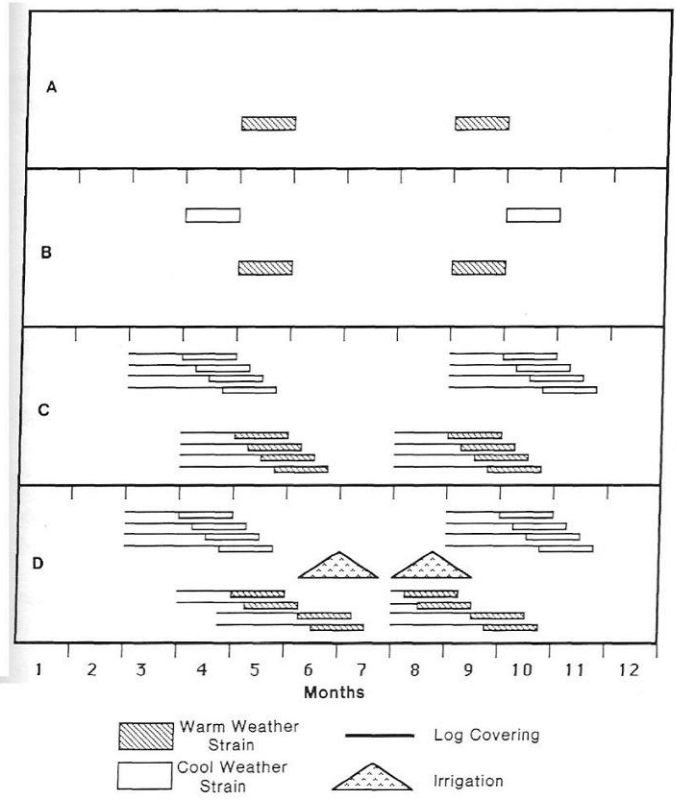


Figure 9–4. Log management for extending natural outdoor fruiting season. A. Warm weather strain, B. Warm and cold weather strains, C. Delaying induction with log covering. D. Extending fruiting season with log covering and irrigation.

could be similarly induced. This would result in continuous production during the fruiting period. Fall fruiting could be extended in the same way (Fig. 9–4 C)

The fruiting season could be further extended by keeping half of the warm weather strain logs dry to prevent fruiting during May. These logs could then be irrigated to fruit in June. The logs which were fruited in May could be irrigated again during late August to lengthen the fall fruiting period (Fig. 9–4 D).

Year-Round Indoor Forced Fruiting

Indoor production is characterized by continuous, steady fruiting of fresh shiitake throughout the year. The growing environment is carefully managed to control water, temperature and air movement. Year-round farms are usually located so the mushrooms can be quickly delivered to market.

Depending on climate, the fruiting structures used for indoor production range from special greenhouses (Fig. 9–5) to simple sheds. A typical spawn run greenhouse can also be used for fruiting.

Soaking

Most indoor producers soak the logs to induce fruiting. Soaking results in higher yields (*146*) and concentrated, synchronized flushes. Strains differ in their response to soaking. Some strains are not suitable for forced production because they do not respond rapidly to soaking.

The length of the soaking time depends on water temperature, log temperature, log moisture content, degree of decay, tree species and size of the logs. Larger diameter logs require longer soaking because the surface-to-

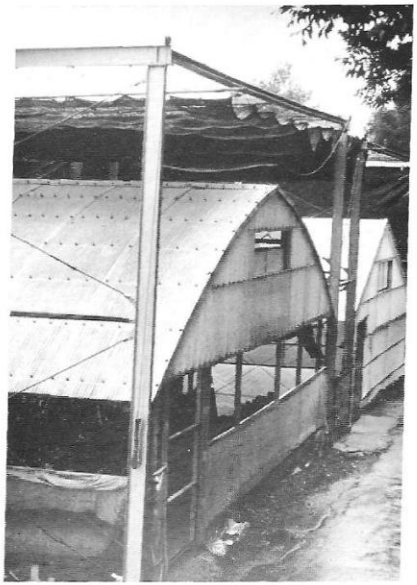


Figure 9–5. Greenhouse used for forced fruiting of shiitake logs (Japan). Note side vents and movable shade cloth.

volume ratio is lower. Generally, tree species with higher wood density require longer soaking periods than lighter woods. As logs become more decayed, less soaking is needed because they absorb water more rapidly.

During warm weather, the soak water temperature should be from 50° and 60°F (10°–16°C). Ground water in temperate regions is often within this range. If fresh water can be left flowing through the tank, the temperature can be kept low. If the water supply is limited or too warm, refrigeration can be used to lower the water temperature. The same water can be reused several times before it is changed.

Two different strategies can provide a temperature shock during cold weather. The first method is used for warm-temperature strains. The logs are warmed for a week or two, soaked in cold water and then placed under pinning conditions.

The second method works well for strains with lower pinning temperatures. Cold logs are soaked in relatively warm water (50°–60°F, 10°–16°C), then pinned. Water heaters are often used with this method.

During soaking, water displaces the air within the logs, causing small bubbles to appear from the logs. When the logs are totally saturated, the bubbles stop forming. Logs should be removed from the soak tank just before the bubbles stop forming. Generally, this requires from 12 to 72 hours.

Logs that have been soaked too long will produce small, thin, overly wet mushrooms. Too little soaking, on the other hand, results in arrested mushroom development and produces small dry-capped mushrooms (*180*).

The soaking tank needs two drains, one at the bottom and one for overflow at the top. It also needs some type of hold-down to keep the logs submerged. New concrete tanks should be filled, allowed to stand full of water and drained. This should be done several times to remove inhibitory compounds (*180*).

Soaking tanks may be above ground, partially buried or totally below ground. The choice depends, in part, on how the logs will be moved to and from the tank. Below-ground tanks work well for logs that are moved by machine. Another advantage of below-ground tanks is that they are insulated by the soil.

In contrast, above-ground tanks are easier to unload by hand. In addition, above-ground tanks can be portable; the grower saves labor by moving the tanks rather than the logs.

Logs can be placed into the tank by hand or in racks lifted by a tractor, forklift or overhead hoist (Fig. 9–6). A number of different rack designs are used. Racks can be built to pick up crib stacks, eliminating the need for restacking. The most common types of racks hold 50 to 100 logs in horizontal bulkstacks.

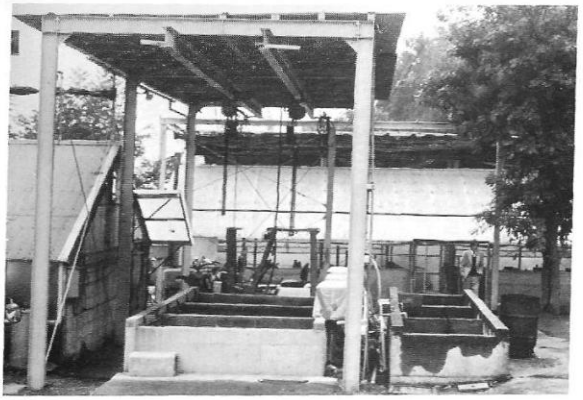


Figure 9–6. Concrete tanks with overhead hoist for soaking shiitake logs (Japan).

Pinning

Pinning has two distinct phases: drying the soaked log until its moisture content (LMC) reaches the optimum pinning range (55% to 65%) and maintaining the LMC and temperature within that range.

Careful management during pinning results in higher yields and better quality mushrooms. The relative humidity of the air surrounding the logs affects the drying rate, which, in turn determines the length of the pinning period. Pinning generally requires three to ten days depending on the temperature and strain. If the pinning stage is short, the number of primordia which emerge will be limited. If there are fewer pins, the resulting mushrooms will be larger. Conversely, more primordia will yield a greater number of smaller mushrooms.

Drying to pinning conditions. Shiitake logs absorb water like a sponge. During soaking, well-colonized logs can reach LMCs as high as 80% to 90%. This high LMC provides an adequate moisture reservoir for mushroom development and induces the mycelium to fruit. Nevertheless, it is too high for pinning. Pinning occurs as the logs dry.

One way to dry the logs is to place them in an open stacking pattern in a well-ventilated area. As water evaporates from the logs, it also cools them. Although this may be desirable during the warm season, it can be a problem during cool weather. Therefore, some farms have special warm areas for drying the logs after soaking.

Another method of rapidly drying the logs is to shake them against one another. Shaking squeezes excess moisture out of the bark, similar to squeezing a sponge. This method is rapid and does not change the log tempera-

ture—an advantage during the cold season. Japanese growers often use large shakers that hold an entire rack of bulk-stacked logs.

Maintaining optimum pinning conditions. The next phase in pinning controls the evaporation potential to maintain the logs between 55% and 65% LMC at a suitable temperature for the primordia to form and push through the bark. Most strains pin between 55° and 65°F (13°–18°C). The beginning of this second stage is signaled by small bumps (primordia) forming under the bark.

The size of the area to be controlled for pinning is a management choice. Pinning conditions may be established in the entire fruiting house, in a smaller area (a pinning chamber), or only in the microenvironment surrounding the logs.

Fruiting structure. The entire fruiting structure can be controlled by managing watering, air temperature and ventilation. An advantage of this method is that the logs need not be moved for pinning. However, since conditions that favor pinning are not ideal for fruiting, this method is not practical if the house contains logs at different stages of mushroom formation. In addition, it is difficult to provide optimum pinning conditions throughout a large structure during the winter months when heating dries the air.

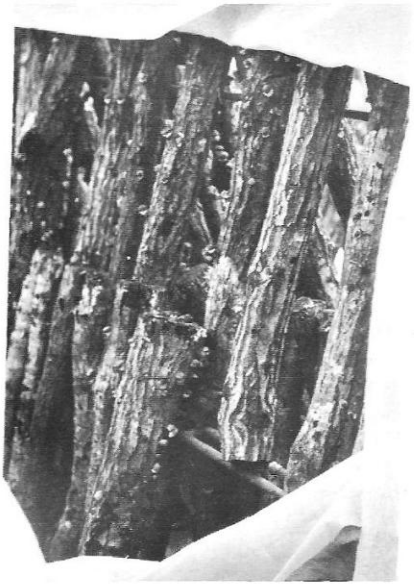


Figure 9–7. Shiitake logs pinning in fruiting racks under humidity blanket (Japan).

Pinning chamber. A special pinning chamber which is designed to allow temperature, ventilation and humidity to be closely controlled may be used. Less energy is required to maintain optimum conditions in a pinning chamber than in an entire fruiting house. This is an important consideration during colder weather. After the logs are removed from the pinning chamber, they fruit rapidly, thus decreasing the time they remain in the fruiting racks.

Some Japanese growers use partially or totally buried concrete tanks as pinning chambers. These tanks maintain relatively constant conditions and can be heated or cooled as needed.

Log handling can be reduced by using the same stacks for soaking and pinning. Then, after pinning, the logs must be placed into fruiting position. During this step, the logs need to be handled carefully because they are covered with fragile primordia which are easily damaged.

An efficient layout for soaking and pinning uses two rows of tanks: one for soaking, the other for pinning. An overhead hoist on tracks can efficiently move logs between the two rows of tanks. The hoist can pick up a rack of logs from a cart or trailer, place it in a soak tank, remove it, place it in a pinning chamber and, finally, place it back on the cart.

Log microenvironment. Another method of maintaining pinning conditions around the logs is to cover them with humidity blanket or plastic tarps after they have reached the optimum LMC range. Humidity blanket can be used to control both temperature and humidity. The blanket absorbs irrigation water, which then evaporates, lowering the temperature and raising the humidity around the logs. By careful watering, the conditions inside the "humidity tent" can be regulated to maintain logs within a desired pinning range.

This method works well to control conditions around logs that have been stacked for fruiting directly after soaking (Fig. 9–7). It eliminates handling logs with delicate primordia and carries out pinning and fruiting within the same structure.

Log Stacking Methods for Indoor Fruiting

Harvesting shiitake is a labor-intensive activity, and when mushrooms are fruited inside, it is carried out in relatively expensive space. Therefore, it is important that both the fruiting area and the stacks be designed to save labor and to use space efficiently. Access for moving logs in and out of the fruiting house is needed, and stacking methods must allow space for the shiitake to develop and to provide access for harvesting.

All of the stacking methods presented for outdoor production can be used indoors as well (Fig. 9–2, 9–8). An advantage of loose crib and triangle stacks is that they can be moved by machine. Thus, logs in these stacks can be incubated, soaked, pinned and fruited all without handling the logs by hand. An alternative is to use a fruiting rack (Fig. 9–9).

The shelf rack holds logs horizontally in tiers, up to five or more high (Fig. 9–9 A). This type of rack is space-efficient and has no log-to-log contact. However, additional handling is required to load the rack, and there can be great differences between logs on the top tiers and those on the bottom, due to temperature stratification in the fruiting house.

The two-tiered A-style rack is also used in indoor fruiting (Fig. 9–7, 9–9 B). This increases the number of logs per square unit of area. However, it has the same problems as other multi-tiered racks: temperature and relative humidity differ between the top and bottom of the rack. These differences extend the fruiting period because the mushrooms mature at different rates. Humidity blanket can be used to minimize this problem.

The pick-through rack is commonly used for fruiting indoors. The rack consists of two parallel rails, about 4 ft (1.2 m) apart and about 2 to 2.5 ft (0.6 m) above the ground. Short rows of nearly vertical logs are leaned against a log or stick placed across the rails to separate the rows (Fig. 9–10).

Before harvesting, all the rows of logs are leaning away from the end where picking will begin. The picker may stand inside the rack while picking,

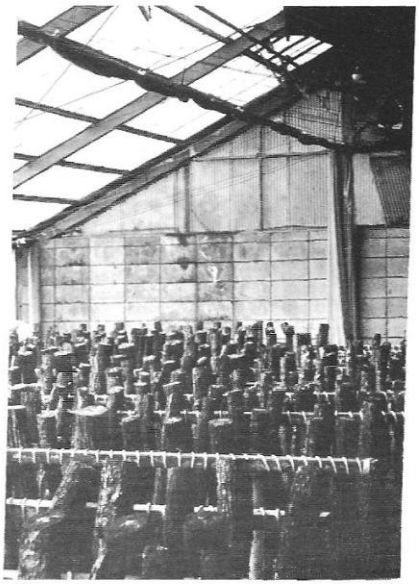


Figure 9–8. A-frame stacking of shiitake logs during forced fruiting in a greenhouse (Japan).

if the rack is wide. After the first row of logs is picked, the horizontal support is moved away from the logs. The harvested logs are then leaned in the opposite direction, against the horizontal support. This forms an opening, exposing a new row of logs for picking (Fig. 9–9 C). Harvesting continues through the rack until all the logs have been harvested and are leaning the other direction.

The pick-through rack has many advantages. It is space-efficient, with little difference between the top and bottom of the logs. By using humidity blanket, the rack can also be used for pinning. Humidity and temperature can easily be controlled because the logs are densely packed.

One disadvantage is that all the logs in a rack must be flushed simultaneously because each log is moved during harvesting. Another disadvantage is that vertical space may be wasted because this type of rack is not suited to stacking several tiers high. However, some growers use logs up to six feet (1.8 m) long in similar racks to utilize more vertical space in the fruiting houses.

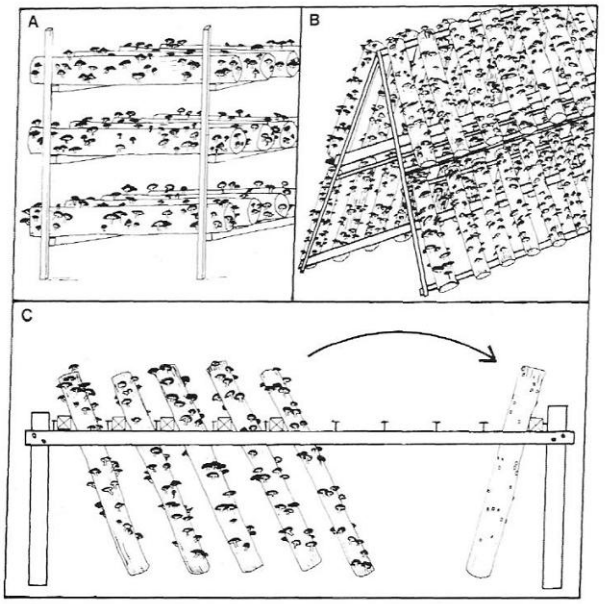


Figure 9–9. Racks for forced fruiting of shiitake logs. A. Shelf rack, B. Two-tiered, A-style rack, C. Pick-through rack.

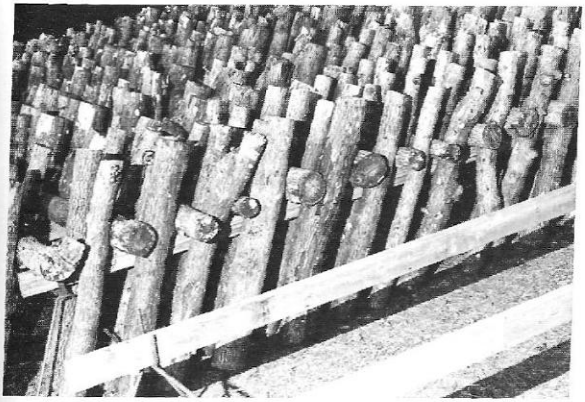


Figure 9–10. *Shiitake logs in pick-through rack during forced fruiting (USA).*

Indoor Fruiting

The fruiting period does not demand the same degree of environmental control as does pinning. Barring extremes, once the mushrooms have begun to expand, they are tolerant of fluctuating conditions. The fruiting period usually lasts from one to two weeks, depending on the temperature and strain.

Optimum conditions for fruiting differ from those for pinning. Once the primordia have emerged and started to expand, the relative humidity may be lowered to 60% to 80% RH and the temperature may be either raised or lowered to produce the desired mushroom quality. Higher temperatures shorten the fruiting period, but result in thinner-fleshed mushrooms.

Temperature and relative humidity are controlled through water management and ventilation. During the summer, evaporation can cool both the logs and the air in the fruiting house. When heat is used during cold periods, the relative humidity drops because the water-holding capacity of warm air is greater than cold. Care must be taken to avoid excessive drying of either mushrooms or logs.

Heat sources may be needed for the fruiting house. A greenhouse utilizes solar heating, but an additional heat source is usually required in the winter. The most common heaters are wood-burning stoves, which are often fueled with old shiitake logs (Fig. 9–11). These stoves are inexpensive, but provide uneven heating. In addition, the high temperature of the stove drastically dries the air.

Hot water heating systems provide more even conditions. Pipes with circulating hot water, buried beneath the fruiting racks, can provide localized heat. Regardless of the heating method, humidity must be monitored because warming the air dries it out.

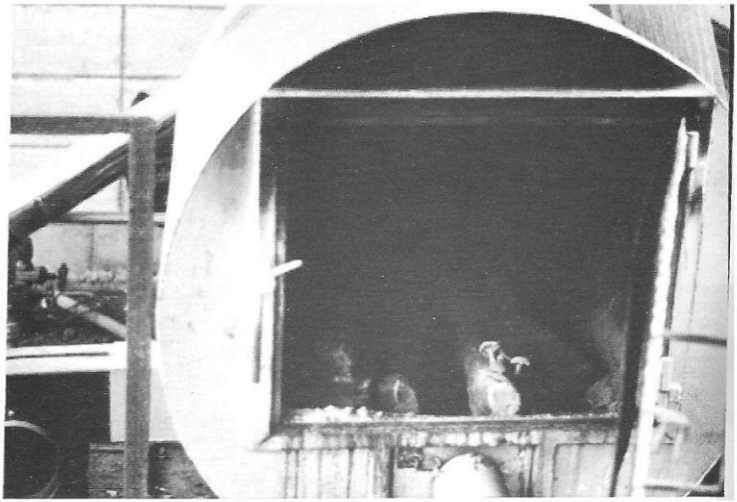


Figure 9–11. Stove fueled with spent shiitake logs for heating fruiting house (Japan).

Cooling. The fruiting house can be cooled in several ways. In summer, the house can be covered with high percentage (85%) shade cloth. Evaporative cooling can also be effective. Indirect watering, irrigating either the top of the house or humidity blanket covering the logs, can lower the temperature while preventing the mushrooms from becoming too wet. Swamp coolers or fan-and-pad cooling systems cool the incoming air by passing it through a water-saturated pad. Air conditioning is seldom used, because of the high expense and the drying effect.

Resting

The recovery period should take place in a warm area with just enough air movement to prevent surface molds—but no more. During the summer, this can be outdoors, but in the winter it should be in a heated area such as the fruiting house. If the logs are dry (less than 30%) they should be watered lightly and then covered with humidity blanket or plastic and incubated at warm temperatures (59°–77°F, 15°–25°C) for four to ten days.

The regrowth period begins after the mycelium recovers. Conditions and stacking methods are similar to those used during incubation. Horizontal bulk stacking is often used. Logs must be kept moist enough to ensure continued growth, but dry enough to prevent untimely fruiting (30% to 40% LMC). The length of the resting period depends on temperature, the production strategy and strain.

The resting area is usually close to the fruiting area and requires conditions similar to the incubation area. Shade structures with windscreens are often used (Fig. 9–12). Access for moving the logs is a major factor to consider.

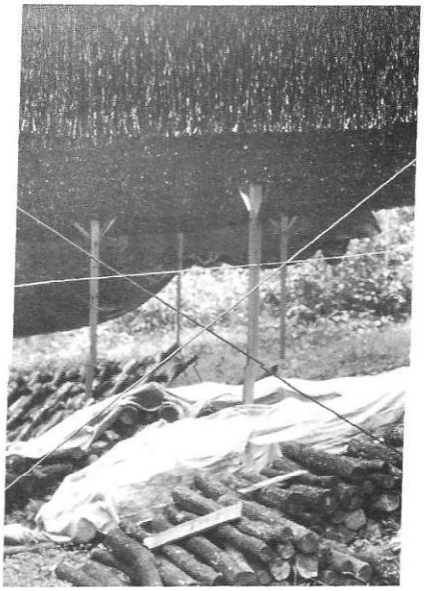


Figure 9–12. Bulk-piled shiitake logs in resting area (Japan).

Continuous Production Schedule

This section examines a hypothetical farm with 10,000 logs, 40 inches (1 m) long, in the fruiting cycle. The goal of continuous production is to produce the same amount of mushrooms each week. This is necessary to develop and maintain regular marketing accounts.

There are several ways to schedule production. This example assumes four fruitings per log, evenly spaced throughout the year. In this example, two wide-range strains are used throughout the farm. These strains respond well to forcing and have similar temperature requirements. Cold-temperature strains could be added to reduce energy usage, but this would complicate the scheduling because they behave differently.

The productive life of the logs in this example is three years. Each year 3,500 logs must be inoculated to maintain about 10,000 logs in production. The spawn run requires one year so incubation space for about 7,000 logs is needed.

