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Casing Materials and Techniques in *Agaricus bisporus* Cultivation

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7.1 General Aspects of Casing and Fruiting

The cultivation technology of *Agaricus bisporus* (Lange) Imbach involves the following stages: preparation of a pasteurized selective substrate (compost), preparation of the spawn on cereal grains, inoculation of the spawn into the compost, and, finally, cultivation in growing rooms (incubation, casing, prefruiting, induction of the fruiting, and harvest).

In commercial cultivation of *Agaricus bisporus*, fructification occurs in the casing layer, material used as a top covering of the compost usually after the substrate is colonized by mushroom mycelium, to induce the transition from vegetative to reproductive growth (Figures 7.1 and 7.2). The casing layer plays a very important role because it conditions the stage of fructification. A compost that is completely colonized by mycelium will not on its own produce mushrooms. It is therefore necessary to modify the compost to initiate fructification. Although the role of casing layers has not been precisely defined, it must have particular physical, chemical, and microbiological properties that determine their function. The ecological modification which implies the beginning of this fructification takes place in this layer and represents the basis of the interest in mushroom as a commercial crop. Full fructification is encouraged so that mushroom cultivation is made as profitable as possible. Moreover, casing layers, made of materials of a very diverse nature, are an important source of variation in terms of the yield, quality, and uniformity of commercial cropping.

The casing layer fulfills several functions (Bazerque and Laborde, 1975; Visscher, 1988); among others, it:

- constitutes the physical support of the emerging carpophores,
- contributes to the maintenance of a moist microclimate to help feed the mycelium and supports the formation of primordia,
- supplies water for the growth and development of mycelium and fruit bodies, providing a reservoir for the maturing mushrooms and supplementing the water provided by the compost,
- facilitates the transport of dissolved nutrients to the carpophores,
- acts as a suitable medium for the development of mushroom mycelium and bacteria that stimulate fructification,
- provides the mycelium with a suitably aerated environment, facilitating gas interchanges,

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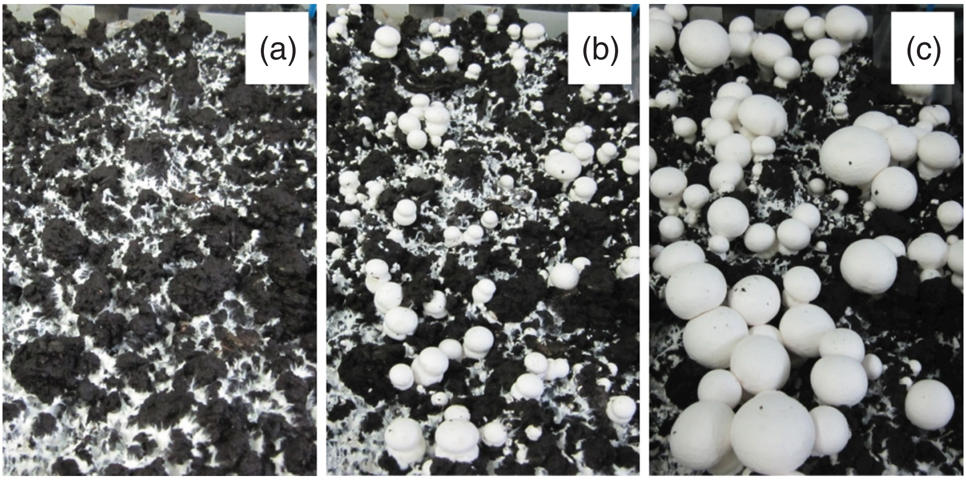

Figure 7.1 Fruiting of mushrooms on a peat-based casing (interval A–B: 5 days; interval B–C: 2 days).

Figure 7.2 Compost (lower) and casing (upper) layers in mushroom production.

- protects the compost surface from drying out and against too rapid disappearance of useful metabolic products,
- has a protective action against antagonistic microflora and induces modifications of the compost microflora (Gillmann et al., 1994),
- provides an environment of low osmotic value unlike compost, whose osmotic value is too high for mushrooms,
- provides a zone where ion exchange may take place (Stoller, 1952b; Kurtzman, 1996a).

In short, casing contains and favors the factors that induce fructification. Many factors are involved in the process. The initiation and subsequent development of the carpophores of *Agaricus bisporus* depend not only on the genetic capacity of the mycelium to fructify but also on physical, environmental, chemical, nutritional, and microbiological factors (Table 7.1). A complex interaction occurs between these factors, impeding the establishment of a definitive theory to fully explain the phenomenon of fruiting. Complete reviews of factors involved in

Table 7.1 Interrelated factors involved in mushroom fruiting.

Factors of Fruiting	Main Aspects to Consider
Physical	Aspects related to structure of materials: • Texture • Density and porosity • Water-holding capacity and water relations • Size and stability of aggregates
Environmental	Aspects related to climate conditions in the growing rooms (environmental transition): • Compost and air temperature • Relative humidity gradient • Aeration (carbon dioxide concentration and inhibitory volatile organic compounds)
Chemical and nutritional	pH Soluble salts (electrical conductivity, osmotic pressure) Chemical reactions in the casing layer Role of calcium carbonate Cation exchange capacity Different nutritional situation between compost and casing Readily decomposable organic matter content
Microbiological	Microbiota present in the casing layer Role of stimulatory bacterial populations (mainly *Pseudomonas* spp.)
Genetics	Genetic heritage of mushroom strains (fruiting ability)

Agaricus bisporus fructification were carried out by Couvy (1972, 1973) and Pardo-Giménez et al. (2002a, 2002b).

