Development and production of *Lentinula edodes* (Shiitake mushrooms) on inoculated logs of a range of tree species

By

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Submitted in total fulfilment of the requirement for the degree of

Master of Forest Science

March 2009

Department of Forest and Ecosystem Science

The University of Melbourne
Abstract

Shiitake (*Lentinula edodes* (Berkeley) Pegler) produces an edible mushroom that has been cultivated for centuries in China, Korea, Japan, Singapore, Thailand and other Asian countries. Shiitake mushrooms grow naturally on decaying wood of hardwood trees and have traditionally been grown on short lengths of freshly-cut logs. Until now, there has been no serious exploration of the potential for Australian forest owners to utilise small logs of native or plantation forest species for shiitake mushroom production, such as eucalypt (*Eucalyptus* spp.).

Logs of six tree species were harvested from farm forestry plantations in Victoria and inoculated with shiitake infected dowels imported from the United States. Over the course of the next 18 months the logs were soaked four times to initiate fruiting. The fresh mushrooms were harvested and weighed to allow a comparison between log species and size. A sample of the mushrooms from each log species produced in the 2\(^{nd}\) and 3\(^{rd}\) fruiting were tested for their protein and fibre content.

*Quercus robur* was the most productive species. Over the course of the trial (four frutings) the oak logs produced almost 1 kilogram of fresh mushrooms per log which was significantly more than *E. cladocalyx* (527 g/log) and *Alnus glutinosa* (465 g/log) and *Eucalyptus nitens* (389 g/log) which were all, in turn, significantly more productive than *Populus* sp. (140 g/log) and *Acacia melanoxylon* (98 g/log). Larger logs produced more fruit although this may have been related to the greater number of inoculations. The protein and fibre content of mushrooms produced from shining gum logs was slightly lower than that from the oak logs but greater than that from alder. Sugar gum mushrooms had the lowest protein content.

The research suggests that there is potential to use eucalypt logs thinned from young fast-grown farm plantations as the basis for a log-based shiitake industry although more work is required to test the marketability of eucalypt grown shiitake and the economic viability of small scale production units.
Declaration

This is to certify that

(i) the thesis comprises only my original work towards the Masters except where indicated in the Preface,

(ii) due acknowledgement has been made in the text to all other material used,

(iii) the thesis is less than 30,000 words in length, exclusive of tables, maps, bibliographies and appendices.

[Signature]
Preface

The research was undertaken in association with a shiitake mushroom production project at the Centre for Education and Research in Environmental Strategies (CERES) in Melbourne. Hundreds of logs of a range of tree species were donated to the project by three private landholders and prepared by CERES staff. The logs selected for this research trial were inoculated prior to the candidate’s involvement.
Acknowledgement

First and foremost praise be to Allah, the One, Almighty, Merciful, and Compassionate God, and ever-loving God for all what He has done, He is doing and will continue to do in my life. Not forgetting His presence, protections and solutions whenever I call to Him in my distress and who is the Source of all revealed Guidance to mankind.

Committing to a Masters study is a challenging undertaking, and can be especially daunting to a non-English speaking person. However, this study would not have happen without the dedicated support of my principal supervisor Mr. Rowan Reid and I would like to express my profound heart-felt gratitude and appreciation for his invaluable academic supervision, guidance, advice, reviews and comments during the experimental process and writing of this thesis as well as for his kindness, patience, assistance and encouragement throughout the years. I will ever remain grateful to him.

I am most grateful to the management of AusAID-Australian Development Program (ADS) Scholarship who has provided the financial support for the length of my study in Australia. The Centre for Education and Research in Environmental Strategies (CERES) in Melbourne provided the site for the research trial. Mr Parsuram Sharma-Luital was the manager of the mushroom project at the time and provided guidance and support throughout the project for which I am very grateful.

I wish to express my deep appreciation and sincere thanks to my other supervisor, Dr. Tina Bell for her inestimable advise, comments, continued support and encouragement during the progress of my study and writing of this thesis. My special gratitude also goes to Dr. Said Ajlouni, for his encouragement, support and providing the workplace in completing the laboratory work on the mushrooms. An award also for the inestimable assistance in the laboratory analysis of my samples goes to Marzieh Hosseini Nezhad. I am gratefully acknowledged the assistance of Raffael Timpano and other staff of the Melbourne School of Food and Land Resources who provided guidance and support.

Many thanks go to all my friends Windu, Rahmat, Wahid, Semi, Inocencio, Ibrahim, Max and others for their invaluable friendship and encouragement in my academic and professional progress. I am especially grateful to those who took time from their busy schedules to sit down with me and review some of my chapters.