All logs in the fruiting cycle will be induced four times per year, which equals 40,000 log fruitings per year. Assuming a 50 week work-year, 800 logs per week will be fruited. This is roughly equivalent to three or four cords.

The logs are stacked in bulk stacks for soaking. After soaking, the logs are placed directly in the fruiting racks where pinning also takes place. The pick-through stacking method is used, with heat pipes under the racks for winter production. The logs are covered with humidity blanket during pinning and fruiting. The humidity blanket is watered periodically to control LMC and temperature. Since the total pinning and fruiting cycle takes three weeks,

fruiting racks for 2,400 logs are needed. A four-foot wide fruiting rack holds about six to eight logs per linear foot.

After fruiting, the logs are bulk-stacked for recovery and resting. Resting space is needed for the logs that are not fruiting: 10,000 minus 2,400 equals 7,600 logs.

Each forcing cycle involves considerable handling of the logs. One critical factor in choosing a handling method is the number of times the logs must be moved by hand.

In this example, the logs are incubated in crib stacks in a greenhouse. The entire stack is then lifted and carried to the soaking area where it is restacked into a bulk stack. After soaking, the logs are hand-stacked into and out of the fruiting racks. During resting, the logs are again bulk stacked. Thus, each log is moved four times by hand in one cycle. This means that 3,200 logs (about 12 to 16 cords) are moved each week.

Using the same stacking pattern for several different steps saves handling. Moving the logs in loose crib stacks by machine throughout the entire cycle would require more space, but would save considerable hand labor.

The yield will be between 0.25 and 0.33 lb (0.1-0.15 kg) per log per fruiting. This equals 200 to 264 lb (91–120 kg) per week or 10,000 to 13,200 lb (4,536–5,988 kg) per year.

10

Diseases, Weeds and Pests of Shiitake on Logs

Like other agricultural crops, shiitake has its share of diseases, weeds and pests. Fortunately, if the grower understands the biology of these organisms, most problems can be controlled through appropriate crop management.

About 150 species of problem fungi can affect the log, mycelium or mushroom during shiitake cultivation (*114*). These fungi can be divided into three categories based on the degree of damage inflicted: disease fungi, competitor fungi, and weed fungi. Disease fungi are capable of attacking and killing shiitake mycelium. Competitor fungi do not actually attack shiitake, but they do diminish the crop by occupying space and withdrawing nutrients from the logs. "Weed" fungi, while not usually a problem, are common and, in severe cases, may act as competitors. Insects and other animal pests also decrease production.

This chapter presents a holistic approach to controlling diseases and pests during shiitake cultivation on logs. It discusses the symptoms, causes, and suggested controls of many problem organisms: fungi, bacteria, insects and other pests.

Holistic Approach to Disease Management

Controlling diseases during shiitake cultivation depends on intelligent management of the log environment to promote shiitake growth while minimizing the impact of other organisms. Although it is impossible to grow shiitake without having a few other fungi occupy some space in the logs, the goal is to prevent large disease outbreaks by understanding the conditions that cause them.

A holistic approach looks at the overall system and solves the problem at the source; it doesn't just focus on the symptoms. Shiitake cultivation is an interrelated process. Each management action has multiple effects which must be considered when attempting to resolve problems.

Air naturally contains many spores of wood-inhabiting fungi, which are ready to land on a suitable substrate, germinate and quickly establish a new colony. Among these, *Trichoderma* spores are among the most prevalent (*76*).

The most potent weapon against this threat is to meticulously provide conditions that encourage strong growth of shiitake at each stage of its life. In addition, disease-resistant shiitake strains can repel many invaders. It is also

necessary to recognize and eliminate conditions that lead to the establishment of shiitake diseases and pests.

Appearance of other fungi on shiitake logs indicates that the shiitake mycelium has experienced stress. However, the stressing factor may have occurred long before the problem is noticed. For example, *Trichoderma* outbreaks are often attributed to overly wet conditions. However, although excessive moisture certainly can induce an outbreak, *Trichoderma* infestations can also be caused by overly dry log conditions, especially during warm periods. Dry conditions stress the shiitake mycelium and allow it to be overrun by *Trichoderma*; however, the characteristic green spores may not appear until much later when the logs are excessively moist. Therefore, this principle must be kept in mind when analyzing a problem situation: The conditions at the time that a problem becomes evident are not necessarily the conditions which caused the problem.

Chemical agents are of limited value for controlling diseases and pests during log cultivation. Not only are chemicals expensive and difficult to apply thoroughly, but their overuse can lead to chemical-resistant strains of the diseases or pests. Furthermore, chemical control usually follows a "band-aid" approach, treating the symptoms while leaving the problem unsolved. Most disease and pest problems are more effectively solved by using crop management techniques.

Although the results of chemical tests are reported in this chapter, the authors do not recommend their use. Moreover, no chemicals are currently registered with the United States Environmental Protection Agency for use on shiitake crops.

Disease Fungi

A number of fungi are antagonistic to shiitake mycelium. They secrete antifungal compounds which inhibit the growth of shiitake mycelium and can parasitize and kill shiitake hyphae (*71, 96, 90, 149*). All of the serious disease fungi are Ascomycetes. Several of the most prominent disease fungi are discussed in this section. However, other fungi not mentioned may be locally important.

If disease fungi infect the logs before the spawn has become established, they can kill the spawn before it can grow into the log. This results in total loss of the log. Once shiitake becomes established, however, it is more resistant to invasion, and infections are usually limited to uncolonized areas of the log. It should be evident, therefore, that disease prevention is of utmost importance.

However, when shiitake is stressed, its ability to resist disease fungi decreases and it may be overrun. As log nutrient levels are depleted toward the end of their productive life, the shiitake mycelium is less resistant to invasion.

Trichoderma

Members of the Ascomycete genus *Hypocrea* and their associated asexual stages, *Trichoderma* and *Gliocladium*, are some of the most serious and widely distributed disease fungi (*87, 92*).

Various species of *Trichoderma* are adapted to a wide range of conditions (*166*). They occur naturally in forest soils around the world. Some species prefer logs in warm, dry locations; others do well under moist conditions, both warm and cool (*88, 91*).

The asexual stage is most commonly seen on logs, but the sexual stage may also occur as logs get older. Initially, the stroma of *Hypocrea* appear as soft white lumps that become hard and turn brick-red to black as the perithecia mature.

Trichoderma and *Gliocladium* are commonly referred to as "Green Molds" or "Forest Green Molds." Colonies of these molds start as white patches or pads of fluffy mycelium. They initially appear on the bark (often in cracks), on the spawn or spawn holes, on wounds, on the ends of the logs and on cut surfaces. As they age and the colored conidia are produced, the colonies usually turn green to rich forest-green. However, conidia may range from almost white to greenish-yellow to dark green (Fig. 10–1).

Later, fruiting bodies characteristic of *Hypocrea* may appear. These fleshy cushions of fungal tissue, called stroma, are circular to irregular in outline. The stroma are small, usually about 1/8 inch to 1/4 inch (3–6 mm) tall and up to one inch across (2.5 cm). Stroma range in color from cream to yellow to reddish-brown. As the fruiting bodies get older, they harden and turn dark-brown to black. (See Chapter 2 for a more detailed description.)

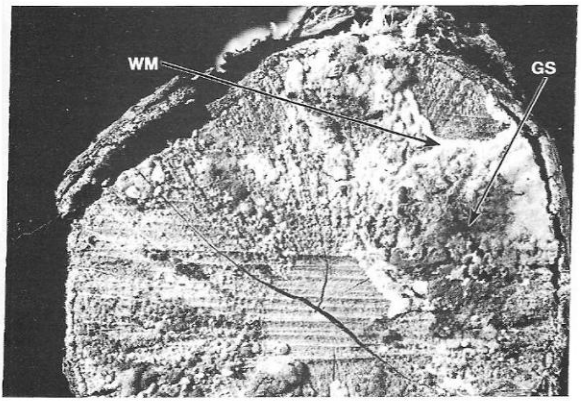


Figure 10–1. *Trichoderma* on end of shiitake log (USA). Note green spores (GS) and white mycelium (WM).

Wood colonized by *Trichoderma* usually appears darkened and discolored, and in advanced cases the bark falls off entirely. *Trichoderma* can cause spawn to turn black, and the shiitake mycelium may disappear, giving the spawn hole a dead look.

Damage to the shiitake mycelium may be slight, or the log may be a total loss. In response to invasion by *Trichoderma*, shiitake forms zone lines, which often limit further invasion. Generally, problems with *Trichoderma* increase as shiitake logs get older and food reserves are depleted. *Trichoderma* produces a number of antifungal compounds which cause the shiitake mycelium to burst open and die (*55, 67, 66*). *Trichoderma* hyphae also may coil around the shiitake hyphae and kill it.

Shiitake is particularly susceptible to *Trichoderma* under certain circumstances: when *Trichoderma* establishes itself prior to colonization by shiitake; when humidity levels surpass 90% RH; when shiitake is weakened by environmental stresses; and immediately following fruiting.

Trichoderma has the advantage if it attacks logs before they are occupied by shiitake. Invasion usually occurs through wounds, exposed log ends or spawning sites. If *Trichoderma* gets into logs while shiitake is not yet well established, it can colonize the wood and kill the shiitake mycelium or limit it to small areas around the spawn holes. Promoting vigorous shiitake growth through the log decreases the chances of *Trichoderma* becoming established in uncolonized areas.

Trichoderma conidia require high humidities (above 90% RH) and free water in order to germinate and invade a shiitake log (*92*). *Trichoderma* infestations can be limited by managing the log environment to let the bark dry while maintaining a high LMC. Drying the log surface can kill *Trichoderma* if it is not well established. Air circulation is very important in controlling these fungi. The surface of infested logs should be allowed to dry, and logs should be restacked, if necessary, to promote air movement.

Conditions which stress the shiitake mycelium should be avoided. Such conditions include prolonged high or low log moisture contents and also direct sunlight on the bark, which can cause high temperature within the logs and stress the shiitake mycelium.

Extra attention should be given during periods when shiitake has been weakened by stress. Critical times are the recovery periods following inoculation and fruiting.

Research has shown that the availability of nutrients influences shiitake's ability to respond to *Trichoderma* attack (*201, 202*). Shiitake can resist *Trichoderma* when glucose levels are high, but when shiitake must grow on other sugars, such as xylose, *Trichoderma* is able to kill it. High nitrogen levels in the wood also favor *Trichoderma*.

Logs that are heavily colonized by *Trichoderma* should be isolated to lower the spore concentration near healthy logs. Also, logs which have large patches of *Trichoderma* should be segregated and soaked separately. Research in Asia has shown that a benomyl (a selective fungicide) suspension

will prevent *Trichoderma* infestations (*43, 109, 147, 191*). However, many disease fungi can rapidly develop resistance to this chemical.

Hypoxylon

Hypoxylon species are a widespread group of Ascomycetes (*124*) which can be a serious problem during shiitake cultivation (*71, 219*). They are antagonistic to shiitake and can stop its growth. *Hypoxylon* invades logs during the early spring months. Its fruiting bodies start as tiny dark spots, usually in cracks in the bark. During the late summer months, they gradually develop into small, hard, brick-red to black mounds, usually less than 3/8 inch (2–10 mm) in diameter (Fig. 10–2). During later stages of infection, the bark falls off the logs (*71*).

Direct sunlight on the bark of shiitake logs can raise the internal temperature to levels which stress the shiitake. At the same time, these temperatures promote the growth of *Hypoxylon*, which grows best between 77° and 86°F (25°–30°C). Very wet conditions also encourage *Hypoxylon*, which grows very rapidly once it becomes established.

Hypoxylon levels can be decreased by shading the logs from direct sunlight. Sheltering the logs to avoid very wet bark during the spring also limits infection. Severely infected logs should be removed to lower spore concentrations.

In Japan a mycoparasite of *Hypoxylon truncatum* has been identified as *Nectria episphaeria*. This promising bio-control agent attacks the fruiting bodies of *Hypoxylon* and prevents spore discharge (*219*).

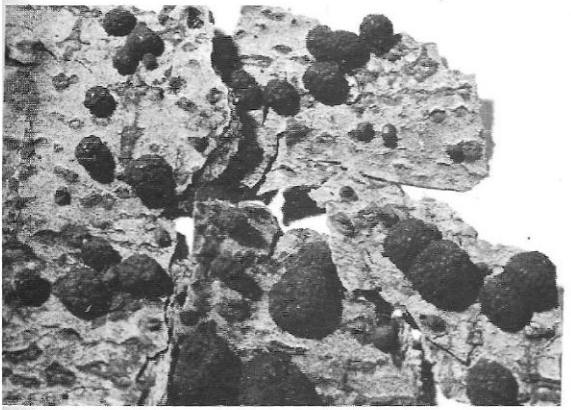


Figure 10–2. *Hypoxylon* fruiting bodies on bark (USA).

Diatrype stigma

Diatrype stigma is another Ascomycete with an associated asexual stage, **Libertella betulina,** which attacks shiitake (*231, 149*). *Diatrype* forms a hard, crusty stroma that initially appears under the bark and eventually pushes the bark off. The stroma ranges from less than one inch (2.5 cm) to three inches (7.6 cm) wide and varies in length from several inches to the entire log. The stroma is light-gray initially, but turns black with age (Fig. 10–3).

Generally, the *Libertella* stage appears first. Fruiting structures at the wood/bark interface produce asexual spores which are extruded through cracks in the bark. If the conditions are wet, the spores form a bright-orange to red sticky ooze on the bark surface. Under dry conditions, the spores form small hook-shaped curls (spore horns), usually less than 1/2 inch (1.3 cm) long. If the bark is removed, additional spore masses are exposed. Stroma of the sexual stage may be visible at the same time or appear later.

The wood is usually dark and discolored, and shiitake spawn holes may be darkened and killed. Dark zone lines appear in the wood along the border of areas where shiitake has become established in the logs (Fig 10–3).

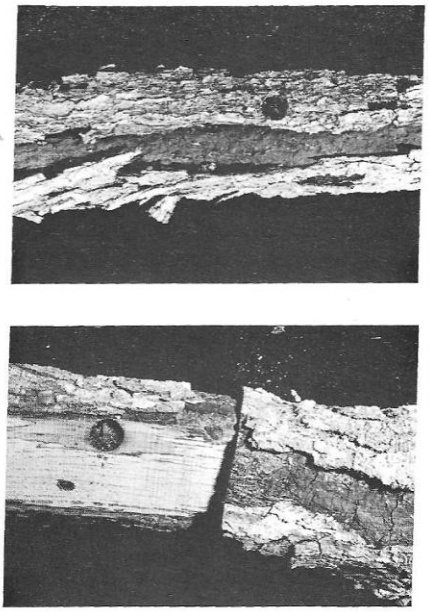


Figure 10–3. *Diatrype stigma* on shiitake logs (USA). Note zone lines in wood surrounding spawn hole in log section, lower left.

Diatrype is a serious competitor and secretes antifungal compounds which inhibit the growth of shiitake (*149*). Severe infections can also result in total loss of the bark.

The occurrence of *Diatrype* varies between tree species. *Diatrype* generally infects the logs while they are in the woods, but it also can be spread in the spawn run area. Sunny, dry conditions, especially during the initial month of the spawn run, favor *Diatrype*. The sexual spores are forcibly ejected, while the sticky asexual spores are transmitted by insects, water or handling.

Diatrype stigma can be controlled by maintaining sufficient log moisture content, especially prior to inoculation. Logs with sig-

nificant amounts of the *Libertella* stage should be segregated during soaking because these spores are dispersed through the water.

Other Disease Fungi

Cephalosporium species and **Phialophora lignicola** are other Ascomycetes which have been identified as antagonists of shiitake during the spawn run (*89*). These fungi have been isolated from areas in shiitake logs where the shiitake had disappeared, but which did not show signs of other disease fungi. When these fungi attack logs, the wood remains hard and moist, similar to fresh logs. In laboratory culture, however, these fungi arrested the growth of shiitake and caused disorganization of the shiitake cytoplasm (*89*).

Slime molds in the genus *Stemonitis* are mobile fungus-like organisms that can spread rapidly over the surface of old shiitake logs (*110, 112*). They appear as dark-brown to black areas consisting of a forest of thin-stalked fruiting bodies up to 1/2 inch (1 cm) in height. Unlike fungi, slime molds engulf their food prior to digestion. Slime molds feed on mycelium, spores and bacteria but their grazing is limited to the log surface. They also consume developing primordia, thereby lowering yields.

Competitor Fungi

In contrast to serious disease fungi, most competing fungi are Basidiomycetes. These fungi do not parasitize shiitake hyphae, but merely occupy space in the logs. This reduces the amount of wood that can be used to produce mushrooms. If these competitors infect the log before the shiitake is established, they can rapidly grow through the log, thus limiting the shiitake to small areas around the spawn holes.

Most of these fungi are members of the order Aphyllophorales, commonly called **polypores** or **bracket fungi** (*155, 184*). These fungi produce leathery fruiting bodies, the underside of which is covered with pores. Under normal cultivation conditions, they often colonize the ends of shiitake logs, where they are not a cause for concern. Their fruiting bodies do not appear until near the end of the spawn run.

Coriolus (Polyporous) versicolor and **Stereum** species are the most common bracket fungi found on shiitake logs. Both have thin, leathery fruiting bodies with pores on the underside. They usually start as solid lumps tightly adhering to the log surface, then extend from the log with age, forming a series of shelf-like structures.

Coriolus, sometimes called "the rainbow fungus," has alternating light and dark stripes on the top of a gray to brown fruiting body (Fig. 10–4). On the pure white underside of the fruiting body, small pores are clearly visible, covering the entire surface. Fruiting bodies of *C. versicolor* may reach several inches in size and extend out from the log.

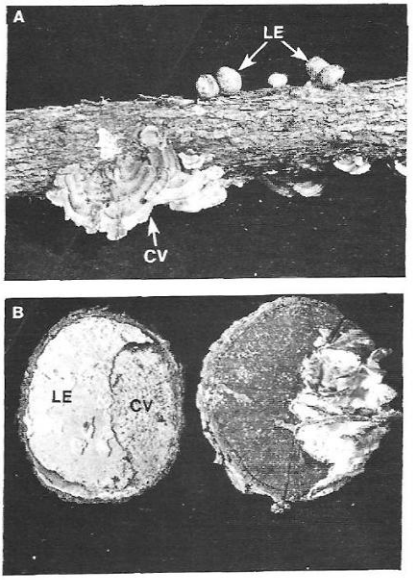


Figure 10–4. *Coriolus versicolor* on shiitake logs (USA). A. *C. versicolor* (CV) and shiitake (LE), B. Cross section showing areas colonized by both fungi.

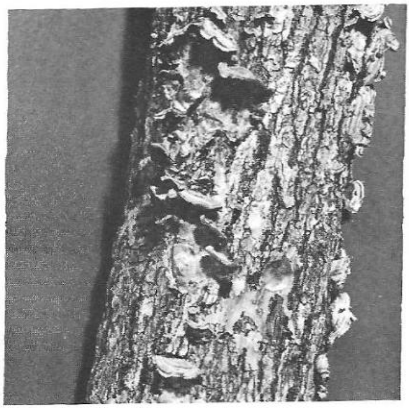


Figure 10–5. *Stereum hirsutum* on shiitake log (USA).

Stereum species appear similar to *Coriolus* except that both the top and bottom surfaces are colored. Colors range from brown, olive and buff, to lilac, to red and orange (*142*). The pores on the underside are very small, almost invisible to the naked eye. Generally, *Stereum* fruiting bodies remain more appressed to the log surface than those of *Coriolus*, and form a small series of shelves (Fig. 10–5). The thin fruiting bodies, which may be cup-shaped, remain attached at the center, with only their margins raised off the log surface.

Schizophyllum commune is a common fungus with small, white, fan-shaped fruiting bodies, usually less than two inches (5 cm) across, which are covered with small hairs or fibers. The underside of the fruiting body has gills which radiate from the attachment point. These gills are characteristically forked near the margin of the fruiting body. *S. commune* is commonly found on very dry spots of second-year shiitake logs.

Lenzites betulina is another common competitor. It forms larger (up to 4 inches), corky-fleshed fruiting bodies with wide pore-like gills on the underside. Generally, the fruiting bodies are white to yel-

lowish-brown. This aggressive, fast-growing fungus is spread rapidly by log-to-log contact. Removing the fruiting bodies will lower the spore concentration, but isolating the infected logs is the most effective control.

Other bracket fungi (Aphyllophorales) which are competitors include members of the genera *Poria*, *Phlebia*, *Phellinus*, and *Polystictus* (*143*).

The bracket fungi have a competitive advantage over shiitake if the logs are too dry during the spawn run and they can quickly grow through the logs. Dry wood, direct sunlight on the logs, and high humidity create the ideal environment for these fungi. Short, frequent waterings encourage bracket fungi because they keep the outside of the log damp, while not adequately wetting the log interior. Moist conditions induce bracket fungi to fruit; however, as with *Trichoderma*, the conditions which allowed them to become established in the logs occurred earlier.

Bracket fungi are controlled by maintaining an ideal log moisture content for shiitake throughout the spawn run. If shiitake colonizes the logs rapidly, competing fungi are limited to small areas near the log ends where the wood is drier. The fruiting bodies can be peeled off to reduce the spore concentration around the logs.

Weed Fungi

Weed fungi include both Basidiomycetes and Ascomycetes which may appear on shiitake logs, but are rarely antagonistic and do not adversely affect shiitake mycelium. Although these fungi may slightly decay the wood or bark, the damage is generally minimal. These fungi usually were already on the logs before they were brought in from the woods; they appear briefly, often in great numbers, then disappear. Only a few species are mentioned below, but there are literally hundreds of fungi that may be weeds during cultivation. Weed fungi are often specific to the tree species and the area.

A common Ascomycete weed fungus is **Bulgaria inquinans.** It is a common canker-forming fungus on living oaks, especially black oaks. The fruiting bodies are from 0.5 inch to 1.5 inches (1–4 cm) in height and slightly less in diameter. The fruiting body is cup or funnel-shaped, tapering from the top to a stout base; it is gelatinous when wet (Fig. 10–6). Its color ranges from rusty-brown to black. The fruiting bodies erupt through the bark surface, often in great numbers.

Bulgaria is a weak wood-decay fungus which can also decay bark (*13, 120*). It can kill the cambium in living oaks, causing a basal canker which may girdle and kill the tree (*119, 120*). This fungus appears during the spawn run, but usually disappears later and is not a problem for shiitake cultivation.

A number of small thin-stemmed Basidiomycete mushrooms belonging to the genus **Mycena** may briefly appear on shiitake logs. The mushrooms are very small, usually with caps less than a half inch (1.3 cm) across, and have brittle, thin flesh.

