

15

Cultivation of Mushrooms in Plastic Bottles and Small Bags

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15.1 Introduction

Glass bottle cultivation of *Lentinula edodes* (Shiitake) and *Flammulina velutipes* (Enokitake) using sawdust was developed around 1928 by a Japanese *Agaricus* grower, H. Morimoto in Kyoto (Nakamura 1983). Subsequently, G. Hasegawa established a method of cultivation of *F. velutipes* on sawdust substrate in glass bottles. In the mid-1930s, commercial cultivation of *F. velutipes* using glass bottles was begun by farmers in northern Nagano, Japan. Though *F. velutipes* production increased in Nagano in the 1960s, farmers suffered heavy damage at the hand of the 1964 Niigata earthquake when glass bottles shattered. Consequently, glass bottles were replaced with plastic bottles.

Bottle cultivation of *L. edodes* began in Fuzhou, China and small plastic bag cultivation of this fungus developed in Taiwan around 1967 (Chang and Miles, 2004). In Japan, small plastic bag cultivation of *L. edodes* and *Grifola frondosa* (Maitake) was started commercially in the early 1980s and around 1975, respectively.

In 1997, button mushrooms (*Agaricus bisporus*) accounted for 32% of world mushroom production (Chang and Miles, 2004). By 2013, it is thought that *A. bisporus* production had reduced to a mere 13%. This eclipse was the result of a remarkable increase of “specialty mushroom” production in China. The main specialty genera cultivated in Asian countries include *Lentinula*, *Pleurotus*, *Flammulina*, *Hypsizygus*, *Grifola*, *Auricularia*, *Pholiota*, *Volvariella*, *Tremella*, and *Hericium*.

It is estimated that around 80% of world mushroom production in 2013 was cultivated in small plastic bags, and 4% of the production was cultivated in plastic bottles. Ninety-two percent of the world production of specialty mushrooms (excludes *Agaricus*) was cultivated using small bags (including use of non-sterilized substrate).

In Japan, 93% of edible mushrooms are cultivated in plastic bottles and bags. Small bag cultivation is the most popular method in Southeast Asian countries. In recent years, large-scale production of *F. velutipes*, *Hypsizygus marmoreus* (Buna-shimeji), and *Pleurotus eryngii* (King Oyster) using plastic bottles increased in China, Korea, and Thailand. Most recently, large-scale bottle production of *F. velutipes*, *P. eryngii*, and *H. marmoreus* by Japanese companies has begun in China, Taiwan, and Malaysia. Significant bag production of *G. frondosa* by a Japanese company has also begun in China.

This chapter covers the cultivation of specialty mushrooms under controlled environments using plastic bottles and small bags containing axenic substrate.

15.2 Characteristics of Mushroom Cultivation in Plastic Bottles and Small Bags

15.2.1 Plastic Bottles

Most large-scale mushroom production companies, as well as the big cooperative farms in Japan, China, Korea, and Thailand, adopt bottle cultivation. These organizations produce primarily *F. velutipes*, *H. marmoreus*, and *P. eryngii*. They utilize fully automated systems with specialized equipment.

A key advantage of plastic bottle cultivation is that it makes it possible to produce mushrooms in areas with limited land availability. In locations where land prices are high, such as Japan and the suburbs of China's big cities, intensive production in limited space is required. Shelving is not typically used during a significant duration of the bottle process and this greatly increases the density of cultivation rooms.

The use of rigid bottles makes it easy to mechanize large-scale mushroom production, and so automated cultivation equipment can be employed. Production costs are further reduced by the introduction of automated cropping and packaging machines. This new cultivation method saves significant labor by automating the whole production process.

Mushrooms cultivated in plastic bottles are generally produced in controlled-environment facilities, where temperature, humidity, concentration of carbon dioxide, and light illumination are maintained and monitored. Mushrooms can be easily grown year-round in these controlled facilities. In bottle cultivation, mushrooms are usually allowed only one flush, under short cycle times. A great deal of precision is required. Excellent cultivation technology and strict hygiene practices are prerequisites to providing high-yield and high-quality fruit bodies in bottle cultivation. Automation and environmental control contribute greatly to the stabilization and consistency of this intensive production model.

On the down side, these automated facilities require high capital construction inputs and the cultivation machines are not cheap. The initial set-up costs may be too high for many. Energy costs are high due to machinery power demands and environmental control needs.

15.2.2 Small Bags

The materials and supplements used in small plastic bag production are primarily sawdust and agricultural byproducts or residues, such as corncob meal, cottonseed hulls, rice bran, wheat bran, and corn bran. These are generally cheap and easily available throughout the world. Typically, the number of supplements for small bag cultivation is lower in comparison to bottle cultivation. As a result, substrate digestion and colonization are prolonged. However, the total yield of mushrooms is high (bags are often repeatedly flushed), and the biological efficiency is relatively higher than bottles.

Small-scale plastic bag cultivation does not require a big initial investment in large facilities and machinery. All processes can be performed manually in simple structures, for instance, plastic greenhouses (Figure 15.1). Bag cultivation is usually seasonal and is the world's most popular method for the cultivation of specialty mushrooms. In cultivation of *G. frondosa* in Japan, two large-scale production companies produce this fungus in small bags in huge facilities equipped with fully automated machinery (Figure 15.2).

15.2.3 Cultivation Containers

In bottle cultivation, heat-resistant polypropylene bottles with a capacity of 450–1100 ml and mouth openings of 52–78 mm are usually used. Each bottle is fitted with a cap equipped with

Figure 15.1 Small-scale bag cultivation of *Auricularia* in Vietnam.



Figure 15.2 Large facility for bag cultivation of *Grifola frondosa* in Japan.

a microporous-filter disk or polyurethane disk. Since mites invade caps and occasionally live in polyurethane filters, caps without such filters are often used. Small (450–550 ml), medium (600–800 ml), and large-sized bottles (850 to 1100 ml) are used. Figure 15.3 shows a tray containing bottles and several sizes of polypropylene bottles.



Figure 15.3 Polypropylene bottles and tray for cultivation.

In small bag cultivation, the growers generally use plastic bags filled with 1.0–3.0 kg (wet weight) of the mixed substrate. Large-scale mushroom companies use microporous-filtered polypropylene bags and the small-scale growers in Southeast Asia generally use polyethylene bags without a microporous filter that cannot withstand the high temperatures that polypropylene bags can (Figure 15.4).

15.2.4 Substrate Materials

Sawdust, cottonseed hulls, ground corncobs, and straw are the most popular basal ingredients in mushroom cultivation using plastic bottles and bags. A common substrate for specialty mushrooms is composed of the basal ingredients combined with starch-based supplements, such as rice bran, wheat bran, corn bran, millet, maize, and sorghum meal. The mushroom growers of Japan, China, and Korea sometimes use soybean skin, soybean meal, *tofu* refuse, malt feed, sugar cane bagasse, and beet pulp.

15.2.5 Mushroom Cultivation Processes in Plastic Bottles and Small Bags

The common cultivation processes of mushrooms in plastic bottles and small bags are as follows.

- 1) Preparation of substrate.
- 2) Mixing of substrate materials.
- 3) Filling into bottles or bags.
- 4) Sterilization.
- 5) Cooling the substrate.
- 6) Inoculation.
- 7) Spawn run (incubation).
- 8) Scratching.