Finally, I wish to express my special thanks to my wife Olivia, daughter Anisah and son Abdurrohman, for letting me put my studies ahead of the need to spend time with them. I am also indebt to my mother Alin, my father Deddy, my brother Akbar and my sister Sari, for their invaluable moral and material support and encouragement in my academic and professional progress.
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Chapter 1
Introduction

1.1 Background
Shiitake (Lentinula edodes (Berk.) Pegler) produces an edible mushroom that has been cultivated for centuries in China, Korea, Japan, Singapore, Thailand and other Asian countries (Ciesla 2002). The word ‘shiitake’ was originally derived from Japanese words: shii which means oak and take which means mushroom, reflecting the importance of oak wood as the natural host of the fungus (Davis 1993; Royse 2001). It is now grown in many other countries including Brazil, Canada, the Netherlands, the United Kingdom and the United States (Slee 1991 cited in Royse 2001) and is the second most commercially marketed mushroom in the world after the button mushroom (Agaricus bisporus (J.E. Lange) Imbach) (Przybylowicz and Donoghue 1988; Oei 1996).

Over the last three decades, the commercial production of shiitake mushroom has increased from an estimated 350,000 tons in 1965 to about 7.5 million tons in 2000 (Royse 2001). Whilst China accounted for approximately 90% of the world’s shiitake mushroom production in 1997 (Chang 1999), increasing production in developed countries including Japan and the US, highlight the potential of the industry. In Japan, shiitake cultivation is now a significant non-wood forest enterprise and has been an important factor underlying the recent increase in the contribution of non-wood forest products to total forest earnings. For example, between 1960 and 1980 the contribution of non-wood forest products to total forest earning increased from less than 4% to more than 13% (Ciesla 2002). Similarly, shiitake production in the US increased following the lifting of a ban by the US Department of Agriculture on importing live shiitake cultures in 1972. Between 1986 and 2000, the total US production of shiitake rose from less than 1 million pounds (less than 500 tons) to 8.6 million pounds (approximately 3900 tons) (Royse 2001), much of it coming from non-industrial forest owners.
Since the 1950s, mushroom production in Australia has been one of the fastest growing food crop industries and is currently regarded as the second most important fresh vegetable type (Davis 2000; Australian Mushroom Growers Association 2005). The industry is dominated by the production of the white button mushroom (*Agaricus bisporus*) (Department of Primary Industries and Fisheries 2004; Australian Mushroom Growers Association 2007). Shiitake production is very small by comparison and has only recently gained recognition as a commercial alternative. In 1994, the total Australian production of specialty mushrooms (of which shiitake and oyster mushrooms are the main types) was thought to be less than 300 tons (Richards 1995). By 2005, the domestic market for specialty mushrooms had increased to 1000 tonnes and was expected to continue to grow, albeit at a modest rate (AMGA 2005). With particular respect to this study, a company in Tasmania is currently producing shiitake mushrooms on a eucalypt-based substrate and selling the product into mainland markets (Hyde 2000).

Although much is known about the life cycle and cultivation of shiitake mushrooms almost all the published research pertaining to the commercial production of shiitake mushrooms originates from the northern hemisphere, particularly northern Asia and the US, and almost none involves Australian tree species as growth substrates. In the early 1990s, the Australian Government’s Rural Industries Research and Development Corporation (RIRDC) funded a review of the specialty mushroom industry in Australian with a view to identifying research and development priorities (Richards 1995). This review highlighted the lack of continuity of supply and the lack of customer awareness as the most important factors limiting market development.

The RIRDC subsequently funded a research program at the University of Sydney which examined the prospects for producing shiitake mushrooms on an artificial substrate (Cho and Radford 1999). The researchers suggested that, although the mushrooms produced on a “synthetic sawdust log” were inferior to those produced on logs, the lack of logs of the appropriate hardwood species and a high labour costs meant that the potential of developing an industry based on the use of the log method in Australia was
limited. Since this initial research was done there has been little development of the commercial production of shiitake mushroom in Australia.

1.2 Shiitake production

Shiitake mushrooms grow naturally on decaying wood of hardwood trees and have traditionally been grown on short lengths of freshly-cut logs. Modern commercial production has proved successful on both logs and alternative substrates such as wood shavings and peat moss (Royse 2001). Although large commercial growers tend to favour more intensive substrate production systems that rely on controlled climate facilities, log-based production systems are widely promoted amongst private forest owners in the US as a means of utilising small logs from forest thinning and diversifying forest production (ibid). Until now, there has been no serious exploration of the potential for Australian forest owners to utilise small logs of native or plantation forest species for shiitake mushroom production, such as eucalypt (*Eucalyptus* spp.).