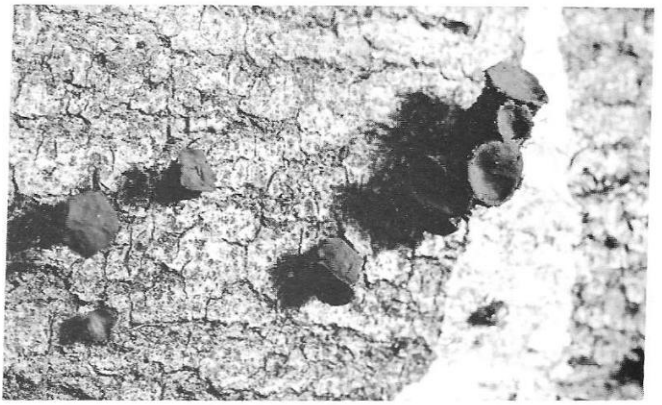


Figure 10 – 6. *Bulgaria inquinans* on shiitake log (USA).

Post-Harvest Fungi

These fungi attack mushrooms, either on the logs or during storage. Usually, only over-mature and excessively wet mushrooms are affected, but healthy mushrooms may be attacked by contact with infected ones. Post-harvest fungi can be controlled by maintaining proper conditions during fruiting and storage and by culling affected mushrooms.

Gliocladium deliquescens forms green colonies with slimy conidia, similar to some species of *Trichoderma* (*95*). This fungus is a problem only at relative humidities above 95%. The affected mushrooms fail to open fully, then turn brown and become soft and shriveled. The conidia appear on the mushroom only during the latter stages.

A number of fungi can decay over-mature mushrooms on the logs or harvested mushrooms in storage (*95*). **Didymocladium ternatum** forms loose, white, cottony mycelium and white conidia on the mushroom surface. **Cephalosporium mycophilum** colonies appear yellow-green to brownish-green, due to the color of the mucilaginous spore mass. **Choanephora cucurbitara** can cause a soft-rot of shiitake, usually under warm moist conditions (*135*).

Bacteria

Bacteria are seldom a problem in shiitake cultivation on logs. However, browning disease of mushrooms, which is caused by the bacterium **Pseudomonas fluorescens,** may be severe under warm conditions (*93*). Affected mushrooms turn brown from the base upwards. The bacteria may cause the fruiting bodies to develop abnormal shapes or abort (*136*). Researchers in

Japan have found that spraying the logs with s-octylisothiouronium chloride provides effective control (*191*).

A number of different bacteria cause a soft-rot of mushrooms during storage. High relative humidity, warm temperature, wet mushrooms and poor ventilation favor these bacteria. They can rapidly turn shiitake into an unappealing slime.

Rickettsia are microorganisms similar to bacteria. Severely misshapen mushrooms with incomplete caps have been found to be infested with small bacteria-like organisms similar to rickettsia (*137*). However, there was no direct evidence that these organisms caused the distortions.

Viruses

Viruses are sub-microscopic particles that are visible only through an electron microscope. In the common button mushroom, viruses are known to cause diseases such as "die-back" and "La France-disease" (*227*). Devastating outbreaks of these diseases have been due, in part, to the fact that mushroom spores carry the virus.

Viruses have been found in shiitake mycelium and spores (*224, 226, 245*). Currently, no definite link has been established between the presence of the virus and disease symptoms. Viruses have been isolated from both healthy and deformed mushrooms (*224*). Other research, however, suggests that abnormal, slow mycelial growth was due to the presence of a virus (*245*).

Insects and Other Pests

Mobile pests may cause problems during shiitake cultivation by affecting either the logs or the mushrooms. In addition to direct damage, some pests indirectly decrease production by acting as vectors for disease organisms.

A wide variety of pests attack shiitake, but relatively few cause large losses. It is rarely—if ever—necessary to use chemical sprays. Most pests can be controlled through cultural means.

Insects

Insects may cause problems by attacking either the logs or the mushrooms. A brief description of some insect pests follows. For identification of suspected pests, an insect field guide should be consulted (*15*).

Termites are the most serious insect pests of shiitake logs (*114*). Termites eat wood and can consume shiitake logs. They can be controlled by chemical treatment or by keeping the logs off the ground.

Some beetles bore into shiitake logs during the initial stages of cultivation. **Bark beetles** (family Scolytidae) are small hard-bodied beetles whose

larvae feed on the cambium layer. These beetles leave small pin-holes in the bark, and the tunnels of their larvae are apparent underneath the bark. Heavy bark beetle infestations can make the bark fall off.

Ambrosia beetles (family Scolytidae) bore into the logs. They leave small holes in the bark, often with a small pile of sawdust beneath it. These beetles are attracted to freshly cut logs and may appear during the early part of the spawn run.

Fungus beetles (Erotylidae, Endomychidae) feed on mushrooms, both as larvae and as adults. They are most prevalent during cool weather when the mushrooms grow slowly and remain on the logs for longer periods. Although isolated mushrooms may suffer considerable damage, these beetles are seldom a problem. Mushrooms should be examined during harvesting, and the adult beetles should be removed.

Both **mushroom flies** (family Phoridae) and **fungus gnats** (families Mycetophilidae and Sciaridae) damage mushrooms. The adults lay eggs on the mushrooms. When the larvae hatch, they damage the mushroom by tunneling through the cap and stem (Fig. 10–7). Other spoilage organisms may enter through these tunnels. Flies may cause problems during cool seasons when large numbers of wild mushrooms are present and fly populations are high. Because shiitake grows slowly during this period, the flies have enough time to develop.

Chemicals are rarely needed to control flies on shiitake logs. Daily picking and sanitation measures which eliminate mushroom debris around the fruiting area are usually sufficient. In the button mushroom industry, however, synthetic pyrethrin dusts and sprays are used for fly control.

Springtails are tiny, dark-colored insects which appear on the mushroom gills during the spring. They get their name from the springing motion they make when disturbed. In large numbers, they can significantly damage

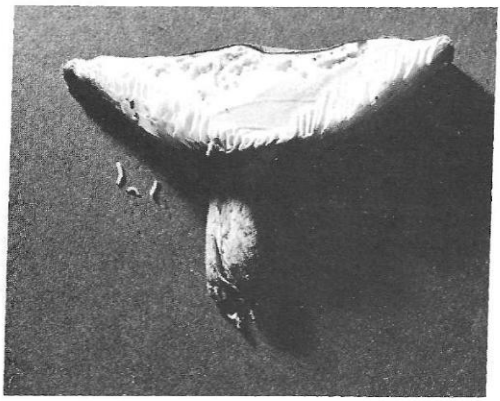


Figure 10–7. Cross section of shiitake fruit-body showing fly larvae and their damage (USA).

the cap and stem, leaving circular depressions in the mushroom tissue (*220*). In severe cases, the entire cap appears "pebbled."

These primitive insects hatch in the fall and mature in the spring. The juveniles are incapable of feeding on fruit bodies, but do eat spores. When their diet includes the spores of disease and competitor fungi such as *Hypoxylon*, *C. versicolor*, *Lenzites betulina*, *Schizophyllum* and *Stereum* (*220*), springtails may spread diseases. When large numbers of adults appear on the gills in the spring, they can be a problem. Controlling competitor fungi will reduce the available food source for these pests.

Sowbugs, Mites, Slugs and Snails

Sowbugs are primitive arthropods, more closely related to crabs and shrimp than to insects. Sowbugs are usually less than 0.5 inch (1.3 cm) long, grey, and many-segmented with numerous legs. They roll into a ball when disturbed. They feed on mushrooms and can cause serious damage to primordia. Control of sowbugs is difficult because they hide under stones or logs and reproduce quickly.

A number of **mites** feed on fungal mycelium and spores. These minute, eight-legged creatures are usually visible only with the aid of a magnifying glass. Generally, mites are not a problem, but large populations may build up during warm weather. Primordia that has been heavily grazed by mites will develop into misshapen mushrooms. Mites can also carry diseases.

Slugs and **snails** are among the most serious pests of shiitake cultivated on logs. These small mollusks often cause serious damage by feeding on the developing primordia or on mushrooms (*Fig. 10–8*). They are a problem primarily in moist climates. The smaller slugs can build up to high levels

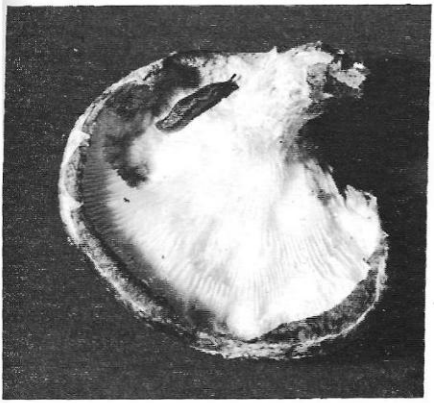


Figure 10–8. Slug damage to shiitake fruit-body (USA).

before they are noticed. The damage they cause is two-fold: degrading the mushrooms directly and surprising the consumer.

Slugs and snails can be controlled by spreading gravel under the fruiting logs, keeping vegetation low so the ground stays dry and using a slug and snail bait. Ducks and geese will effectively reduce the slug population near the logs – but their use is restricted to periods when the mushrooms are not fruiting.

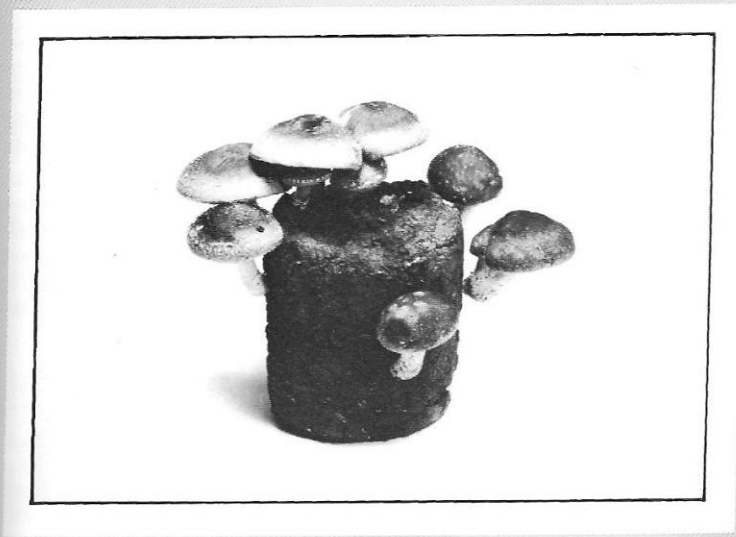
Birds and Mammals

Birds can be a problem in natural spawn run areas where they may peck through the bark and spawn holes, looking for insects. Large birds, such as woodpeckers and flickers, can de-bark a large number of logs in a short time.

Some mammals can also be pests. **Deer**, **squirrels** and **mice** will eat shiitake fruiting bodies. Damage caused by deer can be serious in some areas. Mice and squirrels will nest in bulk-piled logs, removing and shredding the bark to build their nests.

Firewood cutters can present a serious problem in some areas. Stacks of shiitake logs may be seen as easily available firewood. These pests usually cause total loss of the logs.

Section III
Shiitake Cultivation on Sawdust



Cultivation on heat-treated particulate substrates is a relatively new method of shiitake production. Many terms are used to refer to this method: cultivation on bulk substrate or substrate cultivation, cultivation on lignocellulosic materials and growing in space bags. Sawdust is the primary ingredient most commonly used in particulate substrates. In this section, "shiitake cultivation on sawdust" and "sawdust cultivation" will be used as inclusive terms, referring to all formulations of particulate materials.

In many ways, shiitake cultivation on sawdust is like cultivation on logs. The response of shiitake to the environment is identical. The fungus goes through the same stages of growth: inoculation, incubation, induction, pinning, fruiting and resting. As with log cultivation, the grower can control temperature and humidity to provide optimum conditions for shiitake growth during each stage.

In other ways, however, sawdust cultivation is different than logs, due to its particulate nature. This results in higher yields (biological efficiency) in a shorter period of time (6 to 9 months).

This section describes methods for shiitake cultivation on sawdust and other particulate substrates: substrate formulation and preparation, strain selection, inoculation techniques and incubation conditions, necessary facilities and management during fruiting. Controlling diseases and pests during inoculation, incubation and fruiting are discussed in the appropriate chapters.

11

Shiitake Substrate Preparation

Shiitake can be grown on a wide variety of particulate materials composed of cellulose and lignin. Because these materials have been chopped up, their nutrients are more readily available to a wide range of organisms. Consequently, microorganisms – including competitors – rapidly invade these materials. Therefore, the substrate must be sterilized or pasteurized prior to inoculation to kill these microorganisms, creating a selective substrate. It then must be protected so that it is not reinvaded.

This chapter examines various ingredients used to formulate a substrate, specifies the components of some common mixes, and discusses the mixing and bagging of particulate substrates. Finally, it compares two forms of heat treatment: sterilization and pasteurization.

Substrate Ingredients

Unlike logs, the mix of components in particulate substrates can be adjusted by the grower to increase the nutrients available for shiitake growth. Higher nutrient levels speed mycelial growth and increase yields. Generally, sawdust is the main ingredient in substrates used for shiitake production.

During shiitake cultivation on logs, the rate of decay and the yield of mushrooms vary with nutritional and physical characteristics of the wood. These same characteristics also influence the growth of shiitake during sawdust cultivation.

Wood is primarily composed of cellulose and lignin, with lesser amounts of hemicelluloses, pectins and simple sugars. The nitrogen levels in wood are low (between 0.03% and 0.3%) and may be a limiting factor for decay (*123*). Cellulose and lignin occur in a wide variety of other materials, in combination with differing amounts of other nutrients. When cultivating shiitake on sawdust, the choice of substrate ingredients influences the growth rate and yield.

Sawdust

Generally, the preferred type of sawdust is from broad-leaved trees (hardwoods). Hardwood sawdust is the primary component of most mixes used to grow shiitake, usually making up 60% to 90% of the total weight of the dry substrate (Fig. 11–1). Sawdust from oak or maple is commonly used, although sawdust from beech, alder, birch or other less familiar species is also used (*52, 60, 121, 125, 141, 168*).

Figure 11–1. Mixing hardwood sawdust with supplements for shiitake cultivation (Taiwan).

Sawdust from needle-leaved trees (softwoods) is used in areas where hardwood sawdust is scarce. Shiitake can be grown on a substrate composed entirely of supplemented softwood sawdust (*60, 75*). More commonly, however, softwood sawdust is blended with hardwood sawdust (*7*).

Many softwoods contain resins and phenolic compounds which inhibit fungal growth. These compounds must be degraded or removed before the sawdust is used for shiitake cultivation. Softwood sawdust can be chemically altered with sodium carbonate to remove a portion of these compounds (*60*). Aging softwood sawdust outdoors for one or more years results in partial degradation of these compounds. Aging is an aerobic biological process, so sawdust piles must be small to allow thorough gas exchange.

In addition to reducing inhibitory compounds, the degradation which occurs during aging increases the accessibility of complex wood components such as cellulose. The levels of some nutrients, such as nitrogen, may even be increased. In addition to concentrating the nitrogen already in the wood, some microbial populations can also convert atmospheric nitrogen into cell material (*28*). This nitrogen becomes available to the shiitake fungus after the microbial cells are killed.

The time required for shiitake to degrade the substrate is related to particle size. Large particles are degraded more slowly than smaller ones because of their lower surface area to volume ratio. Small particles are easily degraded; however, if the particle size is too small, gas movement in the substrate is restricted. Particles about 1/8 inch (2–3 mm) in size are colonized fastest by shiitake. Mycelial growth in particles less than 1/16 of an inch (1.5 mm) is significantly slower (*141*).

Shiitake has been cultivated on a number of other materials either alone or in combination with sawdust. These include straw, corn cobs and other agricultural wastes such as sugar cane bagasse, citrus-peel wastes and grain chaff (*60, 70, 78, 102, 244*).

Supplements

Supplements are added to the substrate to speed growth and increase mushroom yields. They contribute additional nutrients or alter the chemical or physical conditions of the mixture.

Nutritional supplements are added to increase levels of nitrogen and useable carbohydrates. Adding nitrogen will increase yields; however, above a certain level it inhibits fruiting (*105, 246*). Nitrogen levels of 0.5% have been reported to give maximum shiitake yields (*70*). Sugars and starches provide easily available carbohydrates. These speed colonization and degradation of the medium, reducing the time until fruiting. Because the mycelium readily converts these carbohydrates into reserves for fruiting, they also increase yields. Fats provide a concentrated source of energy which is less available to competing organisms. Fats are degraded slowly, providing a "time-release" source of carbohydrates.

Higher supplementation of nutrients results in higher shiitake yields, but also encourages the growth of disease and competitor fungi. The amount of supplementation that can be used successfully depends on the cleanliness of the shiitake farm. Farms with very low spore concentrations in the fruiting rooms can safely use more supplements than farms with high spore levels.

Grains or similar materials are commonly used as nutritional supplements. They contain a mixture of protein, carbohydrates and fats. Proteins are the nitrogen source in these materials. The carbohydrate content in Table 11–1 was estimated by subtracting the crude protein (nitrogen content X 6.25),

Table 11–1
Analyses of Common Materials Used as Supplements

			Percent of Wet Weight			
Material	**Total Dry Matter**	**Acid Deter. Fiber**	**Total Minerals**	**Fats**	**Carbo-hydrates**	**Nitrogen**
Corn	89.0	3.0	1.3	3.8	71.3	1.5
Millet	90.0	15.0	2.6	3.5	57.3	1.9
Oats	89.0	14.0	3.1	4.8	55.3	1.9
Wheat	89.0	7.0	1.7	1.8	64.3	2.3
Wheat bran	89.0	14.0	6.1	3.9	49.8	2.4
Rice bran	91.0	16.0	11.6	13.7	37.0	2.0
Rape seed meal*	94.0	15.5	6.8	7.0	29.5	5.6
Soybeans	*92.0*	*9.0*	*5.1*	*17.2*	*21.5*	*6.3*
extract meal *	90.0	3.4	5.8	0.9	30.2	7.9
Yeast, brewers *	93.0	3.7	6.6	0.8	38.9	7.0

* Figures for acid detergent fiber were not available for these supplements. Crude fiber is given instead.
Reference: US-Canadian Tables of Feed Composition. 1982.

fat, minerals and fiber from the dry matter. The acid detergent fiber estimates the amount of lignocellulose.

In addition to the nutrients discussed above, nutritional supplements contain minerals and vitamins which also influence mushroom growth. For example, calcium and tin have been reported to stimulate fruiting of shiitake (*106*).

Other supplements may be added to change the acidity (pH) or to improve the physical structure of the medium. The most common buffer is lime or calcium carbonate. It is used to keep the pH of the medium above pH 4 during the latter stages of decay when it would otherwise become increasingly acid. Gypsum is widely used in the button mushroom industry to improve the physical structure of the compost substrate and alter the pH. It can also act as a calcium source.

Peat moss can be used to increase the acidity of the substrate (*158*). The growth of many competing organisms, especially bacteria, is inhibited at pH values below 5.

Substrate Formulations

The substrate formulas used in different areas vary depending on what materials are readily available. A widely used, "standard" formula is 80% hardwood sawdust and 20% supplements on a dry weight basis (*125, 168*).

This standard formula, composed of 80% sawdust, 20% bran (roughly 4:1 on a volume basis), is widely used in Asia (*60*). It is used for the cultivation of a number of different wood-inhabiting edible fungi, including enoki mushrooms (*Flammulina velutipes*), oyster mushrooms (*Pleurotus* spp.) and nameko (*Pholiota nameko*).

The standard formula has many variants. In the U.S., 80% sawdust, 10% bran, 10% grain (usually wheat or millet) is common (*125, 168*). In Taiwan, a medium containing 84% sawdust, 5% rice bran, 5% wheat bran, 3% soybean meal, and 3% lime is used. Swiss researchers have reported success with a medium containing 75% spruce sawdust, 24% wheat bran and 1% lime (*75*).

Some growers use formulas with lower supplementation levels, sacrificing higher potential yields for lower contamination levels. Two examples of such formulas follow: 90% sawdust, 10% rice bran and 0.2% lime (*52*); and 95% sawdust, 5% rice bran and 0.4% corn starch (*218*).

Mixing

Usually, the ingredients are mixed thoroughly while dry; then water is added to bring the moisture content to the proper level. A variety of mixers are used; the most common are cement mixers, auger mixers and ribbon-type mixers. Cement mixers are large drums with fixed blades inside the drum. During mixing, the entire drum is rotated. Auger mixers consist of a bin with two augers which run in opposite directions. Ribbon mixers have a fixed container with mixing blades attached to a central rotating shaft (Fig. 11–2).

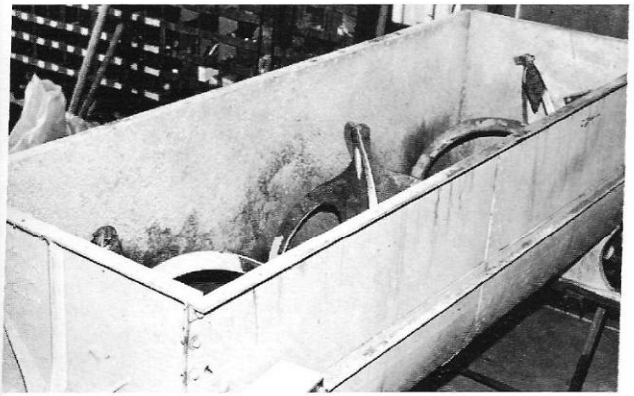


Figure 11–2. Ribbon mixer for blending substrate ingredients (USA).

Some ribbon mixers also have an auger for mixing and unloading the substrate.

Substrate Moisture Content

Proper substrate moisture content is important for good growth. The optimum moisture content is between 55% and 70% before heat treatment (*52, 125, 168*). The desirable level is determined by the substrate formula, the particle size and the amount of water that will be lost during incubation.

Moisture content can be precisely measured using moisture content determination techniques as outlined in Chapter 3.

Many growers estimate the proper moisture content by squeezing the mixture in one hand: Drops of free water should begin to appear while squeezing, and the mix should stay in a clump when released.

Another method is to use a hand-held garlic press, such as is commonly used in cooking. The press is filled with substrate and then firmly squeezed. If the substrate is at the proper moisture content, a drop or two of water will appear.

Bagging

Containers

During shiitake cultivation on logs, the wood is protected from drying and invasion by competing microorganisms by its structure and by the bark. Sawdust substrates lack this protection, therefore the grower must provide it. Generally, the sawdust mix is enclosed in a bag or other heat-resistant container prior to heat treatment.

The ideal container must be resistant to breakage, stand up to heating, be of a suitable shape and have a provision for gas exchange. Several factors influence the container size and shape: the surface area to volume ratio, the length of time needed for heat penetration, the potential for aeration and the handling considerations.

Plastic bags are the most commonly used containers for shiitake cultivation on sawdust, although a number of specialized bottles and other containers have also been developed (*44, 45, 46, 75*).

There is no ideal shape for bags used to hold sawdust substrate. However, most growers use cylindrical bags with volumes ranging from one quart to two gallons (about 1–6 liters). Plastic bags are available in a variety of different materials which differ in transparency, gas permeability, and ability to withstand heating. The maximum temperature reached during heat treatment determines which plastics can be used.