It is well-known that some physical and chemical properties, such as porosity, water-holding capacity, salinity, and pH, can affect both the vegetative and reproductive growth of mushrooms. In fact, for an adequate profitability in commercial cultivation, the casing material must fulfill certain conditions (Hayes, 1981; Stamets and Chilton, 1983a; Flegg and Wood, 1985; Rainey et al., 1987; Visscher, 1988). For example, it should:

- be sufficiently resistant and deep enough to provide adequate support for mushroom growth,
- have a high capacity to absorb and release water, be able to withstand frequent irrigation without serious structural breakdown and possess a structure and porosity which permits good permeability for water and gases,
- have a low nutritional value and sufficiently low salt concentration to minimize any water deficit which would be unfavorable to the initiation of growth,
- be of neutral or slightly alkaline pH,
- contain calcium carbonate, mainly for its buffering effect against changes in pH,
- have a high cationic exchange capacity,
- have a low magnesium content and low levels of other toxic elements,
- be free of parasites and competitors.

In addition to the practical experience of growers, detailed knowledge of the characteristics of the casing layer involves the need for laboratory tests for the main parameters associated with these features. This is of great importance, especially in preparation of mixtures of materials and in the subsequent handling of the different operations in the growing room, particularly with regard to the timing of the crop cycle management, watering, ruffling, and environmental control. The main parameters and methods used in the analysis of casing soils and their components are presented in Table 7.2.

Table 7.2 Main parameters and methods used in the analysis of casing soils and their components.

Parameter	Method	Standard/Reference
pH	Potentiometric	European Standard EN 13037:1999 (AENOR 2001a)
Moisture content	Gravimetric (drying to a constant weight)	European Standard EN 13040:2007 (AENOR 2008a)
Electrical conductivity	Conductimetric	European Standard EN 13038:1999 (AENOR 2001b)
Total N content	Kjeldahl	European Standard EN 13654–1:2001 (AENOR 2002)FOSS, 2003
Organic matter and ash	Thermogravimetric (calcination)	European Standard EN 13039:1999 (AENOR 2001c)
Particle real density	Calculation from ash content	European Standard EN 13041:1999 (AENOR 2001d)
Bulk density (fresh)	Weight of certain volume of material after compaction	European Standard EN 13040:2007 (AENOR 2008a)
Bulk density (dry)	Calculation from fresh bulk density and moisture content	European Standard EN 13040:2007 (AENOR 2008a)
Total porosity	Calculation from dry bulk density and real density	European Standard EN 13041:1999 (AENOR 2001d)
Particle size distribution	Mechanic sieving	European Standard EN 15428:2007 (AENOR 2008b)
Texture	Densimeter	Bouyoucos, 1962 MAPA, 1994c
Water-holding capacity	Saturation and drainage	Ansorena, 1994
Water release curve	Suction at different water tensions	De Boodt et al., 1974 Ansorena, 1994
Permeability	Measuring of percolation rate	Rangel et al., 1996
Cation exchange capacity	Barium chloride	ISO 11260:1994; UNE 77300:1996 (AENOR 1996) SISS, 1985
Cation exchange capacity	Barium acetate	Harada and Inoko, 1980
Buffering capacity	Change of pH after addition of HCl aliquots	Rainey et al., 1987
Active lime	Ammonium oxalate (Bernard calcimeter)	MAPA, 1994b
Total carbonates	Hydrochloric acid (Bernard calcimeter or Scheibler apparatus)	UNE 103–200–93 (AENOR 1993) MAPA, 1994a ISO 10693:1995; UNE 77317:2001 (AENOR 2001e)
Pathogenic nematodes	Baermann funnel	Agrios, 2005
Mites	Berlèse-Tullgren funnel	Brady, 1969 Krantz, 1986
Competitor molds	Growth in petri dish	Tello et al., 1991

7.2 Casing Materials

Many materials, alone or in combination, have been used as casing both at commercial and experimental level, although only very few have proved to be of practical application. Some of them have only been used at the experimental level to study some of their characteristics or certain aspects of fructification, while many others have been discarded for diverse reasons. The behavior of the material as regards to quality and yield of the resulting mushrooms, its availability, and price, are determining factors when choosing a particular casing material.

Among the numerous materials which have been used are materials of a mineral origin (natural or heat treated and industrial wastes), of vegetal origin (natural or transformed), and even synthetic materials. Of these, peat constitutes the most widely used material as casing for mushroom cultivation throughout the world. Its water-holding capacity and structural properties are widely accepted as ideal for the purposes of casing (Yeo and Hayes, 1979). However, problems associated with its use, especially as regards its availability, the depletion of reserves, and the alteration of ecosystems, have led to the search for alternative materials (Price, 1991). In addition to peat moss, natural materials of mineral origin, such as soil, gravel, and calcium carbonate in different forms, and spent *Agaricus bisporus* compost are the most common (Pardo et al., 1999). Mineral materials are usually combined with different organic substrates, mainly peats, which act as structural and water-holding correctors.

Any material which is to be considered as an alternative to peat for use in mushroom casing should have the following properties: similar performance characteristics at least equal to peat, competitive cost, stable quality, continuity of supply, freedom from pests and diseases, and ease of handling (Border, 1993).