While a range of tree species have been used for shiitake production, the most common species used are of oaks (*Quercus* spp.). Alternative species that have been trialled in the northern hemisphere include Alder (*Alnus incana* (L.) Moench), Birch (*Betula pendula* Roth.), Red Oak (*Quercus rubra* L.), Black Cherry (*Prunus serotina* Ehrh.), Sassafras (*Sassafras albidum* (Nuttall) Nees), Eastern Sycamore (*Platanus occidentalis* L.) and China Fir (*Cunningham lanceolata* (Lambert) Hooker (Shieh *et al.* 1991; Raaska 1992; Shukla 1994; Sabota 1996). The results suggest that the choice of wood species can affect yield and timing of mushroom production although none have reported any impact of species on the food value of the mushrooms produced (Sabota 1996; and Raaska 1992).

1.3 Log-based shiitake production in Australia

Although oaks (*Quercus* spp.) are widely grown as an ornamental garden tree, logs suited to shiitake production are not generally available in Australia (Cho and Radford 1999). The prospects for development of log-based production of shiitake rely on the
identification of alternative species that are widely available and the development of viable production methods suited to the local climate that generate commercially acceptable yields. None of the published trials undertaken in the northern hemisphere have included species of *Eucalyptus*; however, there are reports from South America of shiitake having been grown on eucalypt logs (Stamets 2000). While it is not clear what species of eucalypt were involved, it is likely that they were one of the four commonly grown plantation species being grown in the region: either Sydney blue gum (*E. saligna* Sm), flooded gum (*E. grandis* Hill ex Maiden), shining gum (*E. nitens* Dean & Maiden) or blue gum (*E. globulus* Labill.). All of these species are also widely grown in commercial farm forestry systems in Australia.

There has been a rapid expansion in the area and distribution of eucalypt plantations in Australia over the last decade. Many smaller plantation owners are now committed to thinning their plantations to facilitate sawlog production. As a result, there is a large resource of fast-grown, small diameter eucalypt logs owned by hundreds of small private landholders. With limited options for marketing their thinnings and concerns about the long rotations rates for sawlog production, landholders may be interested in the prospect for growing shiitake mushrooms as a form of diversification. Alternatively, if eucalypts prove to be a suitable substrate for mushroom production, specialist growers may source logs from plantation owners.

**1.4 Aim and objectives**

The overall aim of this study is to investigate the potential for log-based cultivation of shiitake mushroom on a range of wood species currently available as thinnings from farm forestry plantations in Victoria by comparing them with species commonly used in the northern hemisphere.

More specific research hypotheses have been formulated to reflect the aims of this research:
1. Log-based shiitake mushroom production can be successfully adapted for use in Australia based on the well-established techniques used in the northern hemisphere.

2. Logs of Australian native plantation species (e.g. Eucalypts, Acacias) are just as suitable for commercial shiitake mushroom production as the species of genera being used by growers in the northern hemisphere (e.g. Oak, Poplar and Alder).

3. The dietary and market quality of shiitake mushrooms grown on alternative substrates is equivalent to those grown on traditional substrates.

Chapter 2 of this thesis provides a review the biology of *Lentinula edodes* including discussion of the environmental factors that may affect mycelium growth and mushroom development. Chapter 3 provides a detailed description of the methods used for fungal cultivation and Chapter 4 outlines the methods used for measurement of growth and analysis of nutrient content. The results are presented in Chapter 5 and discussed in detail in Chapter 6. Conclusions and implications of the development and production of shiitake on logs from range of tree species will be presented in Chapter 7.

The research was undertaken in association with a shiitake mushroom production project at the Centre for Education and Research in Environmental Strategies (CERES) in Melbourne. The CERES project was largely funded by the Victorian Government Adult Multicultural Education Services (AMES) with the aim of supporting newly arrived Karen refugees from Burma and Myanmar by providing training and employment in the production of a range of organic mushrooms (Australian Multicultural Education Services 2006).