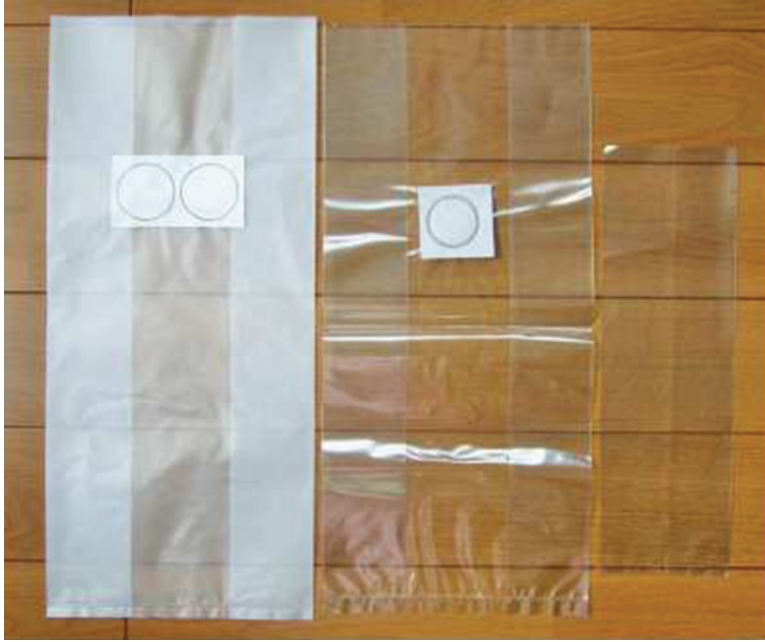


Figure 15.4 Polypropylene small bags with and without microporous filter.

- 9) Sprouting.
- 10) Growing.
- 11) Harvesting.
- 12) Packaging and shipment.

The mixing, filling, sterilization, and inoculation in small-scale cultivation using plastic bags are often entirely performed by hand. In contrast, the main processes in bottle cultivation are performed by automatic cultivation equipment irrespective of production scale. It is estimated that 92% of worldwide specialty mushroom production is cultivated in small-scale seasonal farms using small bags.

15.2.5.1 Substrate Mixing and Filling

Sawdust or cottonseed hulls are mixed with nutritional supplements and water in a mixer in bottle cultivation (Figure 15.5). The moisture content of the substrate is adjusted to 62–68%, depending on the mushroom species. Extended mixing times occasionally result in the rapid propagation of bacteria, especially during warmer seasons.

The moistened substrate is loaded into the bottle filler. Through the filler, bottles are loaded with a preset quantity of substrate. Once bottles are filled, compactors press the substrate in the bottle down to a preset height and then steel rods are used to put inoculation holes into the compact substrate (Figure 15.6). One to five inoculation holes are generally made. Most bottle growers and production companies also use machines to put on and remove the filter caps.

In small-scale bag culture in Asian countries, the mixed substrate is filled into heat-resistant polyethylene bags by manual labor or by a very simple semi-automatic filling machine (Figure 15.7). Large farms or production companies fill the substrate into bags with automatic filling machines. In the large-scale production companies of *G. frondosa* in Japan,



Figure 15.5 Mixing substrate.



Figure 15.6 Automatic filling machine for bottle cultivation.

microporous-filter patch bags are machine fabricated just prior to automatically filling the substrate into the bags. After filling, 1–5 inoculation holes are punched into the firm cylindrical or cubic substrate block.



Figure 15.7 Filling the substrate for small-scale bag cultivation of Shiitake in China.

15.2.5.2 Sterilization

The filled bottles and bags are loaded into a large autoclave (Figure 15.8). Low-tech bag growers will run sterilization cycles of 5–6 hours at 99–100°C without pressure. Bottle systems commonly use a shorter, hotter, pressurized sterilization cycle of 50–60 min at 118–120°C.



Figure 15.8 Large autoclaves for sterilization of substrate. (See color plate section for the color representation of this figure.)

Industrial large-scale pressure vessels are designed with double doors: one for entry and the other for exit of sterile materials.

15.2.5.3 Cooling and Inoculation

The sterilized bottles and bags are cooled to 20–25°C in a cooling room. Slow cooling in a pre-cooling room is advisable because condensation inside the caps occurs when hot bottles are rapidly exposed to cool air. During cooling, the room air flows into the bottles. When the room air is contaminated by microorganisms, the contaminants invade bottles and bags, where they can later propagate. Therefore, the cooling room should utilize strict hygiene and air supplied via HEPA filtration. Cooled bottles and bags are generally transferred from the cooling room through a pass box into the clean inoculation room by a wheel conveyer.

The bottles and the bags are usually inoculated with sawdust spawn using simple or automatic inoculation machines. Some small-scale bag cultivators in Asia inoculate inside low cost wooden boxes in the outdoors. Most mushroom growers using bottles and large-scale companies using small bags usually inoculate using fully automatic inoculation machines in clean rooms (Figure 15.9). The latest automatic machine can inoculate around 7,000–8,000 bottles in 1 h (in a 25 bottle system). Since bottles and bags are opened to introduce the spawn, inoculation rooms must employ high-sanitation HEPA filter systems.

The spawn used by both bottle and bag companies is generally supplied by specialized spawn makers. Large-scale mushroom companies and cooperatives develop hybrid cultivars themselves and make spawn for their own use. Although sawdust spawn is common, recently liquid spawn has gained popularity for large-scale cultivation of *F. velutipes* and *P. eryngii* in Japan, China, and Korea.

Figure 15.9 Automatic inoculation machine for bottle cultivation.





Figure 15.10 Stacking trays containing 25 bottles in incubation room.

15.2.5.4 Spawn Run (Incubation)

In bottle cultivation, 16, 25, and 36 bottles are held in one tray and trays are stacked 8–10 high on a pallet using an automatic palletizer. These pallets are placed on the floor in an air-conditioned incubation room (Figure 15.10). Temperature and humidity in the incubation room is generally maintained between 14–24°C and 65–75%, depending on the mushroom species. The upper limit of carbon dioxide concentration in the incubation room ranges from 2000–4000 ppm. The spawn run (including mycelial maturation) usually takes 21–80 days.

Some of the small, seasonal bag growers incubate in primitive greenhouses or brick houses under natural conditions. They will often organize the inoculated bags directly on the floor of the greenhouse or sometimes place them on shelves in a room. In most year-round Japanese bag cultivation companies, the growers incubate by placing bags on shelves in special environmentally controlled incubation rooms. To save handling, 4–6 substrate blocks are placed in trays, similar to the method of handling bottles.

15.2.5.5 Scratching (Kinkaki) in Bottle Cultivation

Scratching is the mechanical removal of the original inoculum and some surface of the colonized substrate for uniform fruiting in bottle cultivation of *F. velutipes*, *H. marmoreus*, *P. eryngii*, *P. nameko*, and *P. ostreatus*. Automatic scratching machines are equipped with a cap cleaning apparatus for brushing the inside of the caps (Figure 15.11). The scratched surface is sprayed with water to stimulate formation of fruit body primordia and to supply water for sprouting (Medasi).

15.2.5.6 Growing (Seiiku)

The induction of primordia requires low temperature, light, and a low concentration of carbon dioxide. When the scratched bottles are transferred into an induction room that has a lower



Figure 15.11 Scratching machine for fruiting.

temperature and lower concentration of carbon dioxide than the spawn run room, the mycelia shift from vegetative growth to reproductive growth. The environment for pinning and maturation depends on the mushroom species. At the pinning stage, the temperature of the fruiting room ranges from 13–20°C and the humidity from 75–95%. During the growth of the fruit bodies, the colonized substrate is generally maintained at 6–18°C, 85–98% humidity, 1500–2500 ppm carbon dioxide concentration, and 100–1000 lux illumination. For illumination, fluorescent lamps have been traditionally used, but LED technology is spreading.