A list of materials that have been used or evaluated as ingredients of casing layers in mushroom growing, either commercially or experimentally, is presented next. Each one is accompanied by a selection of bibliographic references. Previous reviews of materials were carried out by Pardo et al. (1999), Poppe (2000), and Jarial et al. (2005b).

7.2.1 Materials of Mineral Origin

7.2.1.1 Natural Materials

- Different soil types (Pizer and Leaver, 1947; Pizer, 1950; Stoller, 1952b; Reeve et al., 1960; Edwards, 1974; Hayes, 1978).
  - Clay and clayey soil (Bels-Koning, 1950; De Kleermaeker, 1953; Edwards and Flegg, 1953, 1954; Hayes and Shandilya, 1977; Edwards, 1978; Visscher, 1982, 1988; Stamets and Chilton, 1983a; Shandilya, 1989; Khanna et al., 1995; Kurtzman, 1995a, 1995c; Maas, 2003).
  - Sand and sandy soil (Edwards and Flegg, 1954; Stamets and Chilton, 1983a; Khanna et al., 1995; Angrish et al., 2003; Toker et al., 2007; Yilmaz et al., 2007; Simsek et al., 2008).
  - Clay loam soil (Edwards and Flegg, 1953; Nair and Bradley, 1981).
  - Fargo silty clay soil (Kurtzman 1995a, 1995b, 1997, 1999).
  - Loam soil (Hayes and Shandilya, 1977; Hayes, 1978; Stamets and Chilton, 1983a; Eicker and van Greuning, 1989; Khanna et al., 1995).
  - Marl, limestone, marlstone (Bels-Koning, 1950; Reeve et al., 1960; Hayes and Shandilya, 1977; Hayes, 1978; Hayes et al., 1978; Stamets and Chilton, 1983a; Visscher, 1988; Kurtzman, 1995a, 1995b; Maas, 2003).
  - Diatomite, diatomaceous earth material, charro (Reeve et al., 1960, Huerta et al., 2001).
  - Chalk (Reeve et al., 1960; Allen, 1976; Stamets and Chilton, 1983a; Rainey et al., 1987; Noble and Gaze, 1995; Stamets, 2000).

- Garden soil (Bels-Koning, 1950; Rao and Block, 1962; Vijay et al., 1987; Singh et al., 2000; Om et al., 2008).
- Forest soil, forest litter (Hayes and Shandilya, 1977; Shandilya, 1978; Vijay et al., 1987; Colak et al., 2007; Toker et al., 2007; Yilmaz et al., 2007).
- Muck soil (Reeve et al., 1960).
- Vertisol soil, black swelling clay soil with traces of low-grade coal (Van Jaarsveld and Korsten, 2008).

- Gypsum (Stoller, 1952a, 1979a; Atkins, 1979; Visscher, 1988; Stamets, 2000).
- Gravel (Reeve et al., 1960; Gardner and Davies, 1962; Hayes and Shandilya, 1977; Hayes et al., 1978; Clancy and Horton, 1981; Garcha and Sekhon, 1981; Kurtzmann, 1995a, 1997, 1999).
  - Gravel made of volcanic ash (Kurtzman, 2000).
  - Free of sand aquarium gravel (Kurtzman, 1999).
- Tuffeau (Bazerque and Laborde, 1976; Moguedet and Kaeffer, 1991).
- Crushed basalt rock (“blue metal”) (Clancy and Horton, 1981).
- Pumice soil (Rainey et al., 1987).
- Dolomite (Stamets and Chilton, 1983a; Kurtzman, 1991, 1997).
- Stone grindigs (Maas, 2003).
- Bentonite (Beyer, 2004).
- Zeolite (Noble et al., 2003; Beyer, 2004).

7.2.1.2 Processed Materials and Waste Products

- Vermiculite (De Kleermaeker, 1953; Edwards and Flegg, 1953, 1954; Barnard, 1974; Eicker and van Greuning, 1989; Verbeke and Overstyns, 1991; Noble and Gaze, 1995; Fermor et al., 2000; Stamets, 2000; Noble et al., 2003).
- Perlite (Gardner and Davies, 1962; Barnard, 1974; Lelley et al., 1979; Colak, 2004; Colak et al., 2007; Baysal et al., 2007; Peker et al., 2007; Toker et al., 2007; Yigitbasi et al., 2007; Yilmaz et al., 2007).
- Rockwool (Visscher, 1982, 1988; Noble and Gaze, 1995; Noble et al., 2003).
- Capogro®, mineral wool product spun from molten rock (Wuest and Beyer, 1996).
- Peatwool®. Mineral fibers, made from molten rock, plus 25% (v/v) sphagnum peat moss (Wuest and Beyer, 1996).
- Used horticultural rockwool (Fermor et al., 2000).
- Expanded aluminum silicate (Reeve et al., 1960).
- Turface, flakes of calcined montmorillonite clay (Kurtzman, 1995a, 1995b, 1996b, 1997, 1999).
- Crumbled bricks, brick chips (Bels-Koning, 1950; Edwards and Flegg, 1953; Edwards, 1974).
- Granulated rockwool slabs, waste product of the glasshouse industry (Noble and Gaze 1995; Noble and Dobrovin-Pennington, 2001).
- Pieces of mosaic (Colak et al., 2007; Peker et al., 2007; Toker et al., 2007; Yilmaz et al., 2007; Simsek et al., 2008).