Bag Materials

Polyethylene (PE), one of the most common types of plastic, is of two types: low density and high density. **Low density PE** is a lightweight clear material commonly used for many types of bags. It does not withstand temperatures over 176°F (80°C), but it is the most permeable to gases and water vapor. Shiitake can be grown in sealed, low density PE bags because they allow enough gas exchange through the plastic for mycelial growth (*158*).

High density PE is usually cloudy, translucent and tough. Some high density PE can withstand autoclaving at temperatures up to 250°F (121°C). Because the gas permeability of this material is less than that of low density PE, the bags need a closure which permits gas exchange. Many of the autoclavable biohazard bags used to dispose of laboratory wastes are made of high density PE.

Polypropylene (PP). The most common heat-resistant bags are made of polypropylene (PP), which withstands temperatures of 275°F (135°C). PP ranges from totally clear to slightly cloudy in appearance. Because very little gas moves through PP, some type of breathable closure is needed.

Figure 11–3. Bag closures for shiitake cultivation on sawdust.

Provision for Air Exchange

A small amount of air exchange is needed during the spawn run. This can be accomplished in several ways. Some PP bags are manufactured with a small patch of breathable plastic that lets air through, but excludes contaminants. These bags can be heat-sealed after inoculation (Fig. 5–3, 5–4).

The most common method of allowing air exchange is to close the bags with a ring and plug. After filling, the neck of the bag is pulled through a heat-resistant plastic ring and folded back to create an opening. This opening is then plugged with a cotton or foam plug, which allows air exchange. One variation uses a tie instead of a ring to hold the plug (Fig. 11–3). Another variation uses a ring that accepts a special breathable cap instead of a plug.

Filling

Bags can be filled manually or by machine. Bagging machines especially designed for mushroom cultivation are available in Asia (Fig. 11–4). These machines fill the bag and then compress the sawdust. Often, other bagging machines can be adapted for this purpose.

Compressing the substrate mix increases its density and results in higher yields per bag. Often a hole is pressed down through the middle of the substrate in the bag. This hole is filled with spawn during inoculation, ensuring distribution of the spawn vertically in the bag. This speeds colonization and, because even-aged mycelia reach the outer parts of the substrate, produces more uniform flushes.

Heat Treatments

After the sawdust mix has been prepared, it is heat-treated to eliminate or reduce populations of competing organisms that are present in the sub-

Figure 11–4. Bag-filling machine for mushroom cultivation (Taiwan).

strate. This creates an environment where shiitake can grow without competition for nutrients. Heat treatments require that a specific minimum temperature be reached throughout the substrate.

The rate at which the substrate heats up depends on a number of factors. Heat moves through wet sawdust faster than through air. Thus, the amount of air in the sawdust mix influences the amount of time required for heating. A compressed mix has less air space than a loosely packed mix and, consequently, requires less time to reach a given temperature. The initial temperature of the substrate also affects the amount of time; colder substrates take longer to heat.

The amount of heat being applied to the substrate is another major factor determining heating time. Steam is the most common heat source. The amount of heat contained in a volume of steam increases as the pressure of the steam increases. Consequently, a cubic foot of steam at 15 pounds (6.8 kg) of pressure contains more heat (250°F, 121°C) than steam under no pressure (212°F, 100°C).

The total mass or volume of substrate to be heated is another major factor determining the amount of time required to reach a desired temperature. Substrate in a container that is tightly packed with substrate will require more time to reach the desired temperature than the same container with half as much substrate.

Dry heat may be used instead of steam, but the rate of heating is slower. If temperatures are raised to speed heating, the higher temperature can cause oxidation and other chemical changes in the substrate (*172*). For these reasons, dry heat is seldom used as a heat treatment for sawdust substrates.

Sterilization

Sterilization is the removal or destruction of all living organisms. It can be achieved using heat treatments, radiation, filtration, and gas or chemical treatments (*172*). Sawdust substrates for shiitake cultivation are commonly sterilized with steam, often under pressure.

The time required for sterilization is a function of temperature: the higher the temperature, the shorter the time needed for sterilization. The minimum sterilization temperature is the boiling point of water (212°F, 100°C).

For example, highly heat-resistant bacterial spores which are killed after five minutes at 250°F (121°C) require 60 minutes at 212°F (100°C). Some thermophilic bacteria can survive 5 hours at 212°F (100°C), but are killed after 25 minutes at 250°F (121°C). (*172*).

The total time required for sterilization equals the time required for heat penetration to the center of the substrate plus the length of exposure at the temperature needed to kill all the organisms. Therefore, although thirty minutes at 250°F (121°C) would ensure sterilization, the total cooking time could be two to five hours.

Sterilization under pressure (15 lb, 250°F, 121°C) is done in autoclaves or retorts, which are large, steam-heated pressure cookers designed to withstand high pressures (Fig. 11–5). A high-pressure boiler supplies the steam. The initial cost of such equipment is high; however, complete sterilization can be achieved in two to five hours at these temperatures. This method is commonly used in the U.S. for sterilizing sawdust substrates for shiitake production.

Low temperature sterilization occurs in unpressurized vessels heated with steam to 212°F, (100°C) (Fig. 11–6). Low-pressure boilers can provide

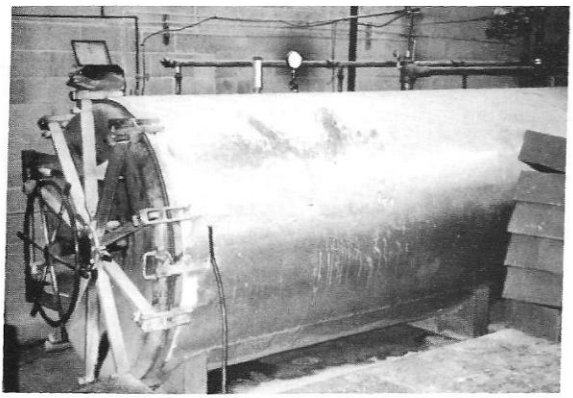


Figure 11– 5. High-pressure retort for sterilization of sawdust substrate (USA).

Figure 11– 6. Unpressurized vessel used for low-temperature sterilization of sawdust substrate (Taiwan).

the necessary steam; however, high temperature steam from a high-pressure boiler will heat the substrate faster. Although low-pressure equipment is cheaper than that used for sterilization under pressure, sterilization may not always be achieved. In addition, because the cooking time is longer (6 to 12 hrs) energy may cost more per cook. This method is widely used in Asia.

Generally, the substrate is bagged first, then autoclaved. Alternatively, a large amount of substrate can be sterilized, cooled, then inoculated and bagged under sterile conditions (*168*). This greatly decreases handling, but requires specialized facilities.

Pasteurization

Pasteurization selectively kills a portion of the microorganism population, but may not affect thermo-tolerant organisms. Pasteurization occurs at temperatures from 140°F to 176°F (60°–80°C). An advantage is that these temperatures allow readily available, less heat-resistant containers to be used.

Although pasteurization is widely used when cultivating other mushrooms, such as button mushrooms and oyster mushrooms, it is seldom used for shiitake cultivation. Experiments have shown that lower yields were obtained with pasteurized substrate (heated to 176°F, 80°C for 30 minutes), than with comparable substrate that was sterilized (*171*).

This difference has two possible explanations. Yields may have been lower because the microorganisms that remained after pasteurization competed with shiitake for available nutrients. Another possibility is that the

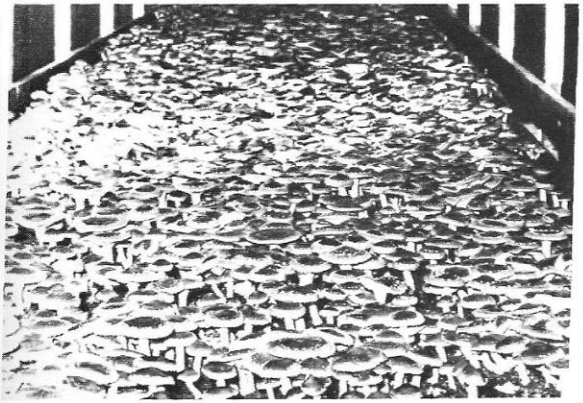


Figure 11–7. Shiitake fruiting on pasteurized sawdust substrate in standard button mushroom growing room (Netherlands).

higher heat of sterilization broke down wood components and released additional simple sugars to be utilized by shiitake.

Another method of shiitake cultivation uses equipment and techniques similar to those used for growing button mushrooms. Beds (4.5 ft wide by 50 ft long, 1.4 m by 16 m) are filled with a four to six inch layer of sawdust-based substrate. The entire growing room is then heated with steam to pasteurize the substrate in the beds and make it selective for shiitake. The spawn run and fruiting stages take place in these same beds. Environmental conditions are precisely controlled throughout cultivation, resulting in a crop of shiitake only four weeks after inoculation (Fig. 11–7) (*228*).

12

Inoculation and Incubation of Sawdust

Inoculation is the process of introducing actively growing shiitake mycelium into a bag or container of sawdust medium. Once the substrate is inoculated, it is placed in a warm environment for incubation to speed mycelial growth.

The substrate's selectivity for shiitake must be maintained during inoculation and incubation by excluding all other microorganisms. If fast-growing contaminants are introduced during inoculation or before shiitake has completely colonized the substrate, the crop may be a total failure. Fortunately, appropriate techniques can minimize contamination.

This chapter examines the characteristics of shiitake strains and types of spawn used for sawdust cultivation. Methods of avoiding contamination during cooling and inoculation are discussed and the inoculation process is described. Incubation conditions and facilities are outlined, followed by a discussion of common contaminants and their causes. Finally, indications that the colonized substrate is ready for mushroom production are summarized.

Strain and Spawn Selection

Strain Characteristics

Not all shiitake strains are suitable for cultivation on sawdust. Each strain possesses specific traits: shape, color, disease resistance, length of the spawn run, and optimum temperature for spawn run and fruiting. However, the expression of these traits is influenced by growing conditions and the substrate formula. Therefore, a successful grower must match the proper strain(s) with the best substrate formula for the specific environmental conditions at the farm.

Temperature range. During their vegetative stage (spawn run), most shiitake strains grow best at about 77°F (25°C). Many farms maintain the spawn run area at this temperature. However, to decrease costs, some growers do not actively regulate incubation temperatures. Although some strains can tolerate a range of temperatures and still grow well, even these strains achieve their maximum growth rate at 77°F (25°C) (*127*).

Different strains of shiitake will fruit on sawdust between 50° and 77°F (10°–25°C). The optimum fruiting temperatures of strains differ; some strains

fruit at warm temperatures, while others prefer cold temperatures. Most growers maintain fruiting temperatures at 55° to 68°F (13°–20°C). However, some growers do not attempt to control fruiting temperatures, in which case they need strains that can fruit under fluctuating or extreme temperatures.

Spawn run time. The length of incubation prior to fruiting is determined by the substrate formula and the strain. Incubation has two phases. During the initial colonization phase, the mycelium grows through the substrate. Following colonization, the mycelium accumulates the resources needed for fruiting. The duration of the spawn run is the sum of these two phases.

Even if a strain is a fast colonizer, it may require a long period to accumulate the resources needed for fruiting. Conversely, slow-growing strains may be ready to fruit soon after they have totally colonized the substrate.

Mushroom quality. Mushroom size, shape and flavor intensity are strain-related traits. Strains also differ in the number of aborted and misshapen mushrooms they produce. Although relative humidity in the fruiting room modifies mushroom moisture content, different strains grown under identical conditions can produce mushrooms with different moisture contents (*160*). This difference affects storage life since mushrooms with a high moisture content do not store as long as those with a lower moisture content.

Cropping characteristics. Shiitake strains differ in their responses to environmental conditions during fruiting. Some strains react to soaking and/or temperature changes and produce a flush of mushrooms within a short time. Other strains do not respond as well to this type of induction; they produce mushrooms continuously at a low level.

Potential yield is also strain related (*169*). Although yield measurements are usually given for the entire fruiting period, the yield per unit time should also be considered. Strains that produce most of the total crop in the first few flushes can be cycled through the system faster, thus giving a higher yield per unit area per unit time.

Types of Spawn

Mycelium must survive the inoculation process and recover to colonize the substrate. During inoculation, the physical structure of the spawn is disrupted, and the mycelium on the surface is damaged and "disappears." Rapid recovery and a fast initial growth rate are desirable because these decrease the length of the spawn run. Different types of spawn recover and begin colonization at different rates.

Generally, the amount of spawn used equals from 1% to 5% of the sawdust volume, though higher spawn rates can speed colonization. Three types of spawn are generally used to inoculate sawdust substrates: grain spawn, sawdust spawn, and, occasionally, liquid spawn.

Sawdust spawn consists of shiitake mycelium grown on a sawdust/bran mixture similar to the sawdust substrate (Fig. 5–4). Since the shiitake mycelium in the spawn is already adapted to growth on sawdust, it quickly becomes acclimated to the new substrate. Sawdust spawn is bound together with mycelium, so it must be broken apart before spawning. This makes it harder to handle than other types of spawn. Spawning rate is usually high, up to 5% (v/v).

Grain spawn consists of intact particles of grain (usually wheat, millet or rye) which are colonized by shiitake. (Fig. 12–3) Grain spawn breaks up easily into individual grains which can be evenly distributed through the substrate, thus decreasing the colonization time. Each kernel of grain is full of mycelium, which is protected during inoculation. The spawning rate is generally less than 2% (v/v).

Because grain spawn has a much higher nutrient level than sawdust spawn, it can act as a supplement. The nutrient base of grain is very different from that of sawdust, so the mycelium must undergo a metabolic change as it grows into the sawdust. However, the nutrient reserve in the grain gives the mycelium a rapid start. The high nutrient content of grain spawn also makes it more prone to colonization by contaminants if the heat treatment was insufficient. Under the same conditions, sawdust spawn may not encounter any problems.

Liquid spawn is a slurry of mycelium in a nutrient solution. It can be produced by blending intact mycelium or by culturing in liquid fermentors. Advantages of liquid spawn are the large number of inoculum particles put into the sawdust and the ease of inoculation.

Liquid inoculum is very different from the sawdust medium into which it is injected. The mycelium must recover from inoculation, then turn on the enzymes needed to colonize wood. Inoculation success is improved if the *mycelium has been adapted to wood extracts prior to inoculation (107).*

Fortifying the inoculation solution with sugars increases initial growth, but it also increases the risk of contamination. The extra nutrients may act as a reserve for the mycelium, but—unlike grain spawn—this reserve is not already colonized by shiitake. The purity of cultures used for liquid inoculation must be absolute because a small amount of contamination is spread much further than with conventional methods. However, under proper conditions, many containers can be inoculated rapidly.

Cooling

The temperature of the substrate must be at or below 86°F (30°C) before inoculation. A higher temperature may weaken or kill the shiitake mycelium. Sterilized substrates are most susceptible to contamination before they are totally colonized by shiitake. Contamination can occur during cooling, but it can be minimized through a combination of techniques and facilities.

After heat treatment, the substrate can be left to cool in the autoclave or other vessel used for heating. This keeps the surface of the bags relatively free from spores, but may delay inoculation for a day or two.

The substrate will cool more quickly if the bags are removed from the vessel, but contamination problems will increase. As the substrate cools, the air inside of the bag decreases in volume, drawing in outside air. If the closure does not filter this air completely, contaminant spores may be pulled into the bag. In addition, spores which settle on the surface of the bag can infect the substrate during inoculation.

These problems can be eliminated by using filter-sterilized air to rapidly cool the bags. Sterile air is created by filtering the air through High Efficiency Particulate Air (HEPA) filters. These filters, which consist of pleated sheets of a microporous material, can remove particles as small as 0.3 microns in diameter with an efficiency of 99.99%. This effectively removes all spores and bacteria from the air and creates a sterile air flow.

After the bags are removed from the heat treatment vessel, they are placed into this sterile air stream either in the inoculation room or in a special cooling tunnel. Moving air, whether at room temperature or refrigerated, will rapidly cool the substrate.

Inoculation

During inoculation, spawn containing actively growing shiitake mycelium is introduced into a bag or container of sawdust substrate. The container must be opened, the spawn added, and the container closed or sealed. The substrate is briefly exposed to the air, a potential source of contaminants. Ideally, no other organisms are introduced into the sawdust substrate during inoculation. Precautions against the introduction of contaminants include disinfecting the inoculation area and working in a "clean room" or sterile air. In addition, limiting exposure of the substrate and developing good sterile technique can decrease contamination.

Inoculation Facilities

Many growers inoculate in a special clean room that is sealed to limit air movement. The entire room can be wiped down with a 10% bleach solution or other disinfectant to sterilize the surfaces prior to inoculation. This reduces the number of spores that might otherwise get into the substrate.

A glove box is a smaller version of a clean room. This enclosed box has a glass side for viewing and two holes for the worker's hands to reach into the box. After the inside of the box has been disinfected, closed containers of sawdust and spawn are placed inside. The containers are then opened, and inoculation and sealing take place within the box where the spore concentration is low and there is little air movement (Fig. 12–1).

HEPA filters are often used to create a flow of sterile air in order to prevent contamination during inoculation. Air from the filter(s) is directed onto the workspace from above or behind to create a steady stream of sterile air as wide as the workspace. This flow of sterile air flushes all of the spores out of the work area and prevents them from reaching the exposed media.

Figure 12–1. Glovebox used for inoculation of sawdust substrate (China).

When working in a stream of sterile air, possible sources of spores are always kept downstream from the open containers. HEPA filters are used to create a wide variety of sterile work areas: pressurized glove boxes or rooms, sterile counter tops in laminar flow hoods, and sterile rooms where an entire wall or ceiling is composed of HEPA filters.

Inoculation Techniques

Exposure of the substrate to contaminants can be limited by opening the bag, quickly dumping in the spawn and immediately reclosing the bag. However, rapid movements often create air currents which can carry spores into the bag. In some areas, rapid inoculation and the use of sawdust spawn are the only precautions taken against contamination during spawning. In areas where production costs are low, growers may be able to tolerate a higher contamination rate. Usually, these growers arrange 12 to 16 bags upright in a metal crate or basket prior to sterilization. After cooling, they rapidly inoculate and reclose these bags in the open air (Fig. 12–2).

Sterile technique. The person doing the inoculation is one of the main sources of contamination. Successful inoculation requires the grower to master a series of skills collectively referred to as sterile technique. Sterile technique combines an awareness of potential sources of contamination with movement that minimizes contamination. Good sterile technique is an art. Movements are done smoothly to limit air currents near exposed substrate or media.

The tools needed for inoculation depend on the type of spawn used. The spawn container and inoculation tools can be a source of contamination

Figure 12–2. Open air inoculation of sawdust substrate (Taiwan).

during inoculation. Consequently, they must be flame-sterilized periodically. An alcohol lamp or a Bunsen burner is commonly used for flame-sterilization.

An example of inoculation with grain spawn in a laminar flow hood is presented below. Prior to inoculation, the jars of spawn are shaken to break the mass of colonized grain into individual grains. Then the outside of each spawn jar and all the surfaces in the hood are disinfected with a 70% alcohol or a 10% bleach solution. A metal funnel, which replaces the jar lid during inoculation, is flame-sterilized and allowed to cool in the sterile air flow. After the spawn jar is opened, its lip is flame-sterilized and the funnel is screwed into place.

Figure 12–3. Inoculation of sawdust substrate with grain spawn in laminar flow hood (USA).

Sterilized bags of substrate are placed into the hood and carefully opened, making sure that no contaminantes are blown into them from hands, tools, etc. Between an eighth and a quarter of a cup (30–60 ml) of grain spawn is poured into each bag and then the bag is closed. (Fig. 12–3). The funnel is reflamed before placing it on a new jar of spawn.

Spawn distribution within the substrate influences the length of the spawn run. The shorter the distance the mycelium must grow to completely colonize the substrate, the quicker the spawn run. Although mixing the spawn

evenly throughout the bag ensures the fastest spawn run, shaking is practical only with heat-sealed bags. For ring-and-plug-sealed bags, the most practical method of distributing solid spawn is to fill a central, cylindrical hole in the substrate with spawn. This distributes the spawn vertically in the bag. Bags that are spawned only at the top require a longer spawn run, but require less time to inoculate.

Sawdust substrates that were heat-treated in bulk can be inoculated in bulk as well. Generally, the container that held the spawn during heat treatment has a provision for introducing the spawn. The spawn is then thoroughly mixed with the substrate and the mixture is bagged under sterile conditions and placed into incubation.

Incubation

During incubation (also called the spawn run) the vegetative mycelium grows through the sawdust medium, digesting it and storing reserves for fruiting (Fig. 12–4).

Incubation Environment

The optimum temperature for vegetative growth is 77°F (25°C). Fluctuating temperatures may cause problems if the bag closure is imperfectly sealed, because, as they cool, the containers draw in air which could carry contaminants.

Exposure to light is required before shiitake is able to fruit. The light requirement can be met during the incubation period by providing a light source (*5, 125, 168*). The most effective wavelengths are in the blue range

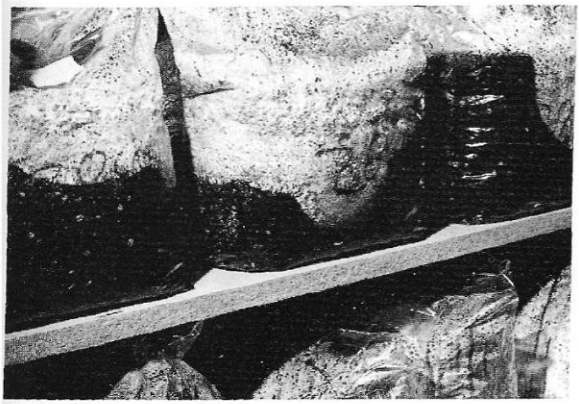


Figure 12–4. Shiitake mycelium colonizing sawdust substrate during incubation (USA).

(370-420 nm) at an intensity of 180 to 500 lux (*46, 52, 102, 125*). Cool white fluorescent lights provide light with the proper wavelengths and intensity.

An alternative method is to incubate the substrate in total darkness, then subject it to a light treatment for 10 to 20 days (*158*). It has been suggested that, initially, mushrooms are formed only from those areas which received light during incubation (*46*).

Oxygen is needed during the spawn run because shiitake is aerobic. Carbon dioxide levels rarely limit vegetative growth, provided there is a small amount of gas exchange.

Incubation Facilities

A range of facilities—from very primitive to highly sophisticated—are used for the spawn run on sawdust substrates. In areas where the climate is favorable, bags can be stacked in rows on the floor and incubated in unheated buildings (Fig. 12–5). In less favorable climates, insulated, temperature-controlled facilities are used.

Incubation rooms for sawdust cultivation are usually equipped with an air circulation system to maintain even temperatures throughout the room. The bags are often packed closely together on shelves to conserve space (Fig. 12–6). This can lead to overheating from metabolic heat if the room does not have sufficient cooling or ventilation.