15.2.5.7 Harvesting and Packaging

While the mushrooms cultivated in small bags can be harvested for approximately 3–5 months by repeated flushing, only one flush of mushrooms is usual in bottle cultivation. Harvested mushrooms are weighed and packaged into plastic bags or containers. In some Japanese large-scale production farms of *F. velutipes*, *H. marmoreus*, and *P. eryngii*, automated harvesting, weighing, and packaging machines are used to save labor costs, the highest of the production expenses (Figure 15.12).

15.2.5.8 Emptying the Substrate

After harvest, the spent substrate is removed from the bottles using automatic emptying machines. These emptying machines operate either by rotary blades or compressed air pressure. Recently, the compressed air emptying machine has become popular in Japan and China. The emptied bottles are washed and reused, while the used bags are thrown away or sometimes washed and recycled. The substrate is usually reused as compost for agriculture. For several years, the spent substrate of *F. velutipes* or *P. eryngii* has been recycled as substrate.



Figure 15.12 Automatic harvesting machine in *H. marmoreus* cultivation.

15.3 Cultivation Methods

15.3.1 *Flammulina velutipes* (Enokitake)

The commercial cultivation of *F. velutipes* began in Japan using 700 ml bottles with an opening of 52 mm in diameter. Later, 850 ml bottles with an opening of 58 to 65 mm became popular. More recently, 1100 ml bottles with an opening of 75–78 mm (16 bottles per tray) and 600–700 ml bottles with an opening of 65–70 mm (25 bottles per tray) are now generally used. While small-scale growers and most large-scale production farms in Japan, China, and Korea cultivate *F. velutipes* in plastic bottles using automated machinery, seasonal small-scale growers in China typically use plastic bags.

In China, large *F. velutipes* farms in the Shanghai area imported Japanese and Korean cultivation machinery at the end of the 1990s. *F. velutipes* production in China has dramatically increased more than 18-fold over the last 16 years from about 150,000 tons in 1997 (Chang and Miles, 2004) to 2,730,000 tons in 2013 (CEFA, 2015). The largest single company produces 160,000 tons annually. In Japan, there are no large companies cultivating *F. velutipes*, and the production by Nagano's cooperative farms is the largest. The annual production in Japan and Korea is 133,000 tons (Japanese Forestry Agency, 2015) and 33,000 tons in 2013, respectively. Nearly all of the 2,930,000 tons global production is cultivated in Asia and so *F. velutipes* is certainly an Asian mushroom.

Up to the mid-1980s, *F. velutipes* growers had to use brown-colored cultivars that turned light brown or brown when exposed to light during growth. However, the market demanded “whiteness” in fruit bodies. Therefore, growers had to cultivate the mushrooms in the dark to

prevent the coloration of caps and stems. Eventually, a novel white strain was developed in 1985. At present, most *F. velutipes* growers use white strains (Yamanaka, 1997).

15.3.1.1 Substrate and Filling

The substrate for *F. velutipes* production was traditionally based on the sawdust of Japanese cedar (sugi, *Cryptomeria japonica*). Most Chinese growers of *F. velutipes* use cottonseed hulls and ground corncobs rather than sawdust. Sawdust, corncob meal, and cottonseed hulls are used as the base ingredients and mixed with nutritional supplements. The recommended formula of substrate for *F. velutipes* cultivation in Nagano is corncob meal (35%: ratio in total dry weight of supplements), cottonseed hulls (5%), rice bran (33%), wheat bran (5%), sorghum meal (5%), dried *tofu* refuse (3%), beat pulp (10%), shell powder (4%), and sugi sawdust. The moisture content of the substrate is adjusted to 65–66%. The wet weight of the substrate filled into the bottles is 68–70 g per 100 ml of bottle capacity. For example, in 700 ml bottles, 470–500 g of the mixed substrate is mechanically filled into the bottle. After filling, 1–5 inoculation holes are punched vertically into the substrate. In small bag cultivation of *F. velutipes* in China, 1–2 kg cylindrical substrate blocks are in general use.

15.3.1.2 Inoculation and Spawn Run

Generally, growers purchase inoculum from spawn manufacturers with the exception of the large-scale company and cooperative farms that often produce spawn themselves. The growers within these cooperatives purchase fully myceliated bottles of liquid spawn grown in the incubation centers of the cooperative. Fifteen to twenty ml of liquid spawn is aliquoted to each bottle.

In bottle cultivation, after sterilization and cooling of the substrate, spawn is inoculated on the substrate surface by automatic inoculation machines. Approximately 9–11 g of sawdust spawn is used for 700–850 ml bottles and these are usually inoculated in strictly clean inoculation rooms. One 800 ml bottle of spawn can inoculate 45–55 bottles.

The inoculated bottles are transferred to a conditioned incubation room kept at 14–16°C, 65–75% humidity, and less than 3000 ppm of carbon dioxide. The spawn run is 21–26 days. Defective sprouting occasionally occurs in *F. velutipes* when the temperature of the substrate exceeds 21–22°C.

15.3.1.3 Scratching and Sprouting (Medashi)

After incubation, both the inoculum and the surface of the colonized substrate are removed by a scratching machine for uniformity of sprouting and fruiting. The scratched surface is also sprayed with water. Scratching should be performed before full mycelial colonization in the cultivation of *F. velutipes*. Waiting until after this time results in reduced number of fruit bodies and yield.

The scratched bottles are placed in sprouting rooms controlled at 13–15°C, 93–95% humidity, and about 1000 ppm of carbon dioxide concentration for 8–12 days. At 6–8 days after scratching, the primordia begin to form. When the young fruit body pilei grow to 1 mm in diameter and the stipe length is 3–5 mm 2–3 days later, the bottles are transferred to the acclimation room.

15.3.1.4 Preceding Period of Growth

15.3.1.4.1 Acclimation (Narashi)

The young fruit bodies dry and abort if, after growing at 13–15°C in the sprouting room, they are abruptly placed into cold conditions of 3–5°C. To counter this, bottles are placed in an

acclimation room maintained at 7–8°C and 90% humidity for 3–4 days to acclimate the fruit bodies in a step-wise manner to the target temperature of 3–5°C, known as restriction.

15.3.1.4.2 Restriction (Yokusei)

After acclimation of the young fruit bodies, the bottles are placed in the restriction room controlled at 3–5°C, 85–90% humidity, and 1000 ppm of carbon dioxide concentration for 5–7 days to restrict the elongation and to equalize the stipe length. Air circulation and illumination are also used to restrict irregular elongation of the stipe.

15.3.1.4.3 Applying the Plastic Collar

When the stipe has elongated about 1–2 cm above the mouth of the bottle, a permeable plastic collar is placed around the bottle neck and secured with a Velcro strip. The collared bottles are moved into the growing room. This collar serves to hold the mushrooms in place so that the development of the pilei is inhibited and the elongation of the stipes is promoted as a result of increased carbon dioxide concentration (Figure 15.13).

15.3.1.5 Late Period of Growth

The growing rooms are maintained 5–7°C, 75–80% in humidity, and 1000 ppm of carbon dioxide concentration. 150–300 lux of illumination for 30–60 minutes per day is required when the stipes reach 6–7 cm long. Excessive humidification in the growing room causes watery mushrooms.