7.2.2 Materials of Vegetal Origin

7.2.2.1 Natural Materials

- Peats of different types and origin (Stoller, 1952a; Edwards and Flegg, 1954; Reeve et al., 1960; Rao and Block, 1962; Edwards, 1974; Ganney and Richardson, 1974; Caron, 1987; Visscher, 1988; Bellmont, 2005; Baysal et al., 2007; Pardo-Giménez et al., 2012).
- Sedge peat (Clancy and Horton, 1981).

- Pine bark (Allen, 1976; Rainey et al., 1987; Visscher, 1988; Shandilya, 1989; Pardo-Giménez et al., 2012).
- Sawdust (Allen, 1976; Hayes et al., 1978; Nair and Bradley, 1981).
- Gum sawdust (*Eucalyptus* spp.) (Eicker and van Greuning, 1989).
- Pine sawdust (*Pinus* spp.) (Eicker and van Greuning, 1989).
- Coconut coir pith (Border, 1993; Labuschagne et al., 1995; Gupta, 1997; Fermor et al., 2000; Kurtzman, 2000; Noble and Dobrovin-Pennington, 2001; Noble et al., 2003; Dhar et al., 2003, 2006; Suman and Paliyal, 2004; Rangel et al., 2006; Afewerki and Korsten, 2008; Van Jaarsveld and Korsten, 2008; Pardo and Pardo, 2008; Pardo-Giménez et al., 2012; Chandra et al., 2014).
- Rice husk/rice hulls (Nair, 1976; Clancy and Horton, 1981; Rangel et al., 1996; Cai et al., 2002, 2008).
- Barley fines (Hayes et al., 1978).
- Poppy straw (Clancy and Horton, 1981).
- Cottonseed meal (Nair et al., 1993).
- Soyafodder (Van Jaarsveld and Korsten, 2008).
- Lignite, brown coal. Soft brown combustible sedimentary rock that is formed from naturally compressed peat (Fermor et al., 2000; Noble et al., 2003).
- Wood charcoal (Noble et al., 2003).
- Anthracite coal (Noble et al., 2003).

7.2.2.2 Processed Materials and Waste Products

- Wood and bark wastes:
  - Fermented tree bark (Shandylia, 1983).
  - Composted pine bark (Rainey et al., 1987; Shandilya, 1989; Hodgkinson et al., 2002).
  - Composted fine bark (conifer and broadleaf bark) (Noble and Dobrovin-Pennington, 2001).
  - Composted conifer bark (Noble et al., 2003).
  - Composted wattle bark from timber industry (Afewerki and Korsten, 2008; Van Jaarsveld and Korsten, 2008).
  - Actilex, ground and composted bark (D'Hardemare, 1985).
  - Composted sawdust (Clancy and Horton, 1981; Nair and Bradley, 1981).
  - Fiber-mix, by-product of a process involving the extraction of polyphenolic resins from the bark of *Pinus radiata* (Rainey et al., 1987).
  - Wood waste from the debarking operations at a pine pole treatment plant, treated in an explosion digester system (Mamers and Menz, 1981).
  - Defibrated pine wood and bark (Clancy and Horton, 1981; Nair and Bradley, 1981).
- Waste products of the sugar industry:
  - Spent lime. Co-product from the processing of sugar beets (Visscher, 1982, 1988; Dergham, 1992; Huerta et al., 2001; Maas, 2003; Beyer, 2004; Pardo, 2008).
  - Sugarcane bagasse (Clancy and Horton, 1981; Nair and Bradley, 1981; Afewerki and Korsten, 2008; Van Jaarsveld and Korsten, 2008).
  - Sugarcane press mud (Dhar et al., 2006).
  - Filter mud, filter cake (Nair and Bradley, 1981; Afewerki and Korsten, 2008; Van Jaarsveld and Korsten, 2008).
- Sisal waste (Hayes, 1978).
- Ground coconut husk (Hayes, 1978).
- Wheaten chaff (Clancy and Horton, 1981).
- Active charcoal (Stoller 1979a, 1979b; Atkins, 1979; Verbeke and Overstyns, 1991; Fermor et al., 2000; Noble et al., 2003; Dobrovin-Pennington et al., 2008).