Humidification is not generally required. Thus, buildings that are not moisture resistant can be used for incubation. Excessive drying of the substrate through the bag closure can be controlled by decreasing air movement.

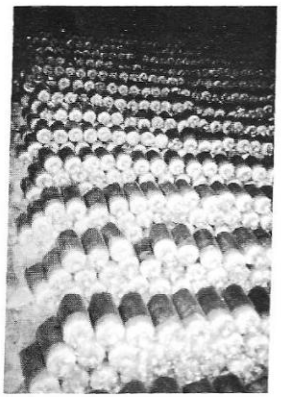


Figure 12–5. Incubation of shiitake on sawdust substrate in unheated building (Taiwan).

Figure 12–6. Incubation of shiitake on sawdust substrate in temperature-controlled building (USA).

Contamination

A wide variety of fungi and bacteria and a few pests can cause problems during incubation on sawdust. These organisms inadvertently get inside the container and quickly grow through the substrate.

Rapid-growing molds, such as *Trichoderma, Mucor* spp., *Penicillium* spp. and *Neurospora* and many others (*185*) can rapidly colonize the substrate. Shiitake will rarely grow into areas occupied by these fungi. A zone line often appears where the shiitake mycelium encounters these fungi (Fig. 12–7). Initially, the colonies often appear white and wispy, as compared to shiitake. They grow rapidly, visibly increasing in size in one day. As conidia are produced, the colonies become colored and appear dusty.

Mature *Trichoderma* colonies vary in color from white to light–green to deep forest–green. Initially, the conidia appear in small, irregularly shaped clusters. *Penicillium* spp. are a pastel green to yellowish green. Sporulation usually progresses outward from the colony center, often appearing very dusty. *Mucor* spp. appears initially as thin white mycelium which rapidly grows over the substrate surface and extends into the air space. As conidia appear, the color changes to grey or black.

Bacteria are harder to spot than mold fungi. Often there is little or no spawn growth in substrate colonized by bacteria. Bacteria multiply rapidly in water drops inside the containers. This fluid may be colored, but is usually clear. Bacterial contamination makes the fluid turn cloudy or murky and become viscous. A sour smell is another sign of bacteria.

Pests. Very few pests of shiitake appear during the spawn run. However, mites, which are very small, translucent and almost invisible to the naked eye, can be a problem under certain conditions. They eat mycelium

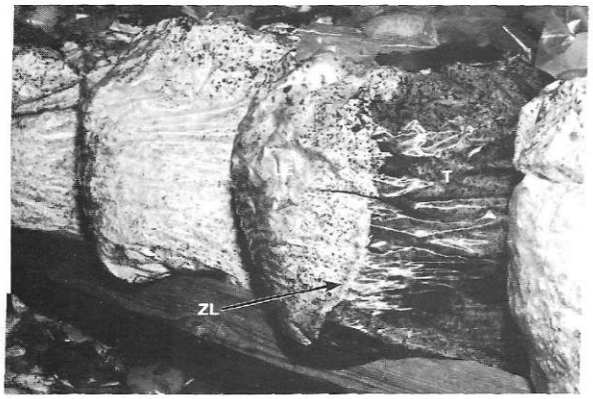


Figure 12–7. Contamination of sawdust substrate by *Trichoderma* during incubation of shiitake (USA). Note zone line (ZL) between shiitake (LE) and *Trichoderma* (T).

and carry mold spores on their bodies, thereby introducing contaminants into the substrate.

Mite infestations may be noticed when contaminants appear on the surface of areas colonized by shiitake. These colonies may take the form of a line or path. When examined closely, the surface of the mycelium often looks honey-combed with small holes. The mites may be visible as slow-moving white specks.

Mites are a problem only when the containers are insufficiently closed. Once mites have become established, however, they are hard to control. Proper closures are the best control, but it may be necessary to apply commercial miticides to all the surfaces in the incubation room for heavy infestations.

Determining causes of contamination. When diseases appear during incubation, the grower must track down the cause of the contamination. Disease organisms can get into the media at several stages. Careful observation of the distribution of the contaminants can reveal clues to how the organism entered the substrate.

Insufficient heat treatment is a common cause of contamination. If the heat was not high enough or did not reach the center of the bags, the surviving contaminants will appear in a large proportion of the bags. Contaminants may grow out from the bag center or be evenly distributed throughout the substrate. Colonies of mold around each particle of grain spawn is often a sign of undercooking. Mold appears around the spawn because the grain is high in available nutrients.

Poor sterile technique during inoculation is another common cause of contamination. Contamination may affect a smaller proportion of the bags, but where it does occur the contaminants are localized near the opening of the container, unless the container was shaken.

Leaks in the bag closures and holes in the bags are other avenues of contamination. Since fungal colonies grow symmetrically, the origin of the colony can often be located. If the container is closely examined near the center of the colony, a hole may be found.

Spawn is another possible source of contamination. Because spawn is high in nutrients, contaminants from other sources often sporulate around the spawn and it may seem that spawn is the source. However, it is necessary to culture some unused spawn to see if contaminants appear. Spawn that is contaminated with bacteria may not recover after inoculation.

Length of Spawn Run

The mycelium usually requires from 30 to 120 days before it is ready to fruit (*52, 125, 158*). The length of this period is influenced by the shiitake strain used, the substrate formula, the amount of substrate available, the spawning rate, the spawn distribution and the temperature during incubation (*169*).

For example, some strains fruit sporadically after 60 days incubation, but fruit more steadily and reliably after 105 to 150 days (*105, 168*). Although a longer incubation time may not increase the number of mushrooms harvested, it can increase mushroom size and total yield (*52, 168*). This may be due to a larger mass of mycelial tissue, higher enzyme levels in the substrate or the increased solubility of the wood components (*168, 169*).

Several changes in the appearance of the substrate help the grower to judge when the mycelium is ready to fruit. However, not all strains exhibit these signs. Also, the substrate formula influences which changes, if any, will occur.

After colonization, the substrate appears totally white, and the sawdust particles are no longer visible. As the mycelium matures, lumps of mycelium may appear on the substrate surface (Fig. 12–8). Clear or brownish metabolic fluid may appear in the bags. Areas on the surface may turn brown, especially where the substrate receives light (Fig. 12–9).

The browning phenomenon is an oxidation caused by polyphenol oxidases, reacting to light and oxygen. The brown mycelium forms a protective skin over the outside of the substrate. This skin acts as a moisture barrier and a defense against invading organisms.

Figure 12–8. Shiitake mycelium forming lumps on substrate surface at end of spawn run (USA).

Figure 12–9. Browning of shiitake mycelium on sawdust substrate at end of spawn run (USA)

13

Fruiting Shiitake on Sawdust

After the spawn run is complete, all or part of the protective container must be removed for fruiting. Because the exposed substrate is no longer protected, it is highly susceptible to the effects of its environment.

This chapter presents management techniques for fruiting shiitake on sawdust substrates. It then considers the buildings and equipment needed for different management strategies. The final section examines some diseases and pests encountered during fruiting and recommends methods for prevention and control.

Fruiting Cycle on Sawdust

During incubation, shiitake mycelium binds the sawdust together within the bag. This mass of substrate and mycelium is called a **block**. The same cues (changes in temperature and moisture content) that initiate fruiting on logs also begin the fruiting cycle on sawdust. Although the physical structures of logs and sawdust are very different, the shiitake mycelium responds similarly.

The particulate nature of sawdust makes the wood components more available to fungi. This reduces the incubation time needed for shiitake to accumulate resources for the initial fruiting and also shortens the resting period before subsequent flushes (Fig. 13–1). The stages of the fruiting cycle are outlined below. (See Chapter 8 for a more detailed discussion.)

Induction

As is the case on logs, shiitake must have depleted the easily available carbohydrates in the substrate in order to begin the fruiting cycle. Shiitake requires light prior to induction. Therefore substrate that has been incubated in the dark may require 10 to 18 days of light to fulfill this need (*158*).

Induction can be initiated by shifting from warm incubation temperatures (77°F, 25°C) to cooler temperatures (60°F, 16°C). Some strains respond well to this temperature shift; others produce more mushrooms in response to a cold shock (102). A cold shock can be provided by refrigerating the blocks at 41°F to 46°F (5°–8°) for 5 to 12 days (*60, 105*). Soaking the blocks in cold water (12–24 hours, 41°F–60°F, 5°–16°C) can also provide this stimulus (*60, 158*). Following the cold shock, the blocks are placed under fruiting conditions (60°F, 16°C).

After the first flush, additional flushes will appear periodically without further induction, if fruiting conditions are maintained. The grower can con-

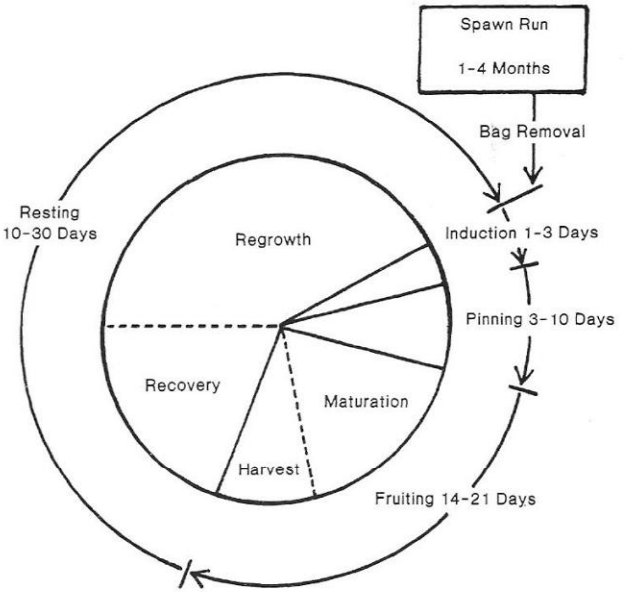


Figure 13–1. Shiitake fruiting cycle on sawdust substrates.

trol the timing of these flushes through management practices. Induction can be synchronized by warming the blocks after each flush, followed by a drop in temperature or a cold shock.

Sprinkling (*78, 171*) or soaking the blocks (*52, 158*) will also induce additional flushes. Sprinkling lowers the block temperature by evaporative cooling and rehydrates the substrate. Soaking results in a thorough water uptake and water temperature can be controlled to regulate the substrate temperature. A water reservoir is needed in the block for mushroom development; therefore, yields are correlated with the water content of the substrate. Blocks can require as long as 72 hours of soaking to become saturated. Adding nutrients to the soak water can increase yields, but may also result in higher levels of contamination (*52*).

Pinning

High carbon dioxide levels inhibit pinning. Therefore, to promote pinning, all or part of the substrate surface must be exposed to air. Exposed surfaces develop a brown "skin" which functions like the bark on logs (Fig. 13–2). This skin forms a favorable microenvironment for pinning, protects the block against molds and retards water loss.

Figure 13–2. Shiitake pinning through protective brown skin on sawdust substrate (USA).

As with logs, if the evaporation potential in the fruiting room is too low, the surface of the block remains wet, and pinning will be greatly reduced. On the other hand, excessively low relative humidities dry the blocks too quickly, and pinning will be aborted. Relative humidities between 85% and 95% RH avoid these two extremes.

Although specific pinning temperatures are strain-related, most strains pin from 50° to 68°F (10°–20°C) in three to ten days. The pinning temperature range of a shiitake strain is independent of substrate. The optimum pinning temperature on sawdust correlates with the fruiting season of the strain on logs (*210*).

Fruiting

Fruiting or maturation of the mushrooms requires specific environmental conditions: temperatures from 55° to 68°F (13°–20°C), relative humidities from 75% to 95% RH, light, fresh air and sufficient nutrient and water reserves in the block.

Temperature affects the speed of maturation and the density of the mushroom flesh. Light is required for the cap to develop color; this need increases as temperature rises. Carbon dioxide levels must be below 1,200 ppm (*168*); high levels cause small-capped mushrooms with elongated stems (Fig. 13–3).

The relationship between relative humidity and mushroom quality is the same for both log and sawdust cultivation. Low relative humidity results in drier mushrooms, which generally are of higher quality. However, blocks do not have a protective bark covering, so low relative humidity can cause rapid

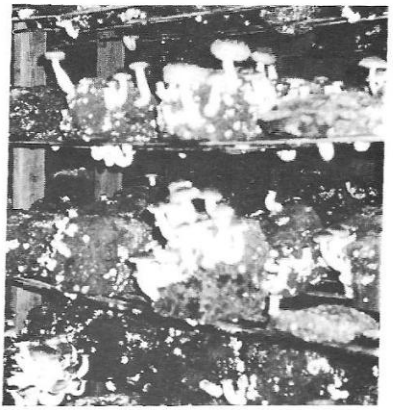


Figure 13–3. Effects of high carbon dioxide levels in shiitake fruiting room on mushroom shape (USA). Note elongated stems and small caps.

loss of the water reserve needed for mushroom development. This loss can be reduced by exposing only a portion of the block surface.

It is not uncommon for the first flush of mushrooms to have a high percentage of aborted and misshapen mushrooms. However, normal mushrooms are usually produced during subsequent flushes (*52, 125, 160*) (Fig. 13–4). Generally, each flush is harvested over a two to three week period.

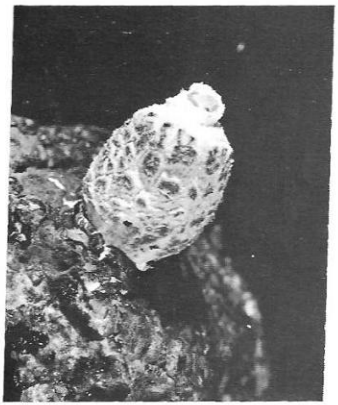


Figure 13–4. Misshapen mushroom produced during first flush of shiitake on sawdust substrate (USA).

Resting

Once a flush of mushrooms has been produced, vegetative growth is resumed and the mycelium accumulates reserves for another flush. The duration of the resting period is from 10 to 30 days.

Resting can occur under a wide range of conditions. In some systems, blocks remain under fruiting conditions. However, they recover faster if the temperature is elevated to 77°F (25°C). Often,

resting is combined with drying, which stops the fruiting process and allows more synchronous fruiting. When conditions are too wet during resting, molds will colonize the blocks because the shiitake mycelium has been weakened by fruiting.

Cropping Cycle

The total cropping of sawdust blocks lasts from three to six months or more. This depends on the environmental conditions during fruiting and resting, substrate formula and size of the block.

Biological efficiencies from 40% to over 100% have been reported for shiitake cultivation on sawdust substrates (*44, 168, 171, 169*). This is higher than on logs due to higher nutrient levels, reduced competition from other fungi for nutrients, increased availability of wood components in the sawdust and controlled conditions during fruiting.

Fruiting Facilities

Buildings

The type of structure required for fruiting depends on the climate. Some areas, such as parts of China and Taiwan, have a climate where fruiting in unheated structures is possible during the winter. Simple buildings made of bamboo mats or plastic retain humidity and shed rainfall (Fig. 13–5). Natural air movement provides ventilation. Some growers use a plastic tent to main-

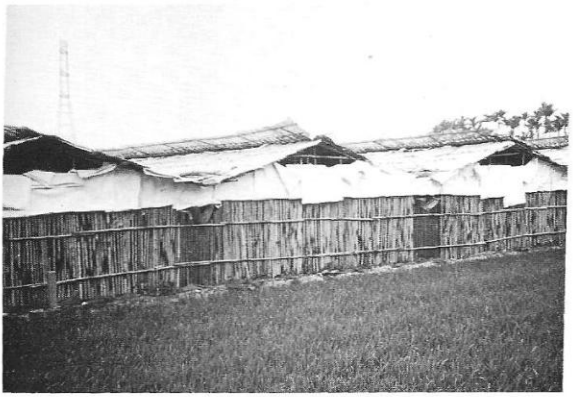


Figure 13–5. Bamboo houses for fruiting shiitake on sawdust substrates (Taiwan).

tain high humidity around the blocks (Fig. 13–6); others use overhead sprinklers to control drying.

As the climate becomes less favorable, fruiting requires air conditioning (heating, cooling and humidification). The rooms must withstand high humidity and free water; floor drains are necessary. The light needed during fruiting can be provided through translucent panels in the roof or with fluorescent lamps.

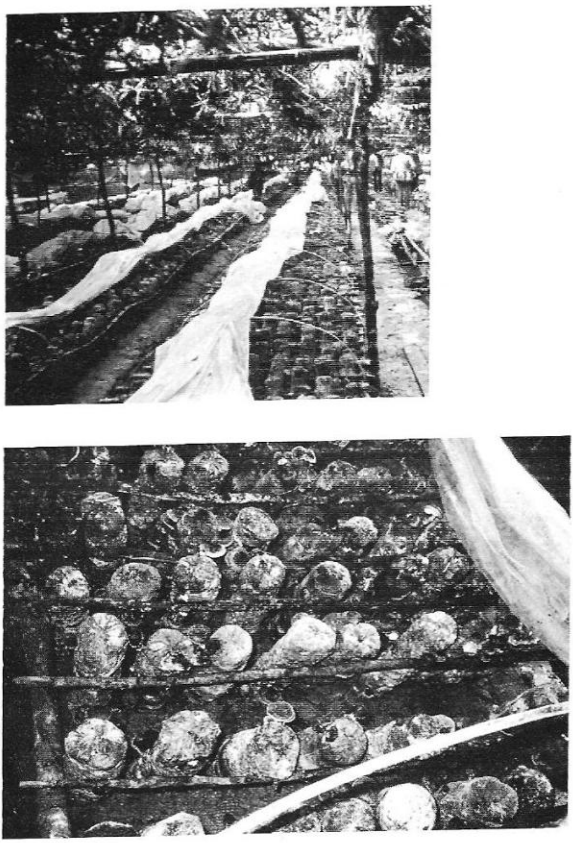


Figure 13-6. Shiitake fruiting on sawdust substrate in simple shade house (China). Note plastic tents used for humidity control.

The arrangement of the blocks in the fruiting area varies. On farms where fruiting space is relatively inexpensive, a single layer of bags is tightly packed on the floor (Fig. 13–7). This keeps the blocks down where the humidity is high and provides plenty of space for heat to dissipate.

As the cost of the fruiting area increases, the number of blocks per unit space must be increased. Several rack designs are used (Fig. 13–3, 13–8). A rack should provide access for picking and allow enough light to reach the block surfaces.

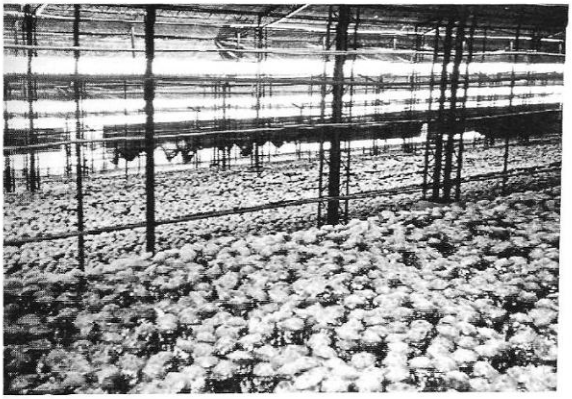


Figure 13–7. Shiitake on sawdust substrate arranged on floor for fruiting (Taiwan).

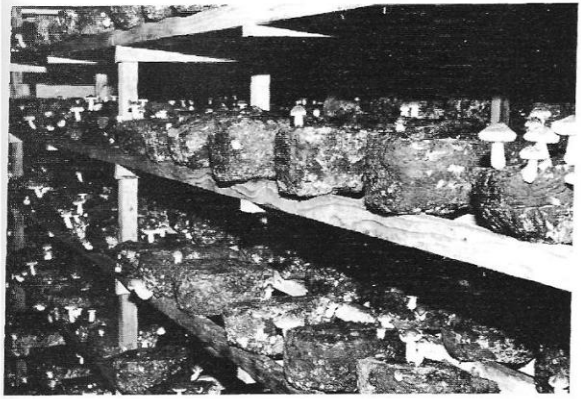


Figure 13–8. Wooden racks for fruiting shiitake on sawdust substrate (USA).

Air Conditioning and Ventilation

In many areas, heating, cooling and humidification are necessary to provide optimum conditions for fruiting. The climate and the design of the fruiting room dictate the type of system needed. Heat can be provided by steam, electricity, gas or other sources. Point sources of heating or cooling in the fruiting room should be avoided because of their drying effect.

Evaporative cooling can be provided by a swamp cooler located on the fresh air intake. However, the outside air must have a low relative humidity for a swamp cooler to operate effectively. Refrigeration is an alternative, but its drying effect must be compensated for.

Steam is a source of both heat and humidity. In situations where heat is not desired, several cool-temperature humidifiers are available. Most cool-air humidifiers create a fog of fine water droplets, either off a rapidly spinning disc or from high pressure water nozzles.

Fresh air is needed to prevent temperature stratification, to keep the surfaces of the blocks dry, and to keep carbon dioxide levels below 1,200 ppm. This requires from one to four air exchanges per hour, depending on the temperature and the amount of substrate per room (*168*). Therefore, tightly sealed rooms need a ventilation system to introduce fresh air. These systems usually recirculate the room air, mix it with fresh air and distribute the mixture evenly throughout the room. Generally, a plastic tube with evenly spaced holes is used to deliver the air to the room.

In most mushroom houses, the air is conditioned before it enters the room. The heater and cooler are mounted in the air stream, followed by the humidifier. All fresh and recirculated air passes through this system and enters the room at the desired conditions (Fig. 13–9). Humidity is especially important with forced ventilation, as the blocks can dry rapidly.

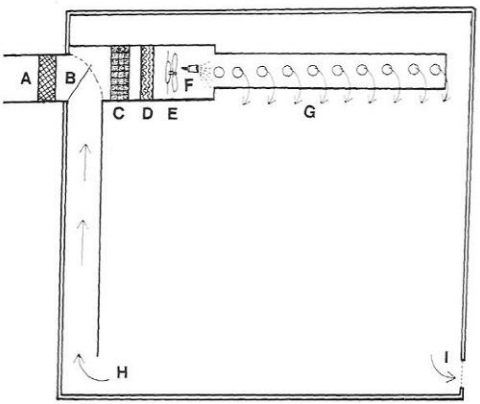


Figure 13– 9. Environmental control system for shiitake fruiting room. A. Fresh air intake filter, B. Air mixing damper, C. Cooling coil, D. Heating coil, E. Air circulation blower, F. Humidification nozzle, G. Air distribution tube, H. Recirculation air intake, I. Exhaust damper.

Crop Management

Crop management is the process of balancing yield, mushroom quality, cropping time and costs with potential income. The proper balance is determined by the market demand. Shiitake are produced from sawdust blocks under a multitude of different situations, from very simple to highly sophisticated. Most of the mushrooms produced under simple conditions are dried, while farms with a large capital investment produce high-quality fresh shiitake.

Examples of two different management strategies are given below. The first is based on a low level of nutrient supplementation, while the second uses a richer substrate. A myriad of variations on these strategies are practiced, leading to many different cultivation systems. Nevertheless, the underlying principles are the same.