15.3.1.6 Harvesting and Packing

About 25–30 days after scratching, the mushrooms are harvested when the stipes are 13–14 cm (Figure 15.14). Mushrooms are usually vacuum packed in polypropylene bags. *F. velutipes* is traditionally packaged and marketed in a 100-g pack, but now, entire bunches of this fungus are vacuum packaged and shipped. The average yield in bottle cultivation is in the range of



Figure 15.13 Fruit body development of *F. velutipes* after scratching.



Figure 15.14 *F. velutipes* fruiting in bottle cultivation. (See color plate section for the color representation of this figure.)

210–240 g per 700 ml bottle containing 480–520 g of substrate and 300–340 g per 1100 ml bottle containing 750–810 g of substrate. Biological efficiencies are 127–145% and 100–130%, respectively. In small bag cultivation of this fungus in China, the total yield in three croppings is around 400 g in 1.0 kg of substrate (Figure 15.15).



Figure 15.15 *F. velutipes* fruiting in small bag cultivation in China.

15.3.2 *Hypsizygus marmoreus* (Buna-shimeji)

The cultivation of *H. marmoreus*, which originated in Nagano, Japan, is now widespread throughout Japan and China. Large-scale mechanized facilities with production capacities of 10 tons per day are found throughout both nations. The present annual production of this fungus is 117,000 tons in Japan and 146,000 tons in China.

15.3.2.1 Substrate and Filling

Commercial production of *H. marmoreus* is commonly on a substrate of sawdust or corncobs contained in 850 ml bottles with an opening between 58–65 mm in diameter. Recently, growers and large-scale production companies use smaller 450–700 ml bottles with an opening of around 60 mm. The inside of the cap typically has a projecting structure that presses the spawn surface lightly.

The substrate for *H. marmoreus* is based on pine wood sawdust and corncobs. In Nagano, a substrate consisting of 20 g (wet weight) of corncobs, 50 g of rice bran, 20 g of wheat bran, 20 g of soybean hull, 10 g of cottonseed hulls, 10 g of dry *tofu* refuse and sawdust in 850 ml bottles is recommended. Around 450 g of the mixed substrate in 700 ml bottles and around 510 g of the substrate in 850 ml are mechanically filled into the bottle. After filling, one inoculation hole is generally punched into the substrate, simultaneously compacting the substrate within the bottle. Some growers punch 3–4 inoculation holes into the substrate to shorten the period for spawn run and mycelial maturation.

15.3.2.2 Inoculation and Spawn Run

Generally, growers of *H. marmoreus* purchase inoculum from spawn manufacturers. Large-scale companies and cooperatives develop hybrid strains and use spawn made in-house. Some growers belonging to farmers' cooperatives purchase pre-inoculated and colonized substrate from the cooperative's incubation centers. All they need do is fruit the bottles for cropping and packing.

After sterilization and cooling the substrate, the spawn is inoculated on the substrate surface with automatic inoculation machines. The amount of spawn for *H. marmoreus* is greater than for other species, because fruiting will take place directly from the surface of that spawn. The usual dose is between 13–15 g of sawdust spawn for a 850-ml bottle and 12–13 g for a 700-ml bottle. Primarily gray-brown strains of *H. marmoreus* are cultivated in Japan, however, one large production company cultivates a white strain too.

The inoculated bottles are placed in an environmentally controlled incubation room conditioned at 21–23°C, 65–75% humidity, and less than 4000 ppm carbon dioxide concentration. After a 28–35-day spawn run, the bottles are incubated for an additional 32–45 days of mycelial maturation. Trays containing 16, 25, or 36 bottles are piled up on a pallet using automatic palletizers to a height of about 8–10 trays (Figure 15.10). When using trays that accommodate 25 or 36 bottles, 1–4 bottles are pulled out of the center of the tray to avoid an undesirable substrate temperature rise.

15.3.2.3 Scratching and Growing

Specialized scratching is performed in the cultivation of *H. marmoreus*. After spawn run and complete mycelial maturation, only the peripheral portion of the spawn that covers the substrate surface is removed (Figure 15.16). This leaves the central area of the spawn untouched. Fruiting will occur directly from this area. The scratched surface is lightly sprayed with water.

Scratched and sprayed bottles are covered with perforated plastic sheeting and are moved into a growing room conditioned at 14.5–16.0°C, 96–98% humidity, around 2000 ppm of carbon dioxide concentration, and 50–100 lux of illumination. Nine to ten days

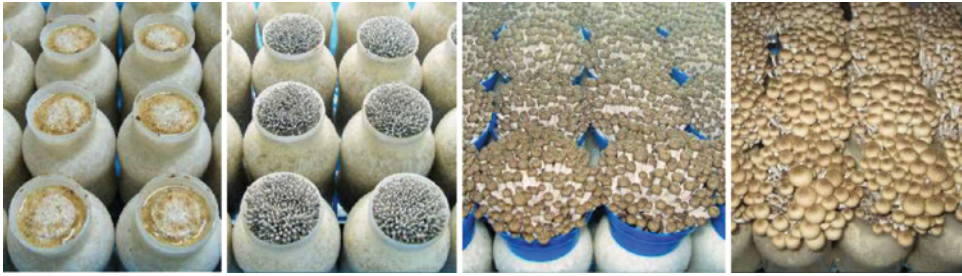


Figure 15.16 Fruit body development of *H. marmoreus* after scratching. (See color plate section for the color representation of this figure.)

after scratching, when the young fruit bodies grow and contact the plastic sheeting, the covering is removed. Then the bottles are kept in an environmentally controlled room at 14.5–16°C, 96–98% humidity, 1500–1800 ppm carbon dioxide, and 800–1000 lux (15–30 min for 12 h per day). Mushrooms are harvested 21–24 days after scratching, depending on strain (Figure 15.17).

15.3.2.4 Harvesting and Packaging

The average yield is in the range of 190–220 g per 700 ml bottle containing 450 g of substrate and the biological efficiency is 120–140%. This fungus has been traditionally packaged in 100 g polystyrene containers by splitting the bunch. In recent years, packaging an entire bunch into plastic bags without splitting has become common. An excellent cultivation technique is required to employ bunch packing because predictably equal bunch weights must be consistently produced.



Figure 15.17 *H. marmoreus* fruiting in bottle cultivation.

15.3.3 *Pleurotus eryngii* (King Oyster)

Bag cultivation of *P. eryngii* by burying the myceliated colonized blocks underground began in the late 1970s or early 1980s in northern Italy (Ferri, 1985). Bottle cultivation of this fungus began in Taiwan around the end of the 1980s. Thereafter, production has increased rapidly in recent years after a large-scale Japanese production company began experimental bottle cultivation in 1992. At present, bottle cultivation of *P. eryngii* has spread in China, Korea, and Thailand because this fungus is delicious and possesses a long shelf life compared to the other *Pleurotus* species. The production of *P. eryngii* in 2013 was about 40,000 tons by bottle cultivation in Japan and 673,000 tons in China, which includes production both in bottles and in bags.

15.3.3.1 Substrate and Filling

Most growers of *P. eryngii* have generally used a substrate of sawdust, corncob meal, and cottonseed hulls contained in 850 ml bottles. The large-scale production companies use small 450–700 ml bottles with an opening of 52–64 mm.