- Composted cotton husks (Eicker and van Greuning, 1989).
- Tea production waste, dried straw and fiber of tea leaves after manufacturing process (Gülser and Pekşen, 2003).
- Composted vine shoots (Pardo et al., 2002c; 2003; De Juan et al., 2003).
- Straw washed with hot water (Kurtzman, 2000).
- Spent hops (Hayes et al., 1978).
- Cotton ginning mill waste (Garcha and Sekhon, 1981).
- Spent paddy straw after its use for *Volvariella volvacea* (Garcha and Sekhon, 1981).
- Burned rice husk (Singh and Saini, 1993; Khanna et al., 1995; Angrish et al., 2003; Dhar et al., 2006; Om et al., 2008).
- Coffee grounds recovered from an instant coffee factory (Eicker and van Greuning, 1989).
- Fly ash, high temperature residue formed during the coal combustion process and collected as a waste by-product (Wuest and Beyer, 1996; Beyer, 2004).
- Composted azolla (azo-compost) (Riahi and Zamani, 2008).
- Cellulosic materials related to paper and paper industry:
  - Crumbled/shredded waste paper (Dergham et al., 1991; Dergham, 1992; Lelley et al., 1994; Dergham and Lelley, 1995; Sassine et al., 2005).
  - Composted waste paper (Sassine et al., 2005).
  - Shredded newspaper (Stoller, 1979a; Atkins, 1979).
  - Paper and pulp-mill by-product (PPMB). Residual lignin and cellulose fibers from the mechanical and chemical treatment of wood in the preparation of pulp for the manufacture of paper (Hayes et al., 1978; Yeo and Hayes, 1979; Hayes, 1981).
  - Filter paper (Bels-Koning, 1950).
  - Paper mulch (Garcha and Sekhon, 1981).
  - Paper pulp leached for at least 2 years (Eicker and van Greuning, 1989).
  - Paper sludge waste (Lelley, 2000; Cai et al., 2002). Waste paper sludge from newsprint manufacture (Noble and Dobrovin-Pennington, 2001).
  - Champyros®. A mixture of crumbled and composted used paper, peat, and calcium carbonate. pH 7.8–8.1, pore space 85.5–87.0, water retaining capacity 336–344 (Lelley et al., 1994).
  - RPC+®. Recycled newspaper, fiberized and formed into roughened pellets (Morris and Wuest, 1995; Wuest and Beyer, 1996).
  - Graypete®. Synthetic casing which is based on recycled cellulosic wastes such as paper and cardboard to which are added a number of ingredients which improve the properties of the raw materials (Clancy and Horton, 1981).
- Byproducts from the mining industry:
  - MRF (Dewhurst, 2003).
  - Coal tailings, by-product from the washing process for coal to be used in burning for energy (Noble and Dobrovin-Pennington, 2004; Beyer, 2004).
  - Mine-dump tailings (Van Jaarsveld and Korsten, 2008).

7.2.3 Synthetic Materials

- Hygromull®, urea formaldehyde foam (Visscher, 1979, 1982).
- Styromull®, expanded polystyrene (Visscher, 1979, 1982).
- Polyurethane (Barnard, 1974; Visscher, 1979).
- Waterstore®, polyacrylamide (Visscher, 1988).
- Hydratex®, polyacrylamide gel (Castle, 1993).
- Non-ionic gels (agarose, acryl-amide-starch, methylcellulose) (Kurtzman, 1996a, 1996b, 1997, 1999).

- Algin®, natural cationic gel (Kurtzman, 1996a, 1999).
- Dowex®, ion exchange resins (Kurtzman, 1996a, 1997, 1999).
- Stockosorb®. A cross-linked polyacrylamide, polymer material that has the ability to absorb many times its weight in water (Dergham 2000; Beyer et al., 2002).
- Chemical soil conditioners. HPAN (hydrolyzed polyacrylanitrite) and VAMA (copolymer of vinyl acetate and maleic anhydride) (Reeve et al., 1960).
- Woven glass fiber (Nair and Hayes, 1974, 1975).
- Silica gel (Noble et al., 2003).

7.2.4 Other Materials

In this group, different materials that, by their nature or origin, cannot be fitted in any of the previous items are included.

- Spent mushroom substrate (Sinden, 1971; Wuest, 1974, 1976; Allen, 1976; Nair, 1976, 1977; Hayes and Shandilya, 1977; Happ and Wuest, 1979; Stoller, 1979b; Brosius, 1981; Nair and Bradley, 1981; Shandilya and Agarwala, 1983; Eicker and van Greuning, 1989; Shandilya, 1989; Szmidt, 1994; Khanna et al., 1995; Riahi et al., 1998; Rinker, 2002; Angrish et al., 2003; Dhar et al., 2003, 2006; Beyer, 2004; Riahi and Arab, 2004; Barry et al., 2008; Choudhary et al., 2009; Pardo and Pardo, 2008; Pardo-Giménez et al., 2010, 2011, 2012).
- Composted sawdust after bottle culture of *Pleurotus ostreatus* (Kim et al., 1998).
- Spawn run compost (CAC) (MacCanna, 1972; MacCanna and Flanaghan, 1972; Nair and Hayes, 1974, 1975; Dawson, 1978; MacCanna, 1983; Samp, 1993; Tschierpe, 1999; Zied et al., 2010).
- Commercial casing inoculum (CCI) (Green, 1990; Markowitz, 1991; Janssen, 1993; Samp, 1993; Romaine and Schlagnhaufer, 1993; Miller et al., 1995; Bodine, 2005).
- Manure spawn (Allen, 1976; MacCanna, 1983).
- Recycled casing (MacCanna, 1972; Flegg, 1975; Nair and Bradley, 1981; Tschierpe, 1982; Nair, 1983, 1985; Jablonsky and Srb, 1989; Farsi et al., 2011; Oei, 2011; Pecchia and Beyer, 2013).
- Cowdung (farm yard manure, FYM) (Hayes and Shandilya, 1977; Shandilya, 1978, 1989; Shandilya and Agarwala, 1983; Saini and Prashar, 1992; Singh and Saini, 1993; Khanna et al., 1995; Angrish et al., 2003; Dhar et al., 2003, 2006; Suman and Paliyal, 2004; Choudhary et al., 2009; Chandra et al., 2014).
- Cabutz, solid fraction of the digested slurry from the cattle manure thermophilic methane fermentation process (Levanon et al., 1984, 1986; Danai and Levanon, 1996).
- Starch (Kurtzman, 1999).
- Starch-based water absorbents (Stamets, 2000).
- Animal charcoal (Noble et al., 2003).
- Vermicompost (Di Fiore and Albarracín, 1998; Dhar et al., 2003; Choudhary et al., 2009; Choudhary, 2011; Chandra et al., 2014).
  - Farmyard manure based vermicompost (Tomati et al., 1989; Jarial and Shandilya, 2005a).
  - Vermicompost prepared by using spent mushroom substrate from *Pleurotus ostreatus* cultivation (García et al., 2005).
  - Castings of earthworms fed with spent composts and sawdust (Shieh and Wang, 1981).
  - Municipal waste based vermicompost (Jarial and Shandilya, 2005).
- Municipal waste compost (Lelley et al., 1979; Visscher, 1988; Dhar et al., 2003, 2006).
- Composted mushroom stalks (Eicker and van Greuning, 1989).
- Orfa coarse fiber. Domestic waste is ground, metals and glass are removed, organic matter is treated with ozone for odor control, and the product is dried and baled (Wuest and Muthersbaugh, 1990; Wuest and Beyer, 1996).
- Digested biogas plant slurry (Khanna et al., 1995; Angrish et al., 2003).