Low Supplementation Model

In this example shiitake is grown on substrate with a low level of supplementation: composed of 90% hardwood sawdust and 10% rice bran. The low supplementation level decreases potential problems with contaminants. Generally, the bags are cylindrical, 8 to 10 inches (20–25 cm) long and about 4 inches (10 cm) in diameter. Often, a bag–filling machine compresses the medium and makes a spawning hole down the middle of the substrate. A ring and cotton plug close the bag.

Figure 13– 10. Bags of sawdust substrate with cotton removed from ring-and-plug closure for pinning of shiitake (Taiwan).

Each bag holds roughly 1 to 1.5 lb (450–680 g) of dry substrate. After filling, the bags are stacked upright into crates or totes which hold 12 to 16 bags. Sterilization and inoculation take place without removing the bags from the totes. Low temperature sterilization is achieved by steaming at atmospheric pressure, 212°F (100°C) for six to ten hours. After being inoculated with sawdust spawn, the bags are tightly stacked several layers high in a horizontal position for the spawn run (Fig. 12–4). Inoculation often occurs year-round, and bags are incubated from two to ten months.

Fruiting is initiated during the cool season. The blocks are stood upright, still closely packed together. This limits fruiting from the sides of the block. The plug is removed from the bag closure to increase ventilation for pinning (Fig. 13–10). After one to two weeks, the top of the bag is rolled down to expose the top of the block, while the sides remain covered. Once several flushes have been harvested from the top, the block is inverted and the bag is cut off. This exposes the bottom portion of the substrate for several additional flushes.

The relative humidity is usually maintained at between 70% and 85%, but the bag protects the blocks from excessive drying. Overhead sprayers are used as needed to moisten the blocks for subsequent flushes.

This is a low-cost strategy because stringent environmental control is not needed. Because of the low costs, a fairly high contamination rate can be tolerated. This strategy is used in many areas to produce seasonal crops during favorable weather. The mushrooms are usually dried for market. However, this fruiting strategy can also be used to produce mushrooms under highly controlled conditions for the fresh market.

Yields between 40% and 50% biological efficiency are commonly realized in a four- to six-month fruiting period.

High Supplementation Model

In this example, shiitake is produced on a rich medium containing 80% hardwood sawdust, 10% wheat bran, and 10% wheat or millet. A variety of bag shapes are used; usually they are large, holding 2.2 to 4.5 lb (1 to 2 kg) of dry media. Either the bags are closed with a ring and foam plug or heat-sealed bags with microporous filters are used. The substrate is sterilized in an autoclave, under pressure, at 250°F (121°C) for two to five hours.

After sterilization, the bags are inoculated with grain spawn, which acts as additional supplementation. The bags are incubated under temperature-controlled conditions with artificial lights on an eight hour on, 16 hour off cycle. The bags are stacked to allow light to reach all sides (Fig 13–11).

Depending on the strain, the blocks may be induced with a cold shock (41°F, 5°C for 12 to 48 hours) or may be moved directly onto racks in the fruiting room (60°F, 16°C, 85% to 95% RH) to begin production. The blocks are pinned with the bags completely removed. The blocks are placed on racks with enough space around each block for mushroom formation and picking.

Eventually, a brown skin forms over the entire block and fruiting occurs from all its surfaces (Fig. 13–12). Overhead misters and/or hand watering may be used to maintain suitable substrate moisture levels.

Additional flushes are produced after resting and drying the blocks under warmer conditions. The temperatures in the fruiting room can be raised, or the blocks can be moved to a drying area (77°F, 25°C, 60%–75% RH) for one to two weeks. The dry blocks are then soaked in water at 50°F (10°C) for 5 to 72 hours (Fig. 13–13). This provides a temperature shift to induce fruiting and supplies a water reserve for mushroom production. Often, the blocks are punctured to speed water absorption. Sprinkling can also rehydrate the blocks. Then, the blocks are again placed under fruiting conditions.

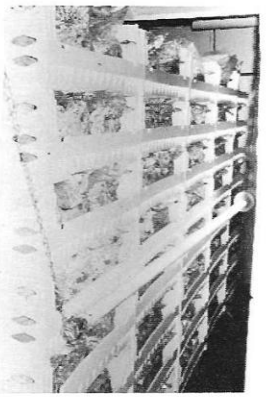


Figure 13– 11. Incubation of shiitake on sawdust substrate with exposure to light (USA).

This strategy of continuous shiitake production requires precisely controlled conditions. The intensive management of fruiting results in a high yield per unit space per time period. Yields of 50% to 80% biological efficiency are generally realized in two to five flushes over a three- to six-month fruiting period. Most of the mushrooms produced in this way are sold fresh.

Figure 13– 12. Shiitake fruiting from all surfaces of sawdust substrate (USA).

Figure 13 – 13. Soaking sawdust blocks to induce fruiting of shiitake (USA).

Diseases and Pests During Fruiting on Sawdust

A limited number of diseases and pests attack shiitake during fruiting on sawdust. Fortunately, most problem organisms can be controlled through cultural methods.

Mold Fungi

The mold fungi which appear during fruiting include species from the genera *Trichoderma, Gliocladium, Mucor, Penicillium*, and *Doratomyces* (*185*). With the exception of *Doratomyces,* these fungi have been described previously.

Doratomyces is commonly known as the black or grey whisker mold. It appears as masses of grey to black spores which easily become airborne when disrupted. Contaminated areas often appear damp or wet. *Doratomyces* spp. often indicate high available carbohydrate levels.

Determining the causes of contamination. The appearance of contaminants during fruiting can be caused by the use of incompletely colonized blocks or conditions that stress the mycelium of fully colonized blocks. Many mold fungi appear after one or more flushes, when the mycelium has been depleted by fruiting. The time of appearance and the distribution of molds, both on the blocks and within the fruiting room, often indicate the source of a mold problem.

High supplementation levels also create a potential for contamination by decreasing substrate selectivity. Contamination due to high supplementa-

tion levels may appear before the first flush, or just after. Several molds may be involved, appearing on most of the blocks, randomly distributed on the block surfaces. This problem can be avoided by reducing the amount of supplementation in the substrate formula. Removing the affected areas and drying the block can also reduce the problem.

Unfavorable environmental conditions. Extremes in temperature and evaporation potential may stress the shiitake and open the way for contamination. Prolonged exposure to temperatures above 95°F (35°C) or below freezing can weaken or kill the mycelium.

Rapid drying of the block surface, combined with a misting system, creates conditions conducive to molds (*171*). Under dry conditions, the shiitake mycelium on the surface becomes inactive or dies. A film of water from periodic misting of the substrate surface then allows molds to become established.

A continually wet block surface can result in mold problems. Often, wet surfaces are caused by large droplets from the humidification nozzles. Large drops fail to humidify the air, but create a fine mist which settles on the blocks. Wet block surfaces can also be caused by high humidity with insufficient air movement or bacterial infection.

The distribution of molds within the fruiting room often can be related to the air flow in the room. Blocks in high air flow areas, such as the top and outsides of the fruiting racks or near walls, are affected first. Once a mold problem becomes established, blocks which would normally not be affected are overcome by the high contaminant spore load in the room.

Control of molds. Most mold problems during fruiting can be controlled through sanitation and by adjusting air movement and relative humidity so that the block surface is neither too wet nor too dry. If the block surface is too wet, the air movement can be increased or the humidity lowered.

Sanitation is aimed at removing both sources of spores and also potential infection sites. The cut-off stumps that remain on the blocks after mushrooms are harvested are likely sites for molds to develop. They should be trimmed very close to the block. Blocks heavily colonized by molds should be removed from the fruiting area to decrease the concentration of mold spores.

Fungicides have been added to sawdust media to control *Trichoderma* and other contaminants during shiitake cultivation on sawdust. These chemicals are added prior to heat-treatment and can provide good control (*109, 198*). However, molds can rapidly develop resistance to these chemicals. Further research is also needed to determine if the mushrooms produced contain residues.

Pests

Flies are the main pests encountered in fruiting rooms. The insect families involved are discussed in Chapter 10. Fly damage appears as pinholes in the mushrooms and on the substrate surface. In addition to decreasing mushroom quality, flies often carry and spread bacteria and mold spores. The bacteria introduced by flies create water-soaked areas, well suited for fly larvae and molds. In this situation, both fly populations and mold problems rapidly escalate.

Fly populations can be monitored with a black light. A standard fly monitor used in the button mushroom industry is an 18-inch, 15-watt black light tube mounted between two strips of sticky paper (Fig. 13–14) (*233*). Flies are attracted to the light and become trapped. This allows the grower to keep track of the number of flies. While large numbers of flies may be caught on these traps, they are not effective as a control measure because many of the flies have already laid eggs before they are trapped.

Flies are best controlled through sanitation and exclusion. Spent blocks and mushroom debris act as fly reservoirs where populations can increase. They should be disposed of at a site well-removed from the farm. Mushroom stumps on the blocks should be trimmed because they also are sites where flies will lay eggs.

Tight-fitting doors and screened openings can exclude flies from the fruiting area. The air intake and exhaust should be covered with 100 mesh screening. However, the air handling system must compensate for the increased resistance to air flow. Exhaust vents are especially important because the scent of mycelium is very attractive to flies. The odor plume from the exhaust attracts flies, which then crawl into the room.

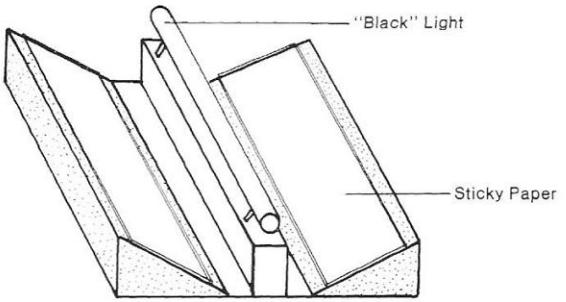
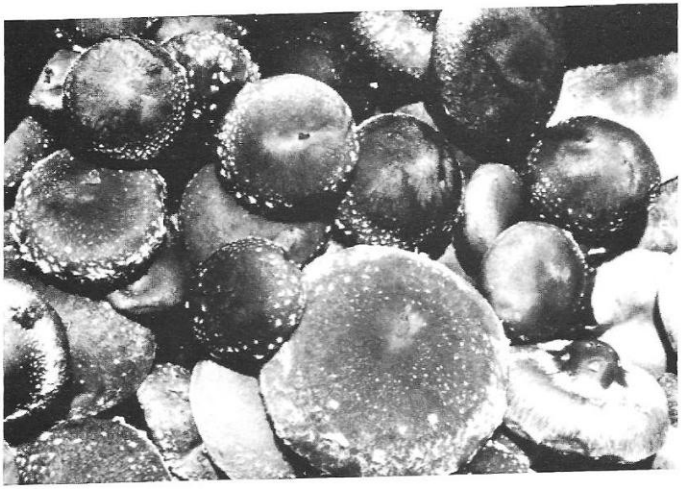


Figure 13 – 14. Black-light fly monitor.

In the commercial button mushroom industry, flies are a major problem. A survey of pesticide usage found that eighty-three percent of the pesticide applications were directed at insect control (*167*). Various chemicals are used to control flies; these include organophosphates, synthetic pyrethrins and growth regulators. Insecticides can help to decrease large fly populations, but no chemicals are currently registered in the United States for use on shiitake.

Section IV
Post-fruiting Aspects of Shiitake



A successful shiitake operation depends on mastering the art and science of mushroom cultivation. Regardless of the production method employed, once the mushrooms have been produced they must be harvested and sold before any income can be realized. Proper post-fruiting management is crucial for a successful farm.

This final section discusses harvesting, marketing, and storage methods that will enable growers to prosper from the fruits of their labor. It then examines the unique nutritional and health-related characteristics that will ensure shiitake's place in the market.

14

Marketing, Harvesting and Processing Shiitake

Marketing

Marketing is the process of moving goods from the producer to the consumer, matching supply with demand. Production is subject to market demands; quality, quantity, product form and volume are all influenced by market demands.

For shiitake growers, this means that the methods of production, harvesting, storage, packaging, processing, and shipping all depend on what the market dictates. Thus, understanding the market demands contributes to wise decision making in these areas. Marketing is a personal aspect of mushroom growing. Customers want reliability and good service. Successful growers must master marketing skills to create demand for their product among both wholesalers and consumers.

Marketing is a very important aspect of the shiitake operation. Inadequate marketing leads to panic selling or "dumping," which drastically lowers the market price. This, in turn, hurts the industry as a whole.

The grower should research marketing alternatives long before mushrooms are ready for picking, and make contact with supermarket produce buyers, restaurant supply persons, or produce wholesalers to determine if and when mushrooms are likely to be needed, how many can be sold and how they should be packed and shipped. Marketing cooperatives can help small producers supply steady markets—a benefit to both producers and consumers (*122*).

The market for fresh shiitake is expanding. New markets can be developed by promoting the distinctive, strong flavor and health benefits of shiitake.

The differences in quality between shiitake grown on logs and shiitake grown on sawdust may become marketing points as consumers become more sophisticated. Mushrooms produced on logs have several advantages. They often are more dense and have a stronger flavor. Generally, log-grown shiitake have a longer storage life than those grown on sawdust. Another advantage of log-grown shiitake is that no pesticides are used. The popularity of this organically grown product is likely to increase in the future.

The potential health benefits of consuming shiitake promise to become an increasingly important marketing aspect. Research has identified a number of compounds from shiitake which stimulate immune system activity and lower blood cholesterol levels. High cholesterol levels are implicated in many circulatory diseases, such as atherosclerosis, whereas immune system disor-

ders may be involved in widespread diseases such as cancer. The prevalence of such diseases emphasizes the need to adopt a preventative attitude toward health. Consequently, consumers are becoming more interested in adding health-promoting foods to their diet.

Harvesting

Harvesting is the process of getting the mushroom off the substrate and into storage for market. This must be done with a minimum amount of damage to both the mushrooms and the substrate.

Stage of Maturity

Commonly, shiitake are harvested after the veil which covers the gills has ruptured, but before the cap has fully expanded. With button mushrooms there is little weight gain once the veil has broken (*34*). Although shiitake are harvested at all stages of maturity, the highest quality mushrooms still have an in-rolled edge on the caps (Fig. 14–1).

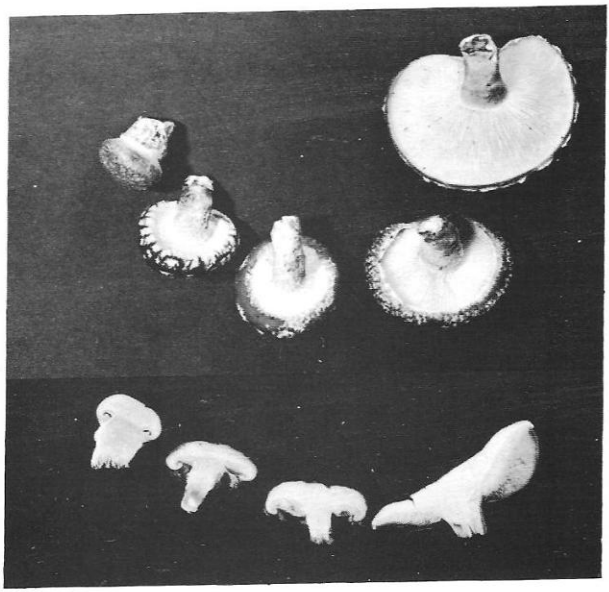


Figure 14– 1. Stages of mushroom development in shiitake (USA). Note veil and cap margin.

There are several disadvantages to harvesting overmature mushrooms. One is that mushrooms continue to develop during storage. This can lead to a drop in value because mushroom quality is partially determined by stage of maturity.

A second disadvantage is that the time between flushes is influenced by the stage of maturity at which the mushrooms are harvested: The more mature the mushrooms are at harvest, the longer the delay before the next flush (*27*). This may be due to the allocation of nutrients in the mycelium. The maturing mushrooms on the substrate command the available resources. If they are harvested earlier, these nutrients will be available to start more primordia.

A third disadvantage is that letting mushrooms mature allows more time for pests and diseases to develop and reach damaging levels. In addition, spores may be shed from the mature fruiting bodies. In the button mushroom (*Agaricus*) industry, several devastating virus diseases are spread by mushroom spores. Although no virus diseases of shiitake are currently known, viruses have been found in shiitake and could become a problem in the future.

Picking

Shiitake are picked by twisting or cutting them off the substrate surface. Generally, the lower portion of the stem is grasped firmly and the mushroom is twisted and lifted off. This does not leave any part of the stem attached to the substrate. However, it may remove some substrate at the base of the stem, thus exposing areas where molds or pests could become established. Only the stem should be touched during picking; bruises on the cap and gills will discolor rapidly. Twisting does not work well with wet mushrooms, since bruising of the stem is unavoidable.

Shiitake can also be harvested by cutting them from the substrate with a sharp knife or scissors. The stem should be cut as close as possible to the substrate surface because the remaining stump can be colonized by molds or pests which may then spread. Cutting minimizes physical damage to both the mushroom and the substrate.

After the shiitake are picked, they may be trimmed to remove debris or to improve the mushroom shape by shortening the stem. The mushrooms will be cleaner if they are trimmed before placing them in the picking containers. If they are trimmed in the fruiting area, the debris must be removed to prevent disease and pest build-up.

Picking containers should be made of plastic or other easily cleaned materials. Air vents are needed so the shiitake can be cooled rapidly. The picking containers should not be filled more than four to six inches (10–15 cm) deep with shiitake. This ensures rapid cooling and prevents bruising caused by the weight of other mushrooms.

Grading

Shiitake mushrooms are usually sorted into different grades after picking. In the button mushroom (*Agaricus*) industry, mushrooms often are sorted as they are picked. This method reduces handling of the mushrooms, but the picker must carry more containers, and picking takes longer.

There is no world standard for grading shiitake. The number of recognized grades varies with the degree of market development in a given area. In Japan, a detailed system of grading is used for fresh shiitake, with a separate system for the dried product. The grade depends on the stage of maturity, size, shape and appearance. Generally, shiitake with pests or disease are not sold.

Fresh shiitake. The Japanese system for fresh shiitake divides the mushrooms into two grades: Grade A is the premium grade: the veil is still partially intact or the cap is no more than 70% open, the mushroom flesh is thick and dry, and the cap shape is round and regular. Grade B is the standard grade: the caps are more than 70% open, the flesh may be thinner or wetter, and the shape may be irregular. Within each grade, the mushrooms are sorted by cap diameter. Large caps are greater than 2–1/2 inches (6–8 cm) in diameter, medium caps are between 1–1/2 to 2–1/2 inches (4–6 cm) and small caps are less than 1–1/2 inches (4 cm) in diameter (*71, 122*).

Although the Japanese grading system for fresh shiitake has been proposed as a standard grading system for the United States (*122*), the current grading system is less sophisticated. Number ones are the premium grade. These mushrooms have an inrolled margin and are medium in size (1–3 inches, 2.5–7.6 cm) with good shape; the ratio of cap diameter to stem length is about 1.6 to 1.

Number twos have fully expanded caps, the mushroom size can be small or large, and misshapen and broken mushrooms are allowed. Pieces and stems are sold for processing and represent a limited market. However, specialty markets exist with unique quality criteria. For example, large shiitake used for stuffed mushrooms and small shiitake for garnishes often bring a premium price.

Dried shiitake are graded under a slightly different system that takes into account the appearance and texture of the cap surface. The grading system for dried shiitake is most fully developed in Japan and China where three major grades are recognized, with numerous divisions within each grade (*69, 71*).

Donko shiitake are the premium dried mushrooms; the thick-fleshed cap is cracked with a deeply inrolled margin and the overall shape is round. These mushrooms are produced during cool, dry conditions, and have an intense flavor due to their slower growth rate and dense flesh (Fig 14–2).

At the other end of the grading system are Koshin shiitake which have a fully expanded cap, thin flesh and longer stems. These are produced during warm temperatures and do not have the intense flavor of Donko shiitake.

Figure 14–2. Dried shiitake, Donko grade (Japan).

Koko grade is intermediate between Donko and Koshin. Koko shiitake have thick flesh, but the cap is not cracked. The caps have in-rolled margins but may be more open than Donko. Most of the mushrooms sold on the fresh market would be graded as Koko, if dried.

Post Harvest Physiology

Mushrooms continue to grow and develop after they are harvested. A number of physiological processes occur within the mushroom during storage. It is important to understand them because they affect the quality of the mushroom presented to the consumer. The rate at which these processes take place is determined by the temperature.

Respiration

Respiration, taking in oxygen and giving off carbon dioxide, is a crucial part of growth and development. Mushrooms, like most fresh produce, continue to respire after harvest. Protein degradation and depletion of carbohydrates occur after harvest and result in changes in mushroom texture (*132, 140*). Respiration rate is a good indicator of storage life. Mushrooms have a high respiration rate compared to other fresh produce items. For example, mushrooms have a much shorter storage life than tomatoes because the respiration rate of mushrooms is up to seven times greater (*116*).

Spoilage

Spoilage during storage can be caused by bacteria, fungi and enzymatic changes within the mushroom. Some of the fungi involved in spoilage have been discussed in Chapter 10 under post-harvest fungi. Low temperature will slow the spoilage rate; nevertheless, bacterial populations and enzyme concentrations continue to increase during cold storage. This results in rapid deterioration when the mushrooms are warmed (*48*).

Enzymes are active during storage and cause changes in mushroom texture. The mushrooms soften and lose their firmness (*12*). Some enzymes digest mushroom proteins, releasing nitrogen during this process of self-digestion (*132*).

Enzymatic browning also occurs. The enzymes responsible for browning (polyphenol oxidases) are contained within the cells, where they are enclosed by membranes. When cells are ruptured, either through bruising or with age, these enzymes are released. They react with oxygen and colorless phenolic compounds in the cells to form brown pigments (quinones) (*138*). The resulting discoloration decreases consumer acceptability. In extreme cases, the gills and flesh turn totally brown.

Bacteria are on the mushrooms during cultivation but are not normally a problem. Bacterial populations increase during storage, however, and eventually cause degradation (*33*). The mushroom texture is altered: It loses its firmness and the flesh darkens (*12*). Free water in the mushrooms favors bacterial growth. Overly wet mushrooms or condensation on the mushroom surface can result in rapid degradation caused by bacteria. The bacteria which cause botulism are not a problem during storage unless the mushrooms are packed in air-tight containers and stored at warm temperatures (*79*).

Moisture Loss

Water loss is detrimental during storage of fresh shiitake, in part because loss of weight results in lost revenue. Mushrooms are 85% to 95% water, and there are no barriers to water loss from the mushroom surface. In fact, evaporation initially occurs at the same rate as evaporation from the surface of an open container of water (*172*).