The substrate for *P. eryngii* production is based on the sawdust of Japanese cedar and corncobs. To the mixed sawdust and corncobs are added 70–80 g (wet weight) of supplement consisting of rice bran, wheat bran, and dried *tofu* refuse. The mixed substrate is adjusted to 65–68% moisture content. The 850 ml bottles contain 510–530 g of wet substrate. In a substrate using only corncobs as the base material, 130 g (wet weight) of corncobs, 20 g of rice bran, 20 g of wheat bran, 20 g of sorghum meal, 30 g of soybean skins, and 15 g of dried *tofu* refuse are mixed and 550–580 g of the substrate fills a 850 ml bottle.

15.3.3.2 Inoculation and Spawn Run

The growers of *P. eryngii* purchase inoculum from spawn manufacturers. Large-scale production companies and large cooperatives develop new hybrid strains for their own use. The 850 ml bottles are usually inoculated with 10–13 g of sawdust spawn using automatic inoculation machines in a clean room. Liquid spawn is usually used in the production of *P. eryngii* in China and Korea. Fifteen to twenty ml of liquid spawn is inoculated in this case.

The inoculated bottles are placed in an environmentally controlled incubation room conditioned at 21–23°C, 65–80% humidity, and less than 4000 ppm of carbon dioxide. After a 23–25-day spawn run, the bottles are incubated for an additional 4–6 days. The environmental management is very important because the colonizing mycelia of *P. eryngii* differentiate easily into fruit body primordia on top and within the bottle under low temperature air flow and bright illumination.

15.3.3.3 Scratching and Growing

After incubation, the spawn and the surface of the colonized substrate are removed. The scratched surface is sprayed with a little water. Then bottles are covered with perforated plastic sheeting or old newspapers and transferred into a growing room conditioned at 14–16°C, 75–95% humidity, less than 3000 ppm of carbon dioxide concentration, and 100–200 lux of illumination. Another method for sprouting is flipping the bottles upside down. The sprouting period required for this flipping method is prolonged compared with the standard method.

When the young fruit bodies grow and make contact with the plastic sheet or newspaper, the covering is removed. Then the bottles are placed in an environmentally controlled room at 14–16°C, 75–95% humidity, less than 3000 ppm carbon dioxide, and 100–500 lux illumination.

Mushrooms are harvested 13–16 days after scratching. Recently, growers began using LED illumination systems instead of fluorescent lighting (Figure 15.18).



Figure 15.18 *P. eryngii* fruiting in bottle cultivation under LED illumination. (See color plate section for the color representation of this figure.)

15.3.3.4 Harvesting and Packaging

The yield of *P. eryngii* is in the range of 160–190 g per 850 ml bottle and 150–180 g per 700 ml bottle. The biological efficiencies are 70–100% and 90–110%, respectively. After trimming the stems near the base with a knife, the fruit bodies are weighed and packaged into plastic containers or plastic bags (Figure 15.19).



Figure 15.19 Packaging of *P. eryngii*.

15.3.4 *Pholiota nameko* (Nameko)

P. nameko is originally from the highlands and high latitudes of Japan and has traditionally been eaten by the people of northern Japan. So, the production of *P. nameko* began with log cultivation around 1921 in the Tohoku district (northeastern Japan). In the 1960s, wooden tray cultivation and small bag cultivation using sterilized sawdust substrate spread in eastern Japan. Bottle cultivation was developed in the early-1980s and at present, this production method is the most popular in Japan. The production of *P. nameko* in 2013 was about 23,000 tons in Japan and 960,000 tons in China. The production of *P. nameko* is rapidly gaining popularity in other Asian countries.

15.3.4.1 Substrate and Filling

The growers of *P. nameko* generally use a substrate of hardwood sawdust using 800 ml bottles with 78 mm openings. Supplements such as corn bran, wheat bran, and dried *tofu* refuse in the ratio 10% of total fresh substrate weight are mixed with sawdust. The mixed substrate is adjusted to a 64–65% moisture content and bottles contain 540–560 g of wet substrate.

15.3.4.2 Inoculation and Spawn Run

The growers of *P. nameko* generally purchase 850–1500 ml spawn bottles from spawn manufacturers. One 1500 ml sawdust spawn bottle can inoculate 100–150 bottles. The inoculated bottles are placed in an incubation room maintained at 16–18°C in the early period (22–23°C in the latter half of the spawn run), 65–80% humidity, and less than 2500 ppm of carbon dioxide. Spawn run of *P. nameko* usually takes 45–65 days, depending on the strain.

15.3.4.3 Growing and Harvesting

The spawn and the substrate surface are mechanically removed with a scratching machine and the scratched surface is sprayed with water. Afterwards, the bottles are placed upside down in a growing room at 14–16°C, more than 90% humidity, less than 2000 ppm of carbon dioxide, and around 200 lux illumination in daytime. Seven to ten days after scratching, primordia begin to form. 25–40 days later, the mushrooms are harvested (Figure 15.20). Average yield is 180–200 g per 800 ml bottle. The biological efficiency is 95–115%.

15.3.5 *Pleurotus ostreatus* (Oyster Mushroom, Hiratake)

It is estimated that 2013 global production of *Pleurotus* spp. (*P. eryngii* excluded) reached 6,340,000 tons including 6,150,000 tons produced in China. Most of the production is cultivated using small bags. *P. ostreatus* production in Japan peaked in 1989 at 35,700 tons (Yamanaka, 1997) and the production was only 2,290 tons in 2013 (Japanese Forestry Agency, 2015), a decrease of more than 16-fold in 24 years. The reason is because *P. ostreatus* growers shifted to *P. eryngii* production. Ninety-nine percent of the total production is by small or big bag cultivation using sterilized or non-sterilized substrate by small-scale growers. Most small-scale *P. ostreatus* growers in Japan and some growers in Korea produce using bottle cultivation.

15.3.5.1 Substrate and Filling

Conifer sawdust is used as the base medium for bottle cultivation in Japan and 850 ml bottles with 58–65 mm openings are generally utilized. Supplements such as rice bran, wheat bran, and hominy feed are mixed with sawdust and the substrate is adjusted to 64–67% moisture content; 480–500 g of substrate is filled into plastic bottles and the bottles are sterilized in an autoclave.



Figure 15.20 *P. nameko* fruiting in bottle cultivation. (See color plate section for the color representation of this figure.)

The small bag growers of this fungus in China and other Asia countries mostly use cotton-seed hulls, wheat or rice straw, corncobs, rice bran, wheat bran, and sugarcane bagasse for the ingredients. The substrate is pasteurized or is nearly sterilized at 98–100°C in a steaming chamber at atmospheric pressure. Bags are typically 1–3 kg wet weight.

15.3.5.2 Inoculation and Spawn Run

In bottle cultivation, an 850-ml sawdust spawn bottle will inoculate about 40–50 bottles. The inoculated bottles are placed in an incubation room maintained at 21–23°C, 65–75% humidity, and less than 4000 ppm of carbon dioxide for 22–25 days. After the spawn run, the bottles are incubated an additional 3–5 days. In small bag cultivation, 20–30 g of spawn is inoculated to the bag and the inoculated bags are incubated at 23–27°C for 35–50 days.

15.3.5.3 Growing and Harvesting

The colonized substrate of *P. ostreatus* in bottle cultivation is usually scratched. After the scratching and water spraying, bottles are placed in a growing room at 14–16°C, more than 90% humidity, less than 2000 ppm of carbon dioxide and 200–500 lux illumination. The bottles are put upside down for sprouting. Five to seven days after scratching, the fruit body primordia form. Around 1 week later, the mushrooms are harvested (Figure 15.21). The yield is 100–120 g per 850 ml bottle. The biological efficiency is 60–80%, which is the lowest of the cultivated mushrooms. In the case of small bag culture, 3–4 flushes are possible. Therefore, the total yield of mushrooms is 800–900 g per 2.5 kg block in bag cultivation and the biological efficiency ranges from 90 to 110%.