7.3 Casing Related Techniques

7.3.1 Reuse of Casing

The limitations of the use of peats, mainly by availability and price, have led to the study, in addition to the use of alternative materials, of the possibility to reuse the casing layer in new production cycles. As a general rule, the regeneration process of casing material involves recovering it from mushroom beds at the end of cropping, leaching it to remove soluble salts, and pasteurizing it to kill disease organisms (Nair, 1985). Another advantage of the separation of casing is the availability of soil-free spent compost for alternative uses, with higher value for fertilizing purposes.

MacCanna and Flanagan (1972) reused a sterilized casing from a previous crop cased with a peat and ground limestone mixture. Although sterilized reused casing material tended to reduce yields, they suggest that the use of such material would give reasonable yields in the event of a temporary scarcity of peat. In the work of Flegg (1975), a peat-lump chalk casing was scraped off after cook-out and reused to case a new crop. It caused a slight delay in the start of cropping, and an overall loss of crop of about 11%. According to this author, washing the casing before the reuse or mixing with fresh casing may improve this performance by lowering the concentration of soluble salts accumulated during the preceding crop.

In Australia, Nair and Bradley (1981) used spent casing peat leached in tap water, mixed with peat moss (1:1, v/v) and pasteurized. This spent casing has very good potential for being developed as casing material to replace imported materials, taking into account that yields were similar to that obtained with peat moss based casing used as a control. Shortly afterwards, Nair (1983, 1985) adopted a technique called *counter current extraction*, originally designed for extraction of sliced sugar beet, for the regeneration of spent peat. Material was collected from a commercial mushroom farm by mechanical means using an automatic commercial machine line. After regeneration, spent peat, leached, and pasteurized, was mixed with fresh peat moss and applied to mushroom beds in a commercial farm. Several tests were carried out and yield was at least as good as that from casing control. The author suggests that the counter current extractor process for regenerating spent peat may find an application in commercial cultivation, with consequent reduction in the cost of mushroom production.

At the same time, in Switzerland, Tschierpe (1982) proposed the reuse of old casing material after one year, though an effective pasteurization or treatment with chemicals is essential, and sufficient land should be available for weathering purposes.

Jablonsky and Srb (1989) studied the repeated use of casing soil. The results obtained point to the possibility of the repeated use of soil after steam treatment (60°C, 12 h) without a reduction in yield being recorded going at least three cycles without the need for washing of the casing soil used. Problems associated with the repeated use of casing soil are a gradually increasing number of pathogens, the salinity of the casing soil, changes in water capacity, the structure of the soil, pH, and the problem of removal of the casing soil from the compost. For practical application in the shelf system, the casing soil intended for repeated use can be placed on the surface of the colonized compost on a nylon net. When the cultures are being removed then first of all the casing soil is separated off on its own net and then the layer of spent compost on its own is removed from shelves on a further net. The method of separating casing from compost with a plastic mesh had been previously described by Hesling (1981) without interference with the mycelial growth into the casing. More recently, Farsi et al. (2011) evaluated the reuse of peat-based casing soil by using a plastic mesh in the block system. After harvesting three flushes, plastic mesh with the used casing was lifted from the blocks. Material was piled for three weeks, leached with distilled water and pasteurized (60°C, 6 h). Finally, recycled casing was applied in a second crop. There were not significant differences between yield of mushrooms from blocks cased with used casing soil and that obtained from original casing material used as control.

In the Netherlands, an installation specifically designed to separate the casing soil from the spent mushroom compost has been recently developed (Oei, 2011). Cultivation trials run using mixtures with the reused casing soil after grinding and treating it in a steam heated auger (70°C) gave good yields of mushrooms when high concentrations were used (50–75% reused), almost matching those of beds using 100% fresh casing soil (Oei, 2011).

Finally, Pecchia and Beyer (2013) conducted experiments to test the impact of different quantities of recycled casing mixed with fresh peat on crop yield. Mushroom yields tend to decrease with an increased rate of recycled casing incorporated into the casing. Taking into account that there was a negative correlation between soluble salt concentrations and mushroom yield, it appears that conductivity plays a key role in limiting the amount of recycling casing material that can be mixed with fresh peat to produce a new crop. A similar situation occurs with the use of spent mushroom substrate as casing (Pardo-Giménez et al., 2012). Methods of inexpensive and effective reduction of the soluble salts content must be investigated for the successful use of recycled casing.