In addition to weight loss, the quality of fresh mushrooms is lowered by water loss. Mushrooms wilt and shrivel as they lose water, thus detracting from their appearance. The degree of wilting depends on mushroom density; dense mushrooms do not wilt as readily (*50*). Mushroom density is influenced by strain (*160*) and also by conditions during fruiting (*85*).

The evaporation potential of the storage facility also affects the rate of water evaporation from the mushroom surface. An additional factor is the degree of exposure of the mushrooms. Mushrooms completely exposed to the air will lose more weight than those protected by a container.

The stage of maturity also influences evaporation rate. Mature mushrooms with exposed gills have more surface area from which water can evaporate.

Temperature, relative humidity and air velocity all influence the evaporation rate. (See Chapter 3 for a detailed discussion of evaporation potential.) For example, *Agaricus* mushrooms stored for five days at 35° to 36°F (2°C), 85%–95% RH, lost 8% of their original weight. Mushrooms stored at 64°F (18°C), 40%–50% RH for the same period lost 50% of their original weight (*139*).

Storing and Shipping Fresh Shiitake

Cold storage extends the shelf life of shiitake by affecting post-harvest physiology. The processes which degrade the mushrooms are slowed or inhibited entirely.

Cold Storage

Cooling the mushrooms results in lower rates of all the physiological processes occurring on and in the mushrooms. The cooling process has two phases: removal of field and metabolic heat, and storage at low temperatures. During the initial phase of cooling, there is a high cooling load. As mushrooms are cooled, however, the amount of metabolic heat produced decreases. Thus, once the mushrooms are cooled, the cooling load is reduced.

Removal of field and metabolic heat. Field heat is the temperature of the mushrooms after picking, which is equal to the temperature in the fruiting area. Metabolic heat is generated by processes within the mushroom and is high during fruiting. If mushrooms were not rapidly cooled, but instead were put into boxes, their temperature would increase due to metabolic heat. To remove this heat rapidly, a large cooling capacity is needed. Ideally, mushrooms should be cooled to storage temperatures of 32° to 36°F (0°–2°C) within five hours of picking (*215*).

Low-temperature storage. Optimum conditions for storage are temperatures from 32° to 36°F (0–2°C) and relative humidities from 85% to 95% (215). Under these conditions, the storage life of shiitake is two to three weeks. Weight loss in open containers is about 1% to 2% per day (*138*). Weight loss of mushrooms in semi-closed containers is less.

The storage life of shiitake is similar to that of *Agaricus* and is dependent on temperature. For example, at 32°F (0°C) *Agaricus* can be stored for 17 to 20 days; at 37°F (3°C) this is shortened to 7 to 10 days, and at 54°F (12°C) the storage life is 3 to 5 days (*215*).

Facilities for Cooling

Initial rapid cooling. Because the cooling demand during the initial phase is much higher than during storage, several methods of rapid cooling have been developed.

The simplest method of rapidly cooling mushrooms is to place them in storage containers that allow ample air movement around the mushrooms. Then these containers are placed in a cooler that is less than two-thirds full and are spaced to allow rapid infiltration of the cold air. This method requires no special equipment. Weight loss is about 3% during this initial cooling (*138*).

Positive ventilation cooling uses special oversized cooling coils (evaporators) in the cold storage area. The evaporator is located where boxes of mushrooms can be placed in front of it. Cold air from the evaporator is blown through the boxes at about two cubic feet per minute per pound of mushrooms (*10*). This method cools the mushrooms faster than the method described above and results in about the same weight loss.

Vacuum cooling uses evaporation potential to achieve rapid cooling. Both the fresh produce industry and some large *Agaricus* growers use this method to remove field heat. The mushrooms are cooled under a vacuum which increases evaporation and cools them to storage temperatures in 15 to 20 minutes. Although vacuum cooling has a higher evaporation rate, the mushrooms lose only 1% to 5% of their initial weight. The mushrooms are then moved to conventional coolers for storage. This method is expensive and requires extra handling; therefore, it is used only for large-scale operations.

Storage cooler. The storage cooler must have sufficient refrigeration capacity to meet the cooling load demands. In addition, the relative humidity should be high (85%–95% RH) to minimize drying. Air in the cooler is chilled by passing it over the cooling coils of the evaporator. Conventional coolers use a small evaporator with very cold coil temperatures. This dries the air considerably by condensing water onto the coils. If moisture is being added to maintain high relative humidities, the evaporator may become coated with ice, which decreases its effectiveness.

To achieve high relative humidities, the temperature differential between the air and the evaporator coils should be less than 2.5°F (1.4°C) (*10*). This is achieved by using a larger than normal evaporator. The same amount of cooling is then occurring over a larger area and can be achieved with a higher coil temperature. Creating a moist microclimate around the mushrooms by limiting their exposure also decreases drying.

Irradiation

Irradiation has been tested as a method of increasing the storage life of *Agaricus* mushrooms. Irradiation with gamma radiation kills bacteria and inhibits respiration and further maturation (*101*). This prolongs storage at temperatures above 50°F (10°C), but does not significantly increase storage life below this temperature (*183*).

Consumer acceptance of irradiated mushrooms is unlikely for several reasons. Irradiation discolors the mushroom flesh, even at low levels (*183*). Unique molecules, known as radiolytic products, which are created during irradiation, pose potential health threats. Furthermore, the process is expensive.

Controlled Atmosphere

The concentrations of oxygen, carbon dioxide and other volatiles immediately around the mushrooms affect storage life. By controlling the concentration of gases, both growth and enzymatic activity can be limited.

Other produce, such as apples, can be stored for long periods in large controlled atmosphere buildings. But because the storage life of mushrooms is so short, the most feasible method of controlling their atmosphere is with packaging.

Overwrapping mushrooms with microporous or perforated plastic films can improve their storage life (*139*). Carbon dioxide levels increase and oxygen levels decrease in overwrapped containers due to mushroom respiration. The relative concentration of these two gases inside the package is determined by the permeability of the overwrap film (*139*). High CO_2 levels suppress stem elongation. In tests with *Agaricus*, CO_2 concentrations of 5% to 10% increased shelf life by two to three days at 50°F (10°C) (*133*).

Low oxygen concentrations limit polyphenol oxidase activity, which is responsible for the browning reaction. However, the danger of botulism poisoning exists if oxygen concentrations become too low (*79*).

Packaging and Shipping

Packaging. The type of packaging used depends on the market. Mushrooms are commonly sold in bulk or prepackaged. Bulk mushrooms are sold in standard mushroom flats (Fig. 14–3). In the U.S., the common sizes used for shiitake hold three, five or ten pounds. These flats are sized to hold the required weight without allowing the mushrooms to be bruised during shipping. Generally, mushroom flats are made of corrugated cardboard, which may be coated with wax to decrease strength loss from moisture. Ventilation holes are needed to allow the mushrooms to breathe during storage.

Shiitake are also sold prepackaged in small quantities: for example, in 4-oz or 100-gram units. Plastic mesh bags are used for this purpose in Japan.

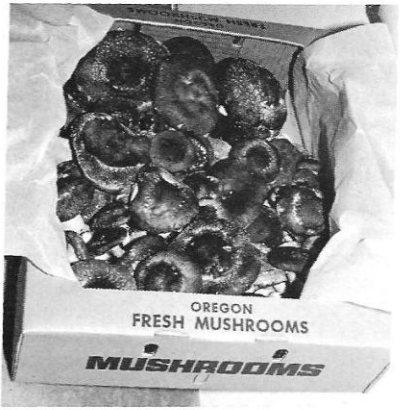


Figure 14–3. Standard mushroom flat for bulk packaging of shiitake (USA).

Prepacks on small trays are often overwrapped with plastic films. Polyvinyl chloride (PVC) films with differing gas permeabilities are commonly used. Overwrap films should be punctured with at least two 1/8-inch holes to decrease the risk of botulism (*79, 140*). Fluctuating temperatures during storage can cause condensation on the inside surface of the film. This can "pump" water from the mushrooms, creating a slimy mess. Hermetically sealed polyethylene bags, commonly used for enoki mushroom storage, have also been used successfully for shiitake (*126*).

Shipping. The most important rule during shipping is to keep the mushrooms cold. Refrigerated transport is best; dry ice can also be used. In addition to cooling, dry ice creates high carbon dioxide levels which extend storage. However, it can freeze the mushrooms if packed incorrectly.

Expedient shipping is very important when shipping fresh mushrooms. In the U.S., non-refrigerated air freight is commonly used to ship fresh shiitake. The major disadvantage with shipping by air is that there is no control over shipping conditions. The mushrooms may remain in a heated cargo area where they deteriorate rapidly (*16*).

Preservation of Shiitake

Preserving fresh shiitake allows seasonal and peak production to be sold throughout the year. Drying is the most common method, although freeze-drying and canning are also used.

Drying

Drying preserves the mushrooms by removing enough water to inactivate the enzymes and microorganisms. Shiitake are usually air dried to a moisture content of about 13%. During drying, shiitake shrink to about half of the fresh size (*197*) and undergo a seven-to-one reduction in weight.

Dried shiitake reconstitute well and retain their color, although they may be slightly tougher than fresh mushrooms. Drying gives the mushrooms a more intense flavor and aroma.

The compound responsible for shiitake's unique flavor has been identified as guanosine–5'-monophosphate (*240*). The levels of this compound increase during drying (*180*).

The distinct aroma of shiitake is due to a sulfur-containing adenine derivative called lenthionine (*118*). It is formed enzymatically from lentinic acid (*240, 241*). Normally, cell membranes separate lentinic acid from the enzymes which produce lenthionine (*240*). When the cells are ruptured by crushing or chewing, the enzymes and lentinic acid mix; the resulting reaction forms the aroma. Drying of shiitake ruptures many of the mushroom cells, and the aroma-producing reaction occurs upon rehydration.

Several potentially hazardous compounds are produced as by-products during lenthionine formation. Carbon disulfide (*69, 217*) and formaldehyde (*40, 239, 242*) have been found in shiitake. Although the concentration of these compounds increases during drying, they are extremely volatile and are lost during cooking (*42*).

To prepare shiitake for drying, they should be sorted by size and grade. This reduces handling of the dried mushrooms and creates uniform drying conditions. The mushrooms should be placed gills down on the drying trays and should not be touching (*180*). Later in the drying process, as the mushrooms shrink in size, three trays can be combined into one.

Sun drying is inexpensive and is practical in some areas. However, sun-dried shiitake are usually lower in quality than those dried in a controlled-air drier. The flavor and aroma are not as strong, and the mushrooms are more shriveled in appearance. An advantage of sun drying is that sunlight causes the conversion of ergosterol to vitamin D in shiitake (*154*).

Air drying uses heated air to dry shiitake. Two types of driers are used, natural convection driers and forced-air driers. Convection drying usually is done in specially designed buildings and uses the temperature differential between inside and outside to circulate the air.

Forced-air driers are common; they use fans to move the air (Fig. 14–4). The high temperatures reached during air drying denature enzymes and kill bacteria (*197*). The rate of drying is carefully controlled to create shiitake's distinctive flavor and appearance.

Phases of drying. There are four distinct phases during drying: the initial drying period, the main drying period, the final drying period and finishing. The temperature is different for each period and is carefully controlled to produce the highest quality mushrooms. The actual drying schedule depends on the drier design and the moisture content of the shiitake.

The initial drying period is a period of rapid water loss, about 12% per hour (Fig. 14–5). The air vents are completely open and the temperature is set between 104° and 122°F (40–50°C). The mushroom temperature during this period is lower than the air temperature, due to evaporative cooling. If the air temperature is too low during this period, the mushrooms will mature

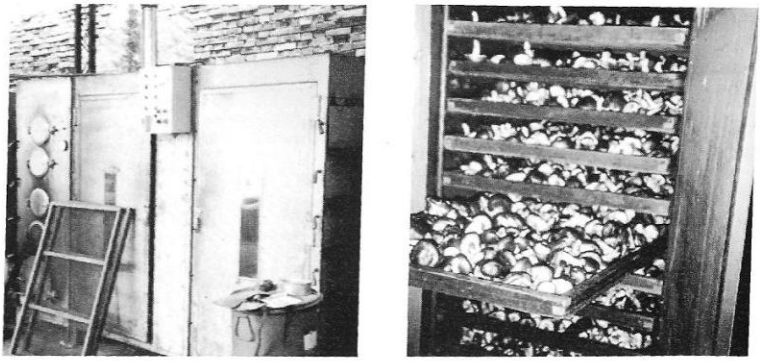


Figure 14–4. Forced-air mushroom drier used for drying shiitake (Taiwan).

and open in the drier. This period lasts from one to four hours, depending on the initial shiitake moisture content. A drop in evaporation rate signals the beginning of the main drying period (*180*). At this point, the mushroom moisture content is about 75%.

The main drying period is characterized by a steady evaporation rate of 6% to 7% per hour. Ventilation is reduced during this period. The temperature is dropped to 104°F (40°C), then raised 2° to 4°F (1°–2°C) per hour to keep the evaporation rate constant. This period lasts from 8 to 12 hours.

Temperatures during the main drying period should not exceed 131°F (55°C) to prevent case–hardening of the shiitake. This occurs when the outside of the mushroom dries so rapidly that the movement of water from inside the mushroom is inhibited. This traps water in the center of the mushroom, which then "cooks" at higher temperatures, degrading the final product. One method for avoiding this is intermittent heating during drying. The heat is cycled on and off periodically to prevent case-hardening.

The final drying period begins when the evaporation rate drops to 4% or 5% weight loss per hour. The temperature is raised to 131°F (55°C) and maintained until the mushrooms are hard and dry.

At that point, the temperature is raised to 140°F (60°C) for the finishing period. Finishing lasts one hour and develops the characteristic flavor and cap luster of dried shiitake.

Irradiation of the shiitake with ultra-violet (U.V.) light from sunlamps during drying increases the vitamin D content by converting ergosterol to vitamin D (*42, 154, 196*). Irradiating the gills is most effective because ergosterol concentrations are highest in the gills and the exposed surface area is greatest. Levels of vitamin D have been shown to increase ten-fold after 40 minutes of U.V. light exposure during drying (*154*).

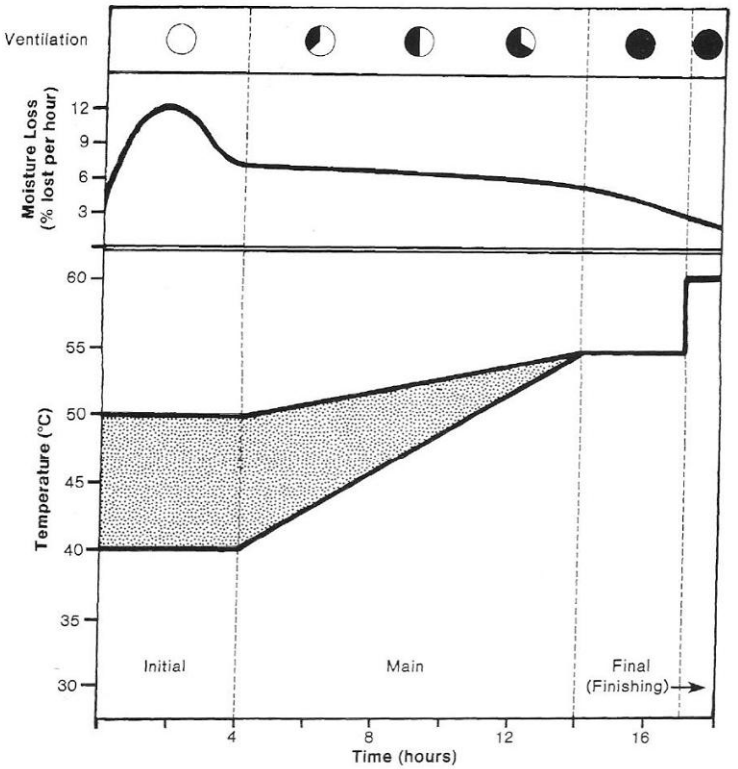


Figure 14–5. Shiitake drying schedule for controlled forced-air drying.

Other Methods of Preservation

While drying is the most popular method of preserving shiitake, freezing, freeze-drying and canning are used on a limited basis. Frozen shiitake brown upon thawing, although chemical pretreatment or blanching will limit browning (*39*).

Freeze-drying involves freezing the mushrooms, then drying them under a vacuum to between 4% and 6% MC. There is little change in shape and color, and the aroma is similar to that of air-dried shiitake (*197*). The quality of rehydrated freeze-dried shiitake is high; appearance and texture are similar to fresh. A unique feature of freeze-dried shiitake is instant reconstitution, which is desirable in instant prepared foods (*197*).

Canned shiitake is less satisfactory, and shiitake is seldom preserved in this way. Canning causes a number of changes in mushroom texture, flavor, color and appearance. However, blanching, microwaving and chemical dips are used to limit these changes (*11, 39*).

15

Nutritional and Health Aspects of Shiitake

In many Eastern cultures, mushrooms are viewed as a separate food group with special health-promoting attributes (*64*). In these cultures, shiitake is considered to be an "elixir of life," and mushrooms are an integral part of the daily diet. Conversely, in Western cultures, mushrooms are valued for their flavor, but are used primarily as a garnish. They are considered vegetables and thought to be devoid of nutritional or other benefits.

In fact, it has been found that shiitake does contain a number of important nutrients. Moreover, recent scientific investigations have isolated many compounds from shiitake and have found evidence of their effectiveness in promoting health. Western consumers are becoming increasingly aware of the role that diet plays in promoting and maintaining health. Therefore, a steady rise in shiitake consumption can be expected.

This final chapter considers the nutritional and medicinal properties of shiitake.

Nutritional Content

Shiitake is nutritious; it contains protein, lipids, and carbohydrates, as well as a number of vitamins and minerals. It should be noted, however, that concentrations of nutrients and biologically active compounds are affected by differences in strains, substrate, fruiting conditions and method of processing. Shiitake has a relatively high nutritional value when compared on a dry-weight basis to vegetables. Nevertheless, because shiitake is from 85% to 95% water, large quantities of mushrooms must be consumed to make a significant nutritional contribution to the diet.

Shiitake ranks above corn, turnips, potatoes, tomatoes and carrots when both the quantity and quality of protein are considered (*170*). The amount of protein in shiitake is less than that found in meat but is comparable to green beans or peas (*235*). Shiitake has a protein content of 10% to 29% (dry wt. basis) (*30, 235*). All nine essential amino acids are present in a ratio similar to the "ideal" protein for human nutrition (*19, 30, 170*). Shiitake is rich in the amino acids leucine and lysine, which are deficient in many grains (*19*).

Shiitake contains from 43% to 78% carbohydrate on a dry weight basis and is considered a low calorie food (*30, 235*). The total mineral content is from 2.6% to 6.5%. Calcium, phosphorus, iron, sodium and potassium are present in significant amounts (*30*).

Shiitake is a good source of vitamins, especially the B vitamins. Vitamins B_1 (thiamine), B_2 (riboflavin), B_{12}, niacin and pantothenic acid are present (*21*). Vitamin B_{12} is synthesized solely by bacteria and fungi and is not available from vegetables.

The processing method affects the vitamin content. Sun drying increases the vitamin D content, but destroys niacin, thiamine and riboflavin. Shiitake cultured outdoors have higher vitamin D contents than those cultivated in a greenhouse (*196*).

Health Benefits

Sixty to seventy percent of the drugs currently used in Western medicine are derived from plant or fungal sources (*232*). Until the 1800s, most medicines consisted of plants or plant extracts. At that time, researchers began isolating the "active" ingredients from plants. Since then, Western medicine has preferred these purified ingredients over plant preparations. However, the activity of medicinal plants is rarely due to one "active" compound (*232*).

The Eastern concept of health focuses on preventative medicine; it is based on a balance of energies within the body (*232*). The Asian pharmacopia contains a large number of medicinal plants and fungi, including shiitake and several bracket fungi (*62*). These materials are used to promote health and maintain resistance to disease.

A number of distinct biologically active compounds have been isolated and purified from shiitake (Table 15–1). Some of these compounds have more than one effect. In addition, there may be synergistic effects between these compounds, and between them and other "inactive" compounds in the mushroom. Thus, consuming shiitake fruiting bodies, either fresh or dried, may give the maximum potential health benefit.

Influence on Cholesterol Levels

High cholesterol levels in the blood are known to contribute to hardening of the arteries in humans. Water extracts of shiitake have been shown to lower blood serum cholesterol (BSC) levels in rats (*77, 236*). Feeding rats dried shiitake along with cholesterol resulted in BSC levels lower than rats fed no cholesterol (*212, 237*).

The cholesterol-lowering activity is associated with the fiber extracted from shiitake, but is higher when the whole mushroom is fed (*99*). Isolation and purification of this active fraction yields the adenine derivative, eritadenine. Also known as lentisine, lentinacin and lentisyne, eritadenine appears to accelerate the metabolism and excretion of cholesterol (*213, 214*).

Table 15–1
Biologically Active Compounds Found in Shiitake

Compound	Effect(s)	Type of Compound	Activity
Eritadenine	lowers cholesterol antiviral	adenine derivative	accelerates cholesterol metabolism and excretion
Ac2P	antiviral	polysaccharide	inhibits viral replication
Virus-like particles	antiviral antitumor	double stranded RNA	induces interferon production
KS-2	antitumor antiviral	polysaccharide	induces interferon production
Lentinan	antitumor	polysaccharide	stimulates T-helper cells in immune system
LAP1	antitumor	polysaccharide	immune system modulator
Polyphenol oxidase	antitumor	protein	unknown
Unknown	reduces blood coagulation	possibly nucleo--sides or -tides	inhibits platelet aggregation
Cortinellin	antibacterial	unknown	broad spectrum antibiotic
Unknown	antifungal	disulfide	unknown
FBP	antiviral	protein	inhibits viral infection in plant

Feeding studies with humans have indicated a similar effect, but further research is needed. One study showed that a diet containing fresh or dried shiitake lowered BSC levels. The addition of cholesterol-containing foods to the diet raised BSC levels, but when fed with shiitake, the BSC levels dropped slightly (*188, 189*).

Effects on the Immune System

Many of the human diseases currently increasing throughout the world have no specific cures. Immune system failure or dysfunction is a common element in cancer, viral and immune deficiency diseases. There is increasing evidence that the health-promoting compounds found in medicinal and edible fungi, including shiitake, stimulate the immune system (*61, 63, 129, 243*).

The biological activity of most of these compounds has been tested on rats and mice with induced diseases or imbalances. However, the physiology of an initially healthy animal with an induced disease is very different from that of a naturally diseased animal. Although many active compounds have been identified, further studies, including ones with humans, are needed to substantiate the effectiveness of these compounds.

Antitumor. Shiitake has been shown to inhibit a number of different transplanted cancerous tumors in mice. Injecting aqueous shiitake extracts or feeding powdered dried shiitake resulted in from 67% to 81% inhibition of sarcoma 180 tumor growth (*51, 63, 129*). Unlike many chemotherapeutic agents, the compounds from shiitake are non-toxic in higher doses.