15.3.6 *Grifola frondosa* (Maitake)

The production of *G. frondosa* began with sawdust cultivation using plastic bags in the Tohoku district of Japan around 1975. Large-scale production of this mushroom by bag cultivation started in 1983, and the production rapidly increased in eastern Japan. The volume sold in 2013



Figure 15.21 *P. ostreatus* fruiting in bottle cultivation.

was 45,450 tons in Japan and 21,000 tons in China. The majority of worldwide production takes place in Japan and China. In Japan, 76% of the production is cultivated in polypropylene small bags by general growers and two large-scale companies. The remaining 24% is cultivated in plastic bottles by a large mushroom production company. Amazingly, 84% of *G. frondosa* production in Japan is cultivated by only three mushroom companies (Yamanaka, 2011).

15.3.6.1 Substrate and Filling

A substrate base of hardwood sawdust and hardwood chips is generally used in bottle and bag cultivation. Companies employing bottle cultivation use bottles of around 750 ml in volume. Supplements such as rice bran, wheat bran, corn bran, and dry *tofu* refuse are mixed with sawdust, and the substrate is adjusted to a 63–66% moisture content.

Small bag cultivators of this fungus in Japan and China mostly use sawdust, wood chips, cottonseed hulls, rice bran, and wheat bran for the ingredients. They usually fill bags with 1–3 kg of substrate. In Japanese bag cultivation, 80% of the production is cultivated in 2.5–3.0 kg blocks and 20% is cultivated in 1.5–1.7 kg blocks. Growers usually use polypropylene bags with heat-sealed permeable filters (Figure 15.4). The moistened substrate is filled into bags with semi-automatic or automatic filling machines, compacted, and reamed with 4–6 inoculation holes.

15.3.6.2 Inoculation and Spawn Run

The typical growers of *G. frondosa* purchase inoculum from spawn manufacturers, however three large-scale companies in Japan have developed new hybrid strains and operate their own spawn divisions. Though commercial strains are popular in gray-black, the two large-scale bag companies also cultivate white isolates. Ten to fourteen grams of spawn is inoculated to a 2.5 kg substrate block.

The inoculated bags are placed in the incubation room maintained at 20–23°C, 65–70% humidity, and less than 2000 ppm of carbon dioxide for 30–50 days, depending on strain, substrate, and cultivation method. Thereafter, a white mycelial mass (primordia) is formed and swells on the surface of the substrate.

15.3.6.3 Growing and Harvesting

When the mycelial mass changes color to a dark-gray or black in the incubation room, the bags are transferred into a sprouting room maintained at 20°C, 75–90% humidity, and 250–600 lux illumination. Upon moving into the sprouting room, the bags are cut to allow the primordial mass to develop. In another sprouting method, the bags are cut after the mycelial blocks are transferred into a growing room controlled at 17–19°C, 92–95% humidity, less than 1000 ppm CO₂, and 600–1,000 lux illumination.

Four to five days after cutting the bags, black and large primordia rapidly develop and differentiate into young fruit bodies by exposure to fresh air. Blue light is used to make the pilei darker during the latter half of development because Japanese consumers prefer dark-black pilei over gray. The fruit bodies are harvested 8–10 days after cutting the bags (Figure 15.22). In Japanese cultivation of *G. frondosa*, only one crop is usual and the yield from a 2.5 kg substrate block is 480–650 g (biological efficiency is 56–76%) and the yield from 1.1 kg of substrate is in the range of 220–300 g (biological efficiency is 60–80%). The best yield of 400–500 g (biological efficiency 74–92%) is obtained from 1.6 kg mycelial blocks cultivated using an excellent hybrid strain and the best substrate. The cluster of fruit bodies of *G. frondosa* are usually sliced, weighed, and packaged.

15.3.7 *Lentinula edodes* (Shiitake)

Dried Shiitake production by log cultivation in Japan has decreased gradually because it requires hard labor, growers are aging, and there is a deficiency of high-quality logs. Additionally, the



Figure 15.22 *G. frondosa* fruiting on colonized substrate in bag cultivation. (See color plate section for the color representation of this figure.)

number of logs produced in the Tohoku district decreased sharply because many oak trees for log cultivation suffered from radioactive contamination following a nuclear plant accident in 2011.

In small bag cultivation of *L. edodes*, the cultivation cycle is short compared with log cultivation. Labor costs are saved by taking advantage of mechanization and the use of small and light mycelial blocks. Year-round production is possible by modestly equipped small plants. For these reasons, production of fresh *L. edodes* in bag cultivation has rapidly increased since 1993 in Japan. Bag-grown product accounts for about 89% of fresh Shiitake and 65% of the total Shiitake production (including the dry Shiitake production converted in wet weight).

On the other hand, *L. edodes* has been produced traditionally by small bag cultivation in China. Chinese Shiitake production in 2013 was about 7,100,000 tons and the quantity is estimated to be 98% of worldwide Shiitake production.

15.3.7.1 Substrate and Filling

Two main types of cultivation methods are popular in Japan. The first model has growers purchase bags, sawdust, and supplements, along with spawn from spawn manufacturers. With the technical guidance of the spawn manufacturers, growers control the entire cultivation cycle themselves from substrate through to harvesting. The second model has growers purchasing fully colonized mycelial blocks from the spawn manufacturers and they then cultivate from sprouting through to harvesting. Lately, there has been a considerable increase in Japanese Shiitake growers purchasing fully colonized mycelial blocks from China.

The substrate is based on 30–40% hardwood chips mixed with hardwood sawdust. Supplements such as rice bran, wheat bran, and corn bran are mixed with sawdust, and the substrate is adjusted to a 62–64% moisture content. In China, cottonseed hulls and corncobs are also popular basal ingredients. The nutritional supplements are generally added to the mix at a 10–12% ratio (wet weight) of the substrate. The moistened substrate is poured into bags with 1–2 microporous filters by semi-automatic or automatic filling machines. Four to six inoculation holes are vertically opened after the substrate is pressed into a cubic or cylindrical form. General weight of the substrate is 2.5–3.3 kg. One spawn manufacturer sells 1.1 kg colonized substrate blocks.

15.3.7.2 Inoculation and Spawn Run

Most growers of *L. edodes* purchase their inoculum from spawn makers. After sterilization and cooling, 15–22 g of spawn is inoculated to a 2.5 kg substrate. The inoculated bags are placed in an incubation room maintained at 18–21°C in the early incubation period, thereafter, in the latter half of the incubation period at 20–22°C, 65–70% humidity, and less than 3000 ppm of carbon dioxide. Mycelia colonize the substrate within 28–35 days, depending on strain and substrate formula, and the incubation requires an additional 50–70 days for mycelial maturation. During this time, the substrate surface changes color from white to dark brown and all, or part, of the bags are shifted to a fruiting environment.

15.3.7.3 Primordia Formation

Fruit body primordia begin to form under the surface of the colonized substrate block in an environment of 18–20°C, 60–80% humidity, less than 2000 ppm carbon dioxide concentration, and 100–300 lux illumination. After maintaining this condition for 10–20 days, the colonized substrate blocks are transferred into the growing room.