7.3.2 Ruffling

With dense materials that are compacted during the vegetative phase, it is necessary to generate an open texture in order to achieve uniform and abundant fructification and to prevent the production of too much carbon dioxide during fructification and harvesting. This fact led to the introduction, in the 1970's, of deep "scratching" or "ruffling." This process consists of mixing all the casing layer with the mushroom mycelium, which is growing in it, about a week after casing. The result of this procedure is a more open structure which facilitates the interchange of CO_2 and O_2 (van Gils, 1988) and ensures that all the mycelium appearing at the surface is at the same development stage, giving a much more even spread of the primordia over the entire bed surface (Vedder, 1989).

The background of this technique is found in the work of Flegg (1967). Different experiments were carried out to study the effect of a vigorous disturbance of casing layer. When the casing layer was almost completely colonized, it was either left undisturbed or emptied out, broken up, and replaced.

The ruffling gives rise to "mountains and valleys," in such a way that the pinheads are preferably formed in the valleys where they will suffer less from the air-streams, especially when they are young and vulnerable (Visscher, 1975). Dawson (1978) observed a more uniform distribution of mushrooms, although there was no significant increase in yield. However, Visscher (1988) obtained a greater stimulation of fruiting and higher yields with casings of compact texture during the vegetative phase and loose during the generative phase.

This technique is particularly effective with strains of difficult fruiting, where the mycelium has a tendency to continue their vegetative development (D'Hardemare and Mazuel, 1986).

Experience shows that good ruffling allows more accurate control of the first picking day (Buth, 2006). Ruffling is an aid for the diffusion of CO_2 and the admission of O_2 when the growing room is aerated, and it also contributes to a more rapid and uniform colonization by the mushroom mycelium, so that a more uniform fruiting in size and time is achieved. The first consequence is that the mycelium is fragmented, the content of a number of cells comes out, and the action of the bacteria in the hyphosphere is stimulated (Visscher, 1988).

In addition to the improvement of the structure, if the casing layer is not the same depth all over the beds, ruffling can aid to even out irregularities in mycelial growth (Tschierpe, 1981;

Figure 7.3 Hand-operated ruffling apparatus.

Flegg, 1989). From the standpoint of hygiene, however, this method can be dangerous (Tschierpe, 1981). Another variable factor during ruffling is the way the soil is compacted (Van Gerwen and Hilkens, 2004).

The ruffling operation can be carried out manually by means of a flat board with nails worked with a back and forth or circular motion over the beds (Visscher, 1988; Flegg, 1989), or with the aid of a hand-operated rod with teeth (Figure 7.3), but also mechanically with fully electric or hydraulically driven machines specifically developed for use with the shelf system of growing (Figure 7.4). In this case, the intensity of ruffling depends on the number of times the machinery passes over the beds, how deeply the pins or teeth penetrate the casing soil, the number of teeth, their size and shape, the configuration of the teeth on the rod, the space around the rod, the speed at which the rod revolves, and how quickly the machine moves over the bed (Van Gerwen and Hilkens, 2004; Buth, 2006). In the case of manual ruffling, the experience of the operators is crucial for a successful operation.

7.3.3 CACing Technique

The CACing technique ("Compost Added at Casing") involves the application of small amounts of compost fully colonized (spawn-run compost) by the mushroom mycelium to the casing layer at casing time. The technique was developed in Ireland during the 1960s and reported for the first time by MacCanna and Flanagan (1972).

This addition has the effect of assisting a rapid and even growth of mycelium through the casing layer (MacCanna, 1983). The CACing technique has several advantages, and the most important are: time saving (earlier first flush by 3–4 d), more uniform distribution of mushrooms over the entire bed surface, elimination of soil-breaking pins resulting in cleaner mushrooms, no requirement for leveling, no need for ruffling, and a reduction in clumping (Ganney and Stanley-Evans, 1973; Vedder, 1989; Samp, 1993).

In the CACing technique, it is vital that the compost mixed into the casing layer is well run and free of possible diseases and pests commonly found in compost (such as weed molds, bacteria, virus, mites, and nematodes). The obvious advantages of using this technique can be

Figure 7.4 Detail of a ruffling machine: rod with teeth and pressure roller.

nullified by hygiene problems: if contaminated compost is used, the contamination will be spread to the areas inoculated with it (Ganney and Stanley-Evans, 1973; MacCanna, 1983). General precautions to be followed are:

- use the same spawn strain for the casing as for the compost;
- avoid the use of compost that has been overheated;
- remove pieces with black spots or that are excessively dry;
- ensure exclusion of pests and diseases and discard material if it is positive for contaminations such as the presence of nematodes; and
- pay special attention to the hygiene conditions of facilities and equipment.

In addition, an even distribution and proper mixing of material on the casing is vital for the success of the CACing technique (Tschierpe, 1999). In practice, amounts between 0.125 and 2 kg m^{-2} of slightly crushed spawn-run compost are used in growing areas (MacCanna, 1983; Vedder, 1989; Tschierpe, 1999). The amount of CAC influences the structure of the casing soil, the pore volume, the gas exchange capacity and the amount of mycelium (Hilkens, 2012).