Hundreds of logs of a range of tree species were donated to the project by three private landholders. The logs were inoculated and housed for the commercial production of shiitake and other specialty mushrooms and the produce was harvested and sold through the CERES market and local food outlets (AMES 2006). With the support of CERES, The University of Melbourne was invited to be involved in the design and implementation of a research trial. The logs selected for this research trial were
inoculated and stored along with other mushroom production logs and treated in the same manner. The University of Melbourne acknowledges the support of CERES, its staff and the trainees involved in the project and the landholders who donated the logs for the project.
Chapter 2
Biology of shiitake mushroom (*Lentinula edodes* (Berk.) Pegler)

2.1. Introduction
In order to cultivate shiitake mushroom, it is important to first understand the biology and environmental and nutritional requirements of shiitake. This section will include the classification of shiitake mushroom and the environment factors affecting mycelial growth and fruit development of *Lentinula edodes*. Physical factors such as temperature, light, relative humidity, moisture availability and nutrition will be discussed. This section will also explain the chemical factors such as pH and gas concentration required by *Lentinula edodes*.

2.2. Classification
Shiitake is a *ligolytic, aerobic basidiomycete* (Andrade *et al.* 2008) known by different names in different parts of the world. For example, in the US shiitake is known as the *black forest mushroom*, while in France it is known as *lentin*. The Japanese called it *shiitake*, whereas in China, different forms of shiitake are known by various names such as *xiang-gu* or the fragrant mushroom; *dong-gu* or the winter mushroom; and *hua-gu*, the flower or variegated mushroom (Chen 2001).

Shiitake has had several scientific names over the years. The following are the scientific names and year assigned: *Agaricus edodes* (1877), *Collybia shiitake* (1886), *Armillaria edodes* (1887), *Agaricus russaticeps* (1888), *Lepiota shiitake* (1889), *Lentinus tonkinensis* (1890), *Mastaleucomyces edodes* (1891), *Pleurotus russaticeps* (1891), *Cortinellus shiitake* (1899), *Tricholoma shiitake* (1918), *Cortinellus berkeleyanus* (1925), *Lentinus shiitake* (1936), *Cortinellus edodes* (1938), *Lentinus edodes* (1941), and *Lentinula edodes* (1975) (Singer 1941; Przybylowicz and Donoghue 1988; Hibbett *et al.* 1995; Chen 2001). Historically, *Cortinellus shiitake* was the most commonly used name (Tokimoto and Komatsu 1978), although a dispute as to whether shiitake was a member of the genus *Cortinellus* resulted in shiitake being placed in the genus *Lentinus*.
(Singer 1941). In 1975, shiitake was transferred into the genus *Lentinula* based on the fact that *Lentinula* is monomitic (fruiting body consists of one type of hyphae only) while *Lentinus* is dimitic (fruiting body consists of two types of hyphae). This classification is also supported by recent DNA research placing shiitake in the genus *Lentinula* (Chen 2005). Regardless of these viewpoints, scientists still frequently refer to shiitake as *Lentinus edodes* (Berk.) Singer (e.g. García-Mena *et al.* 2007; Vetchinkina *et al.* 2008).

There are many commercial strains of shiitake selected and propagated for their adaptability to different log species, the time taken to fruit after inoculation and the size, colour, taste and shape of the mushroom (Stamets 1993).

### 2.3. Shiitake mycelium growth and mushroom development

As the shiitake mycelium spread through a log it secretes exoenzymes that degrade the dead wood in order to obtain nutrients (Andrade *et al.* 2008). The production of mushroom fruiting bodies (sporophores) starts when the logs are fully colonised. Under natural conditions heavy rains and an associated drop in temperature stimulates mushroom production (Shiomi *et al.* 2007).

Shiitake mycelium growth and mushroom development are clearly influenced by environmental factors. Moisture and temperature are the two most important factors (Kaul 1997) although the log itself plays an important role in buffering the environment factors and protecting the fungi from extremes. The availability of nutrients, either as drawn from the substrate or provided as a supplement in the water supply, is also important.

#### 2.3.1. Temperature

Przybyłowicz and Donoghue (1988) indicate that temperature has a strong influence on survival, growth rate, time of fruiting, yield and the shape of the mushroom produced.
There are three temperature zones which are important for mushroom cultivation: the air temperature outside the growing room, the air temperature inside the growing room and the substrate temperature (Oei 1996). Of these, the substrate temperature (i.e. within the log) is the most important parameter influencing mycelial growth and fruiting body formation. Despite the importance of substrate temperature, Oei (1996) does not specify the temperature suitable for *Lentinula edodes* although substrate temperatures above 35 °C can cause thermophilic microflora to grow. This activity generates heat which produces an increase in substrate temperature and eventually causes mycelia to terminate.