The first antitumor compound isolated from shiitake was a large polysaccharide, lentinan (*24*). Lentinan is not toxic to tumor cells, but inhibits tumor growth by stimulating the immune system (*23*). The level of various immune responses which had been depressed by chemotherapeutic chemicals was restored by lentinan injections (*23*).

Apparently, lentinan activates macrophages which engulf the tumor cells (*23, 32, 51*). This activation occurs indirectly by stimulating T-helper cells. These cells increase the effectiveness of macrophages (*32, 51*).

Another polysaccharide, dubbed KS–2, has been shown to have antitumor activity against induced sarcoma 180 and Ehrlich ascites tumors in mice (*38*). This compound is much smaller than lentinan and contains a protein chain. KS–2 stimulates interferon production in both mice and humans and stimulates macrophage activity in mice.

Antitumor properties of virus-like particles isolated from shiitake have been demonstrated. These particles also stimulate interferon production (*193, 194*).

Other unique polysaccharides with antitumor activity have been isolated from shiitake mycelium grown on sawdust substrate (*199*). One active compound, designated LAP1, suppressed liver cancers and ascites tumors in rats (*186*).

Polyphenol oxidase, a protein enzyme involved in the browning reaction of shiitake mycelium, has antitumor activity as well. Inhibition of induced sarcoma 180 and MH–134 tumors was not related to the action of the enzyme itself, but to its protein content (*153*).

Antiviral. Viral diseases, which are widely distributed, do not respond to conventional treatments. The antiviral properties of shiitake have been tested against a flu (influenza) virus in mice. Injection of water extracts of shiitake inhibited viral disease development in influenza-infected mice (*221*).

Two types of antiviral activities of shiitake extracts have been described: inhibition of viral replication and stimulation of host interferon production. A polysaccharide, Ac2P, appears to inhibit virus replication, but does not affect the immune system (*238*).

Another compound isolated from shiitake, RNA (ribonucleic acid), stimulates interferon production (*187*). The isolated RNA was double-stranded, similar to viral RNA. RNA levels were highest in the spores, where high concentrations of viral particles were found. This suggests that the double stranded RNA may be from a virus within the shiitake (*187*). Virus-like particles isolated from shiitake (*128*) have been shown to induce interferon production (*193*).

The polysaccharide, KS–2 which has antitumor activity, also is effective against viral infections (*190*). As previously discussed, KS–2 induces interferon production in the host, but does not directly kill or inhibit the virus.

Eritadenine, the compound which affects cholesterol metabolism, also possesses antiviral properties (*25*).

Other Biological Effects

In addition to compounds affecting the immune system and cholesterol metabolism, a number of shiitake's other properties have been investigated. Shiitake contains compounds that affect not only mammals, but other microorganisms as well.

Shiitake extracts have been shown to reduce blood clotting. Unidentified compounds from shiitake inhibit the aggregation of platelets that begin the clotting process in blood. These compounds have potential for reducing blood clots in the circulation system, which are a factor in cardiovascular diseases (*59*).

Several compounds with antibiotic activity have been isolated from shiitake. Cortinellin is a broad-spectrum antibacterial agent, effective against gram-negative and gram-positive bacteria (*229*). A sulfide compound purified from shiitake has a potent antifungal effect against *Trichophyton* spp. Fungi in this widespread genus cause a number of skin disorders, including ringworm, on man and animals (*192*). Shiitake has also been shown to be effective against other human disease organisms, including *Candida albicans, Staphylococcus aureus* and *Bacillus subtilis* (*229*).

A protein fraction from shiitake fruiting bodies, labelled Fruiting Body Protein (FBP), prevented infection of plants with tobacco mosaic virus (TMV). The binding of the virus to the plant cells was inhibited by FBP (*84*).

Traditionally, shiitake has been prized for its many health benefits. As scientists investigate these claims, a number of unique biologically active compounds have been identified. Further investigations will most likely reveal new compounds with hitherto unknown activities. This promises an exciting future for shiitake as its production and consumption continue to increase.

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Glossary

Absolute humidity. The total amount of water vapor contained in a volume of air, generally expressed in grains (gr).

Acidic. Having a pH value less than 7. See also pH.

Aerobic. Requiring at least a minimum amount of oxygen for activity or life.

Agar. A gelatinous material obtained from a seaweed used to solidify culture media, or any culture media with agar as a base.

Agaricales. The order of Basidiomycetes containing the typical mushrooms with gills.

Amino acid. The basic building blocks of proteins, an organic molecule containing nitrogen in the form of NH_2 and a carboxylic acid group (COOH).

Anaerobic. Life or activity not requiring free oxygen.

Aphyllophorales. The order of Basidiomycetes containing fungi with woody, leathery or corky fruiting bodies with basidia lining pores.

Ascospore. Spores of an Ascomycete which are produced by sexual recombination and a reduction division in an ascus.

Ascus (pl. asci). Sac-like cell of the sexual stage of an Ascomycete where ascospores are produced.

Asexual. The absence of sexual reproduction by reduction division.

Autoclave. An airtight, pressure resistant vessel used for sterilization with super-heated steam under pressure.

Basidiomycetes. Sub-division or class of fungi whose sexual spores are produced by basidia.

Basidiospore. Sexual spore of a Basidiomycete, produced by a basidium.

Basidium (pl. basidia). Club-shaped cell in Basidiomycetes where sexual recombination and reduction division occurs, resulting in basidiospores.

Biological Efficiency. A method of expressing mushroom yields where the fresh weight of the mushrooms is divided by the dry weight of the substrate.

Bracket fungi. Basidiomycetes of the order Aphyllophorales which produce shelf-like fruiting bodies and grow on wood.

Button mushroom. = *Agaricus bisporus, A. bitorquis* or *A. brunnescens.* The white mushroom most commonly found in U.S. grocery stores and the most widely cultivated mushroom in the world.

Cambium. A single layer of living cells found between the sapwood and the bark that repeatedly subdivides to form new bark and wood cells.

Cellulose. Complex carbohydrate consisting of chains of glucose molecules, constituting the major part of the cell walls of higher plants.

Clamp connection. In Basidiomycetes; a hyphal outgrowth which, at cell division, makes a connection between the resulting two cells, ensuring that each cell receives a pair of nuclei.

Conidiophore. Hyphae bearing specialized cells that produce conidia.

Conidium (pl. conidia). A small asexual spore, produced vegetatively by special cells on a conidiophore.

Dewpoint. The temperature at which air becomes saturated and water condenses.

Dikaryon. A mycelium consisting of cells, each containing two nuclei of unlike mating types.

Enzyme. Protein molecules produced by living cells which catalyze or facilitate biochemical reactions.

Evaporation potential. The amount of water that can be vaporized per unit time, as a function of temperature, relative humidity, air volume and movement.

Evaporative cooling. Cooling that occurs due to the transfer of the heat required to vaporize liquid water.

Flush. A synchronized fruiting of mushrooms. Also known as a "break."

Forced fruiting. Deliberate induction of a synchronized crop of mushrooms.

Fruit-body. A specialized structure where sexual spore-bearing cells are produced. e.g. mushroom.

Fruiting. The act of fruit-body formation.

Genus. A group of closely related species.

Gill. A vertical, plate-like tissue covered with basidia on the underside of a mushroom cap.

Glucose. A simple six-carbon sugar that is the basic building block of many complex carbohydrates. Also known as dextrose.

Gypsum. Naturally occurring sedimentary rock containing calcium sulphate.

Habitat. The environment in which a plant or animal lives.

Heartwood. The non-living central portion of a woody stem which often contains gums, resins and other materials that may make it decay-resistant. See also sapwood.

Hemicellulose. A complex carbohydrate found in wood and other plant materials which resembles cellulose, but is easily broken down into simple sugars.

Heterothallic. Having a system in which two or more genetically distinct and compatible mycelium are required to complete sexual reproduction.

Humidity blanket. A white, porous, non-woven fabric used as a log covering to control evaporation potential.

Hymenium. A layer containing fertile, spore-producing cells, either asci or basidia.

Hypha (pl. hyphae). A single strand of filamentous fungal cells.

Immune system. The organs and tissues in the body concerned with the recognition and destruction of foreign substances, such as invading germs.

Inoculation. The act of transferring mycelium to a new substrate or medium.

Life cycle. *The series of stages between one spore form and the recurrence of the same the spore form, including any asexual phases.*

Lignin. A complex, amorphous organic substance containing phenolic molecules which acts as a binder for cellulose fibers in wood, adding strength and stiffness.

Lignocellulose. Any of several combinations of lignin, cellulose and hemicellulose forming the essential part of woody tissue.

LMC. Log moisture content. The amount of water contained in a log, expressed as a ratio of the water weight in the log to the total weight of the log.

Lux. A unit used to express light intensity. One lux is equal the amount of light received one meter away from a burning candle.

Medium (pl. media). A nutrient source which supports growth and may also act as habitat.

Microorganism. A minute living thing.

Monokaryon. A mycelium composed of cells, each containing a single, genetically identical nucleus.

Mycelium. A network of hyphae. The vegetative portion of a fungus.

Nucleus. A specialized body found in cells which contains most of the genetic information.

Particulate substrate. A growth medium composed of fragmented materials, such as sawdust and ground corn cobs.

Pasteurization. Selectively killing a portion of the population of organisms in a substrate, usually by heat.

Pectin. Complex water-soluble carbohydrates present in wood.

Perithecium (pl. perithecia). A flask-shaped fruiting body formed by some Ascomycetes.

Petri plate. A thin, flat, circular container with a lid, used to hold media for culture of microorganisms.

pH. A unit used to express degree of acidity or alkalinity. Measured as the hydrogen ion concentration in a solution. pH values range from 0 to 14. Distilled water is neutral (pH 7), values above 7 are alkaline and below 7 are acid.

Phenolic. Any of the group of aromatic hydroxyl derivatives of benzene. Resistant to degradation and often toxic to fungi. See lignin.

Phialides. Bowling-pin shaped cells which produce asexual spores, usually found on conidiophores

Pinning. The process of primordium formation, the beginning of mushroom development.

Pins. Primordia.

Polyphenoloxidase. Enzymes which act on phenolic compounds in the presence of oxygen, often forming brown products.

Polypore. Member of the Aphyllophorales. See bracket fungi.

Pore. A cylindrical tube lined with hymenium, found on the underside of fruiting bodies.

Primary mycelium. Mycelium arising from germination of a sexual spore in the Ascomycetes or Basidiomycetes. Monokaryon.

Primordium (pl. primordia.) The earliest stage of fruit body development.

Protein. Complex, organic compounds which contain nitrogen, composed of amino acid chains. Found in all living cells.

Pure culture. A colony of mycelium or cells, maintained under sterile conditions, consisting exclusively of one individual strain.

Reduction division. The division of a nucleus which reduces the number of chromosomes in half, resulting in new combinations of genetic information. Meiosis.

Relative humidity. The amount of water vapor in the air expressed as a percentage of the maximum water-holding capacity of air at that temperature.

Respiration. The metabolic processes by which an organism takes in and uses oxygen and gives off products of oxidation, esp. carbon dioxide and heat.

Retort. Autoclave.

Sapwood. The living wood near the outside of a tree stem. Sapwood is generally susceptible to decay due to its high nutrient content.

Saturation point. The maximum amount of water vapor that air at a given temperature can hold. Usually expressed in units of weight.

Secondary mycelium. Dikaryotic mycelium resulting from the fusion of two compatible primary mycelia.

Selective substrate. A nutrient source that limits the growth of some organisms while favoring the growth of others.

Sexual stage. The phase in a fungal life cycle which includes reproduction by reduction division.

Shade cloth. Woven or knit fabric used to block all or a portion of the sun's rays. Generally a black synthetic material.

Spawn. The mycelium of fungi growing on a substrate and prepared for the purpose of propagation of mushrooms.

Spawning. Inoculation using spawn.

Spawn run. Incubation period during which vegetative growth of a cultivated mushroom occurs after inoculation and prior to fruiting.

Species. A fundamental category of taxonomic classification, ranking below genus, consisting of organisms capable of interbreeding.

Specific gravity. A measure of density, expressed as the ratio of the weight of a given volume of a substance to that of an equal volume of water. Used as a standard.

Spore. A general term for the small reproductive bodies found in fungi, bacteria, algae, ferns, mosses etc.

Starch. Easily degraded complex carbohydrate, consisting of chains of glucose molecules.

Sterilization. The removal or destruction of all living organisms.

Strain. Genetically uniform mycelium possessing distinctive characteristics.

Stroma. A mass of vegetative hyphae on or in which fruiting bodies are formed.

Substrate. A source of nutrients which also acts as habitat.

Zone line. A mat or sheet of darkened mycelium which serves a protective function, seen as a dark line in wood sections.

Appendix

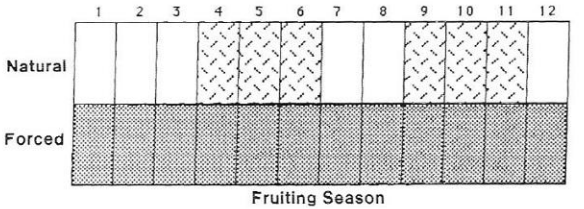
Shiitake Strain Characteristics

To illustrate the range of strain characteristics available for shiitake cultivation on logs, detailed descriptions are given for commercial shiitake strains produced by Northwest Mycological Consultants, Inc., 702 NW 4th St., Corvallis, Oregon, 97330, USA.

Wide-Range Strains

Wide-range strains produce mushrooms under a wide range of temperatures and are easily induced to fruit by soaking following a dry period. They are particularly well suited to forced fruiting and can be produced year-round under controlled conditions. Under natural conditions they fruit during the late spring and early autumn. These strains are fast colonizers and fruit within 6 to 12 months after inoculation. Growth during the spawn run is fastest at 77°F (25°C) but higher temperatures can be tolerated. They rapidly decay the logs and give heaviest yields during the second year of fruiting.

CS–41



All season, rapid fruiting strain.

Mushrooms: Medium-thick to thick caps with minimal white fringe.

Spawn Run: 6 to 12 months, aggressive, rapid colonizer.

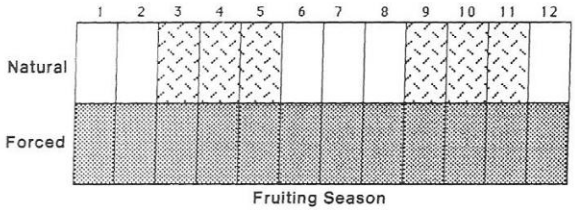
Induction: Soak water temp. optimum = 60° to 68°F (16°–20°C), range = 54° to 75°F (12°–24°C). Drying prior to soaking or irrigation is most effective. In winter, warm logs for two weeks prior to induction.

Pinning: Temp. optimum = 60° to 77°F (16°–25°C), range = 50° to 82°F (10°–28°C).

Fruiting: Temp. optimum = 50° to 64°F (10°–18°C), maximum = 77°F (25°C). Low relative humidity (60%–75%).

Cropping Cycle: Four fruitings can be evenly spaced throughout the year; can be fruited three times per year with 20 to 40 days of resting between fruitings. Three years of intensive fruiting can be expected.

CS–15



All season, rapid fruiting strain.

Mushrooms: Thick caps with heavy white fringe. Thick stems in good proportion to cap. Mushrooms often produced in clumps.

Spawn Run: 8 to 12 months, rapid colonizer, tolerant to low log moisture content.

Induction: Soak water temp. = 54° to 64°F (12°–18°C). Irrigate logs several weeks before induction to activate the mycelium. Logs should be warmed in the winter before induction.

Pinning: Temp. optimum = 60°F (16°C), range = 54° to 72°F (12°–22°C).

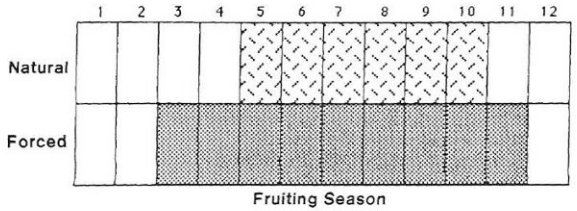
Fruiting: Temp. optimum = 50° to 64°F (10°–18°C), will tolerate higher temperatures.

Cropping Cycle: Highest yields are achieved if 3 to 4 fruitings are spaced evenly throughout the year; however, this strain can be fruited 3 times per year with 30 to 40 days between fruitings. At least three to four years of production can be expected.

Warm-Weather Strains

Warm-weather strains produce good quality mushrooms at high fruiting temperatures. These strains are readily induced to fruit in response to a drop in temperature. They are well adapted to fruiting in warm, moist areas from the spring until the late fall when periodic rains or irrigation induces fruiting. They are aggressive colonizers but typically will require an 8 to 12 month spawn run. Maximum growth during the spawn run is near 77°F (25°C), but growth is good at higher temperatures.

CS–24



Warm-weather, rapid fruiting strain.

Mushrooms: Robust, thick-fleshed caps with a thick white fringe. Stems are thick and well proportioned.

Spawn Run: 8 to 12 months, aggressive, rapid colonizer.

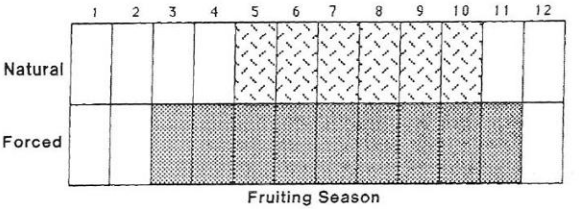
Induction: Optimum soak water temp. = 50° to 60°F (10°–16°C). Irrigation or soaking in tepid water after slight drying also effective.

Pinning: Temp. optimum = 60° to 64°F (16°–18°C), maximum = 75°F, (24°C).

Fruiting: Temp. optimum = 60° to 72°F (16°–22°C), maximum = 77°F (27°C).

Cropping Cycle: Fruitings can be forced two or three times with a 20 to 40 day resting period or fruiting can be spaced evenly throughout the year. Forced fruiting during the winter can be achieved only if logs are heated and irrigated prior to induction. Three to four years of high yields can be expected.

CS–125



Warm-weather, rapid fruiting strain.

Mushrooms: Medium to thick-fleshed mushrooms with moderate white fringe are produced at high temperatures. Stems thin to medium.

Spawn Run: 6 to 12 months, aggressive, rapid colonizer.

Induction: Easily induced by soaking or irrigation, soak water temp. optimum = 50° to 60°F (10°–16°C), maximum = 72°F (22°C).

Pinning: Temp. optimum = 60° to 68°F (16°–20°C), range = 50° to 72°F (10°–22°C). Heavy pinning often results in small mushrooms.

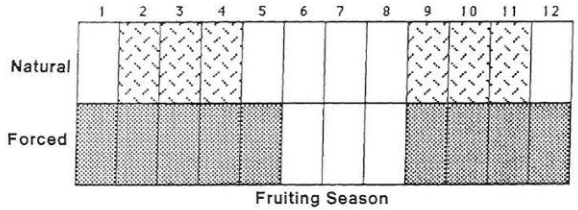
Fruiting: Temp. optimum = 50° to 68°F (10°–20°C), maximum 86°F (30°C). Low relative humidity (60%–75%).

Cropping Cycle: Resting period can be short (20–40 days) with three fruitings in rapid succession if logs are rested the remainder of the year. For winter fruiting, logs need several weeks of vigorous growth at warm temperatures prior to induction. Three years or more of high yields are to be expected.

Cold-Weather Strains

Cold-weather strains require cold or cool temperatures to fruit. With many of these, fruiting is arrested or aborted at high temperatures. Mushroom quality is very high due to cool fruiting temperatures. Induction generally occurs during a warming period following a cold period. Pinning can be slow after induction. These strains are fast colonizers, but many require a long spawn run (16 to 20 months). Fastest vegetative growth is near 77°F (25°C) but growth continues at colder temperatures. These strains have their highest yield after the second year and logs will produce heavily for four or more years.

CS–16



Cool/cold-weather strain.

Mushrooms: Robust, dense, thick-fleshed caps with heavy white fringe, often Donko grade.

Spawn Run: 16 to 20 months, rapid colonizer.

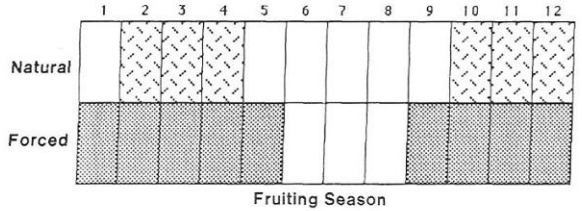
Induction: A cold period is needed prior to induction. Soak water temp. optimum = 39° to 54°F (4°–12°C).

Pinning: Temp. optimum = 45° to 60°F, (7°–16°C), range = 39° to 64°F (4°–18°C).

Fruiting: Temp. optimum = 45° to 64°F (7°–18°C). Low relative humidity (60%-75%). Development is slow due to low temperatures.

Cropping Cycle: Fruiting can be induced twice with only a 20 to 40 day resting period between times. After a longer resting period, this can be repeated. Yields are highest after the second year and logs will produce for four or more years.

CS–11



Cold-weather strain.

Mushrooms: Robust, thick-fleshed with a heavy white fringe and short stout stems, often Donko grade.

Spawn Run: 16 to 20 months, rapid colonizer, grows well at low temperatures.

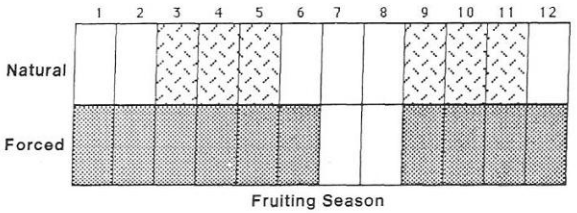
Induction: Responds slowly to soaking, requires a cold period prior to induction, soak water temp. = 39° to 54°F (4°–12°C).

Pinning: Temp. optimum = 39° to 60°F (4°–16°C), slow to pin.

Fruiting: Temp. optimum = 45° to 60°F (7°–16°C).

Cropping Cycle: Logs can be fruited three times each cool season. Fruiting will be most productive after the second year and the logs will continue to fruit for four or more years.

CS–118



Cool-weather, rapid fruiting strain

Mushrooms: Medium, thick-fleshed cap with dense white fringe retains in-curved margin. Stems thin, densely fringed and well proportioned to the cap.

Spawn Run: 6 to 12 months, aggressive rapid colonizer.

Induction: A cold period generally required prior to induction. Soak water temp. optimum = 50° to 64°F (10°–18°C).

Pinning: Temp. optimum = 45° to 64°F (7°–18°C).

Fruiting: Temp. optimum = 45° to 68°F (7°–20°C).

Cropping Cycle: Two or three fruitings can be induced in rapid succession with only 20 to 40 days of resting between fruitings or four fruitings can be more evenly spaced throughout the year. Logs will be productive for at least three years.

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KENDALL/HUNT PUBLISHING COMPANY
2460 Kerper Boulevard P.O. Box 539 Dubuque, Iowa 52004-0539