15.3.7.4 Growing and Harvesting

Although, the optimum temperature for fruit body growth is 15–18°C, growers typically employ a broad temperature fluctuation regimen during which the highest temperature is

22–23°C and the lowest is 12–13°C. Fruit bodies of *L. edodes* develop at 80–85% humidity, less than 2000 ppm and 100–1,000 lux illumination. Ten to twenty days after spraying water over the blocks, the mushrooms are harvested over a 7–20-day period, depending strongly on the strain. After a flush, growers use a rest-dehydration-rehydration cycle to initiate additional flushes – up to five or six over 3–4 months. The yield from a 2.5 kg substrate is 1.0–1.1 kg and the biological efficiency is 105–120%.

Two types of flushing methods for *L. edodes* cultivation have become popular in Japan. One is the traditional flushing from the entire surface of the block after removing the colonized substrate completely from the bag. The other is flushing from only the top surface of the block after cutting or turning down the bag to expose a quarter of the block.

15.3.7.4.1 Entire Surface Flushing Method

Mushrooms are flushed from the top and all side surfaces of the block after humidifying or spraying. The main advantages of this flushing method are early flushing and limiting damage to the colonized blocks. On the other hand, humidifying or spraying requires labor, and cropped mushrooms are sometimes damaged by watering. Occasionally, the colonized blocks are placed at 28°C before removing the bags to harvest fewer, larger, high-quality fruiting bodies (Figure 15.23).

15.3.7.4.2 Top Surface Flushing Method

Water is filled into the bag that has been turned down, and thereafter the mushrooms flush mainly from the top surface of the block. Spraying the blocks and injecting water into them is unnecessary. Therefore, quality degradation by overhydration is avoided. Because growth from the sides and the bottom of the block is prevented, large and high-quality fruit bodies are produced from the top surface. To reduce the number of fruit bodies, the colonized blocks are often placed at 27–28°C before flushing. Recent work has found that further benefit is possible by exposing the top surface 3–4 weeks earlier than usual, spraying with water and keeping at



Figure 15.23 *L. edodes* fruiting on colonized substrate removed from bags (See color plate section for the color representation of this figure.)

20–23°C. Such treatment can encourage flushing 2–3 weeks earlier than the usual high temperature regimen. Discolored water in the bag should be regularly drained away, allowing the water-covered surfaces exposure to fresh air prior to refilling the bags with water.

15.3.7.5 Resting

The mycelial blocks are rested in the growing or resting room maintained at 20–25°C, 60–90% humidity, less than 2000 ppm of carbon dioxide concentration, and 50–100 lux for 12–15 days. In the entire surface flushing system, the blocks are rested at 16–22°C after spray washing the microorganism off the block surface at 23–25°C for 2 days. In the entire surface flushing, it is possible to flush continuously for 5–6 months by spraying water onto the blocks for a few hours per day under fluctuating temperature conditions. The resting period in the top surface system is generally 3–7 days under high temperature conditions of 23–27°C.

15.4 Diseases in Mushroom Cultivation in Plastic Bottles and Small Bags

In mushroom cultivation in plastic bottles and small bags, all living microorganisms in the growth substrate are essentially killed (deactivated) by sterilization autoclaving. Therefore, the fungal and bacterial contamination of substrate is generally caused by use of contaminated spawn, airborne spores, contaminated dust, mites, and contaminated workers. Most contamination is the result of:

- Infection by airborne fungal spores and/or bacteria of the substrate during post-sterilization cooling and inoculation.
- The invasion of mites carrying fungal spores and bacteria into bottles during spawn run.
- Bacteria and the spores of imperfect fungi occasionally contaminate the substrate through pin holes at the bottom of bags.

15.4.1 Fungal Diseases

15.4.1.1 *Trichoderma* Diseases

Mycoparasitic *Trichoderma* (green mold) is a ubiquitous soil fungus and is the common mushroom contaminant in cultivation in bottles and bags. *Trichoderma* spp. proliferated into the substrate degrade not only active mushroom mycelium but the mycelium in spawn. *Trichoderma* can completely prevent mushroom fruiting and cause serious damage to crops. *T. viride* and *T. harzianum* are well known as the most serious and damaging contaminants in bottle cultivation of *Hypsizygus marmoreus* and in bag cultivation of *Lentinula edodes*.

Serious *Trichoderma* diseases are frequently found in association with mushroom mites. The mites feed on the mycelium and the conidiospores of *Trichoderma* and *Penicillium* spp. and then carry these into uninfected bottles. Some mite species have pouches on their bodies that hold mold spores and mycelium. This increases the likelihood that they will “seed” their own food as they travel about. The population of mites increases explosively with propagation of *Trichoderma*. The mites move from contaminated bottles to uninfected bottles until finally *Trichoderma* contamination expands throughout the incubation room. An incubation room heavily populated by mites can result in 100% of newly inoculated bottles becoming lost to contaminant infection in less than 7 days. In general, the fruit bodies of specialty mushrooms are hardly affected by *Trichoderma* spp. and do not develop cap spotting. In bag cultivation of *L. edodes*, *Trichoderma* spp. sometimes infect wet block surfaces and the cut-off stumps that remain on blocks after harvesting.

For protection against *Trichoderma* diseases in bottle cultivation, disinfectant mats should be placed at the entrance of cultivation rooms. Bottles and bags contaminated by *Trichoderma* must be removed from incubation rooms immediately upon finding these bottles. The contaminated substrate should be emptied from bottles and bags after a low temperature autoclaving of about 80–100°C.

15.4.1.2 Cobweb Disease, *Cladobotryum* Infection

Several species of *Cladobotryum* spp. are well known, common pathogens causing cobweb disease in *Agaricus* cultivation (Fletcher and Gaze, 2008). Cobweb disease (“cottony mold disease” in Japanese) has been observed in bottle cultivation of *Flammulina velutipes*. Recently this disease has frequently been found in bottle cultivation of *H. marmoreus* and *Pleurotus eryngii*. The species causing cobweb disease in *F. velutipes* and *H. marmoreus* is identified as *C. varium*. The species *C. mycophilum* found on *P. eryngii* produces a yellowish-red pigment (Back et al., 2012). Occasionally, infected fruit bodies of *P. eryngii* turn a pink color.

Contamination of substrate by this mold is rare. The typical symptom of cobweb diseases in bottle and bag cultivation is a fluffy, down-like growth of mycelium over the young fruit bodies of *F. velutipes* (Figure 15.24a) and *H. marmoreus* (Figure 15.25). Affected fruit bodies are gradually enveloped by a soft powdery mycelium (Figure 15.24b). In *P. eryngii*, *Cladobotryum* colonizes mainly by crawling up the stem. Sometimes, mycelium of the pathogen grows on the stem of *P. eryngii* after packaging, while on the market.

Contaminated mushrooms, *Cladobotryum*-infected debris, and airborne spores in growing rooms are the initial infection sources. To avoid the spread of infection, all primary pathogen sources should be removed from the growing room through strict cleaning. *Cladobotryum* spp. prefer to grow in wet, dead-air pockets such as the corners of the growing room. Environmental control such as absolute humidity control and efficient ventilation in the growing room is very important to prevent cobweb disease in bottle cultivation. Vigorous and healthy fruit bodies are able to escape infection with cobweb disease because *Cladobotryum* spp. are opportunistic pathogens that attack weak mushrooms.

In bottle cultivation of *F. velutipes*, reused plastic collars contaminated with *Cladobotryum* can cause the disease. Contaminated plastic wrapping collars must be washed and disinfected before reuse.