7.3.4 Commercial Casing Inoculums

As an alternative to CACing, commercial products are specifically inoculated for use in the casing to eliminate this risk factor of potential spreading of contamination, although it is more expensive (Green, 1990; Miller et al., 1995). A system of using manure spawn instead CACing was previously suggested by Ganney and Stanley-Evans (1973).

Spawn makers have developed different casing inoculums (CCI) prepared on sterile substrates, ususally granulate, in a form suitable for easy distribution in the casing soil at time of mixing, providing the nutrition required by the mycelium, with the opportunity of gaining all the advantages of CACing with none of the risks (Romanens et al., 1989; Green, 1990; Markowitz, 1991; Samp, 1993; Miller et al., 1995; Bodine, 2005). Thus, growers have a safe, very convenient, and relatively low-cost method to use spawned casing (Green, 1990).

The main advantages of these commercial inoculums are: reducing the risk of contamination, shortening of the growing cycle, no need for ruffling, best quality, and ease of harvesting, without clumps and without appearance of mushrooms in deep layers, and the possibility of immediate casing. Shortening the cycle also reduces the generation of flies and thus the incidence of the diseases they transmit, such as dry bubble.

Regarding the doses used, the rate of use per surface area has steadily declined over the years, up to around 60 g m^{-2} (Green, 2004).

Examples of such products are PAC Casing Spawn (Amycel), CMS CACing Spawn (Hollanderspawn B.V.), Lambert Spawn's Casing Inoculum (Lambert Spawn Co.), and Sylvan CI (Sylvan Inc.).

7.3.5 Other Techniques

7.3.5.1 Supplementary Casing Material Addition ("Patching")

The thickness of the casing layer should be as uniform as possible. According to Stamets and Chilton (1983a), an uneven casing depth is undesirable for two reasons: risk of overwatering in shallower regions and irregular pinhead formation. The operation commonly known as "patching" consists of the addition of casing material on the shallow areas, where the mycelium appears prematurely. Thus, an even mycelial spread is assured (Stamets and Chilton, 1983b).

7.3.5.2 Compacting

Compacting the soil after casing and/or after ruffling is a growing technique that can be used to regulate the fructification and, accordingly, the yield and size of mushrooms. This technique modifies the density of the casing layer, mainly affecting the porosity and the air-water relationship. The effect of compaction is not always the same and depends, among other factors, on the size of air spaces, the materials used, and its moisture content. There is always the chance that the casing soil is compacted too much, with insufficient air filled porosity and poor respiration, obstructing the passage for mycelium. This upsets pinheading and the yield remains low. Furthermore, the negative effect of compaction is increased if the soil is dry (van Gils, 1988).

The work of Den Ouden (2005) describes the use of a leveling axle or rod to correct small differences in the surface of the casing layer after ruffling or CACing, and a pressure roller to free the casing soil surface of any remaining lumps of soil.

7.3.5.3 Double Cropping: Casing Substitution

The weakening of the mycelium in the compost and the deterioration of the sanitary status of casing with the progress of the growing cycle, led Talon and d'Hardemare (1979) to develop a technique to remove the casing layer after the second flush, proceeding to fill back the compost into trays, at the rate of three times the original weight per square meter, and to case it with new fresh casing.

Despite the reduction in cropping area, crop quality was improved and compost productivity was maintained with some white type strains, by reducing the incidence of dry bubble and bacterial blotch. However, "weeping" mushrooms tend to appear with susceptible cream and white strains. In addition, operational difficulties and labor costs constitute another important limitation.

7.3.5.4 Double Cropping: Re-Supplementing and Re-Casing

Removal of the casing layer, re-supplementing spent compost after one or two breaks and re-casing offers an opportunity to growers to obtain additional mushroom production from the same compost. This technique can increase yields by 40% or more (Royse, 2008; Royse et al., 2008). For commercial application, different aspects should be taken into account, mainly the costs of materials and labor, duration of the cropping cycle, sanitary status, and contamination risk.

7.3.5.5 Plastic Film Coverage

The use of a macroperforated polyethylene film (6 mm in diameter) on the casing soil during the first week after casing was proposed by Vedie (1990, 1995), in order to improve the synchronization of fruit body development under the variable climatic conditions of mushroom production in caves. The film was maintained on the casing layer for 9 days. After removing the plastic film, casing was ruffled and a fungicide treatment was applied. This technique resulted in an increase of yield, a shorter first flush, a better timing of the beginning of picking and a higher percentage of smaller mushrooms.

Previously, Gushue (1988) described a technique referred to as *split casing* that involves covering the casing layer with polyfilm to delay pinning on part of the house, in order to regulate production.

7.3.5.6 Sandwich Technique

The sandwich technique is a novel method to shorten the mushroom cropping cycle by about one week with no impact on yield. It overlaps the last week of spawn run with the first week of case hold (Spear, 1998). Spawning and CAC rates are not changed, but a small amount of top-dressing, to provide immediate sustenance for the mycelium in the casing layer, is spread on top of the compost after spawning. The use of CCI for top-dressing at rates of 270 g m^{-2} worked well. Obviously, the technique is not applicable if Phase III bulk compost is used.

7.3.5.7 Ditch Technique

This is a physical separation between the two halves of the bed formed by a shallow ditch running along the middle, in order to improve the performance of manual harvesting (Van Gerwen and Hilkens, 2004). The ditch is pressed down harder than the rest of the casing soil using a wheel mounted to the ruffling machine, so produces fewer or no mushrooms in the first flush. This line sets the limit of the area assigned to each picker.

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