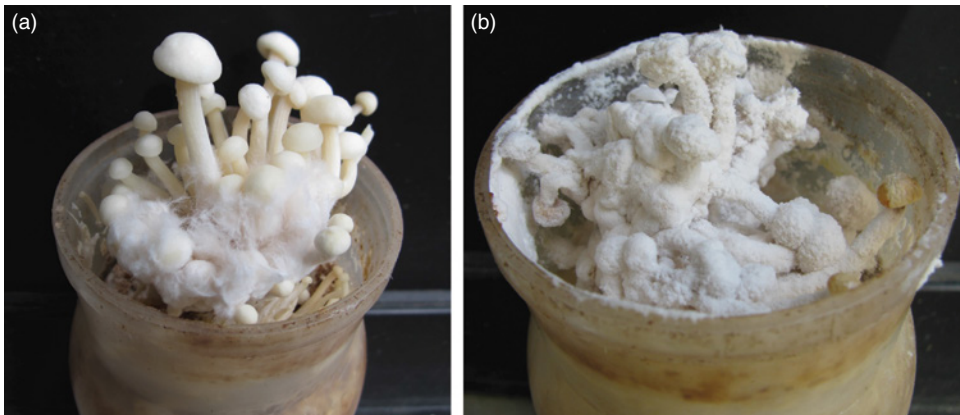


Figure 15.24 Fruit bodies of *Flammulina velutipes* affected by *Cladobotryum varium*. Mushrooms covered with the fluffy mycelium in the early stage (a) and enveloped with soft powdery mycelium at a later stage of disease development (b). (See color plate section for the color representation of this figure.)



Figure 15.25 *C. varium* attacking fruit bodies of *Hypsizygus marmoreus*.

15.4.1.3 Other Fungal Diseases

Imperfect fungi such as *Penicillium*, *Aspergillus*, *Cladosporium*, and Zygomycetes such as *Mucor* and *Rhizopus* are extremely common contaminants in bottle and bag cultivation facilities. These contaminants are not mycoparasitic pathogens for mushroom mycelium but are antagonistic molds or weed molds growing in the substrate. The mushroom's mycelial growth is seriously inhibited by fast-growing weed molds as they compete for food in the confined space of a bottle. Consequently, the molds inhibit mushroom fruiting and cause crop losses.

Zygomycetes rapidly grow in bottle and bag substrates. It is fully possible for mushroom mycelium to grow through a substrate that has been previously colonized by one of these contaminants. When this happens, the contamination is difficult to distinguish from mushroom mycelium by the end of spawn run. Occasionally therefore, spawn contaminated with Zygomycetes is used as inoculum and this is unnoticed.

After autoclaving, condensed dew is usually formed on the underside of the caps during substrate cooling. When the cooling room and the incubation room are contaminated by airborne microorganisms, contaminated dew (including mold spores and bacteria) fall down on the substrate surface. Mold and bacteria inoculated onto substrate in this manner frequently form diamond-shaped or mountain-shaped contamination patches in the bottle neck (Figure 15.26).

Neurospora crassa (pink mold) is one of the fastest growing contaminants and spreads numerous airborne spores. The bottles or blocks contaminated with this mold, though sealed, can spread and infect most nearby bottles or bags placed in an incubation room. All affected bottles and bags should be removed as soon as possible from the incubation room. Once the mold propagates in an incubation room, elimination is very difficult. Remnants of substrate and neglected waste substrate under high humidity and warm temperature conditions frequently become the initial cause of *Neurospora* contamination.

Major contamination caused by the previously mentioned competitor molds can be prevented by standard sanitation and strict hygiene of the cooling, inoculation, and incubation rooms.

15.4.2 Bacterial Diseases

In bottle and bag cultivation in Japan, *Bacillus subtilis* is the most common bacterium that severely inhibits the growth of mushroom mycelium. Mycelial growth is strongly prevented



Figure 15.26 Antagonistic contaminants competing with mushroom mycelium. The diamond-shaped patches show that the contaminants were introduced into the bottles during the cooling of substrate or inoculation.

when substrates are precolonized by *B. subtilis* (Figure 15.27). Endospores of *Bacillus* naturally occur in substrate ingredients (air- or soil-borne). These are able to survive at high temperatures and pressures within an autoclave. Infection of the endospores of *B. subtilis* is also caused by contaminated air flow into bottles and bags during substrate cooling, even when the substrate is a high temperature at 100°C. A principal pathogen of bacterial diseases in mushroom cultivation is *B. subtilis* var. *natto* that produces the Japanese traditional food *natto* (a fermented soybean). In Japan, therefore, workers who have eaten *natto* at breakfast are strictly forbidden

Figure 15.27 Bacterial contamination in the substrate (right). Mushroom mycelial growth is strongly prevented by preceding fully colonized bacteria.



to inoculate spawn. This is especially important for spawn making. *Natto* food remnants can stick to the mouth and hands and as they dry, the endospores form. These can easily then become airborne and cause bacterial contamination transfer to the spawn and general inoculation room.

Pseudomonas spp. and other competitor bacteria occasionally contaminate substrate during spawn run. Unnoticed, negligible bacterial contamination in spawn can expand extensively. Most common pathogenic bacteria, such as *Pseudomonas tolaasii*, cause a bacterial blotch or a discoloration of fruiting bodies of *F. velutipes*, *P. ostreatus* cultivated in bottles, and *L. edodes* cultivated in bags. Mushrooms should be grown under a favorable environment, because fruit bodies grown under wet and poor-air-circulation condition are readily affected by bacteria. Strict hygiene throughout the cultivation facility is most important to prevent infection by bacterial and fungal contaminants.

15.5 Pests in Mushroom Cultivation in Plastic Bottles and Small Bags

Mites are the most serious pests of mushrooms cultivated in bottles, especially *H. marmoreus*. *Tyrophagus putrescentiae*, *Pygmephorus mesembrinae*, and *Tarsonemus* sp. are common mushroom mites in bottle cultivation of specialty mushrooms. These mites preferably feed on spores and mycelia of *Trichoderma* and *Penicillium* molds as well as bacterial contaminants. Figure 15.28 shows patch-like blue-green colonies of *Penicillium* caused by an invasion of mites fed on spores. Such patch-like contamination patterns indicate the moving traces of mites in the substrate.



Figure 15.28 Patch-like blue-green colonies of *Penicillium* caused by an invasion of the mites fed the spores. Patch-like contamination patterns show the moving trace of mites in the substrate.

newly inoculated bottles. If a mite-infested, mold-contaminated bottle is left in the incubation room for a long time, the mite population within the bottle becomes overpopulated. When this occurs, the mites evacuate the bottle all together and invade the incubation room in search of new food – and finding newly inoculated, uncontaminated bottles. At the last stage of serious mite infection associated with *Trichoderma*, the reddish-brown-colored shed skins of mites are frequently clustered on the cap and shoulder surface of bottles and also on the floor.

In bottle cultivation of *H. marmoreus*, it is well known that mites can invade bottles only 1 to 5 days after inoculation, prior to colonization of the substrate surface by mushroom mycelium. Therefore, incubation under a strict, hygienic environment in the early period of spawn run is very important to protect from mite invasion. The thorough removal of dust from the floor of incubation rooms using a vacuum cleaner is the most effective method to decrease the population of mites. Puddles on the floors of incubation rooms accelerate mite propagation because mushroom mites prefer to inhabit dark and wet places, such as floor cracks and underneath pallets.

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