Kuso (1982, cited in Przybylowicz and Donoghue 1988) reported that shiitake have been found to survive in logs at temperatures from -30 to 45 ºC. The impact of high temperature on the survival of fungi is determined by not only by the temperature, but also on exposure time (Przybylowicz and Donoghue 1988). The damage caused by a short period of exposure at high temperature corresponds to prolonged exposure at lower temperature. For example, Tokimoto and Komatsu (1978) report mycelium of shiitake can be terminated at 45 ºC; however, prolonged exposure of mycelium to 35 ºC can also cause mycelial death (Przybylowicz and Donoghue 1988).

There is an optimum temperature for mycelial growth above and below which growth is restricted (Przybylowicz and Donoghue 1988; Miles and Chang 1997). This may be due to the effect of temperature on enzyme activity and the resulting changes in rates of chemical processes (Miles and Chang 1997). Temperature also plays an important role in the initiation and development of fruiting bodies and a sudden shift in temperature may be required to induce fruiting of mushrooms (Komatsu 1961, cited in Przybylowicz and Donoghue 1988).

Suitable temperatures for mycelial growth and fruiting for a number of species are reported in Kaul (1997). For *Agaricus bisporus*, the optimum temperature for mycelial growth is 23-25 ºC, while fruit development is optimal within the range of 14-16 ºC. For *Coprinus cinereus* (Schaeff.) Gray, the optimum temperature for vegetative growth
is 37 °C; however fruiting does not occur at temperatures above 30 °C. The optimum temperature requirements are more complex for *Flammulina velutipes* (Curt.:Fries) Singer, with the optimum temperature for mycelial growth being 22-26 °C while primordium formation occurs at temperatures of between 10 and 20 °C and fruit development is at 10-15 °C.

The optimum temperature for mycelial development of *Lentinula edodes* is variable - reported to be 24-28 °C (Tokimoto and Komatsu 1982) or 15-24 °C (Sabota 2007). The temperature required for fruiting ranges from 5 to 30 °C (Sabota 2007), while the optimum range is from 10 to 25 °C (Przybylowicz and Donoghue 1988). However, the optimum temperature for fruiting may vary with the particular strain used for cultivation. For example, cold weather strains will fruit when temperatures are between 7 and 15 °C (Przybylowicz and Donoghue 1988). In contrast, warm weather strains will fruit when temperatures are between 10 and 28 °C (Sabota 2007) and wide-range fruiting strains will fruit between 10 and 27 °C.

Temperature also affects mushroom shape of *Lentinula edodes* (Ohira et al. 1982, cited in Przybylowicz and Donoghue 1988). Mushrooms developed under higher temperatures tend to form long stems and thin cap, whereas those cultivated under cooler temperature have short stems and thick caps (Tokimoto and Komatsu 1978).

### 2.3.2. Relative humidity and moisture availability

Moisture content of the substrate, relative humidity of the growing environment and the wetness of the surface of the log and fruiting bodies are all important considerations in the cultivation of shiitake (Miles and Chang 1997). Relative humidity is defined as the ratio of the amount of water actually present in the air and the maximum amount of water the air at the same temperature can contain (Oei 1996). According to Miles and Chang (1997), different mushroom species may require different levels of relative humidity for optimal growth. While most species will thrive in an environment with a relative humidity of close to 100% (Wichers 2001), this may not be optimal for shiitake log-based production systems as it may increase the number of competitive organisms.
Shiitake has been found to survive and grow in relative humidity ranging from 50-70% (Brauer et al. 2002), 80-95% (Queiroz et al. 2004) and 70-100% (Campbell and Racjan 1999). Sabota (2007) suggests that the optimal relative humidity for growth is 80-85%, while Leatham (1982) suggests that relative humidity between 85 and 90% is optimal. Stamets and Chilton (1983) provide humidity levels for various stages of production starting with a high humidity (90-100%) during the spawn run which is reduce to 85% during primordial formation and cropping.

2.3.3. Light

The role of light in mushroom cultivation has been investigated by many authors (Webster 1980). For Agaricus bisporus light is not essential for fruiting (Kaul 1997) and may actually inhibit mycelial growth (Tokimoto and Komatsu 1978; Miles and Chang 1997). In contrast, light is necessary for some species, including shiitake, during both the vegetative and fruiting stages (Przybylowicz and Donoghue 1988).

Light intensity during a bright sunny day can be more than 100000 lux (Halsted 1993). The spawn run of shiitake requires light intensities of 180-940 lux with an optimum of 550 lux (Han et al. 1981) and optimal light intensity during fruiting is 50-100 lux (Chen 2005). Excessive light exposure can reduce the number of fruit bodies, whereas a lack of light diminishes the diameter of pilei and the length of stipes (Han et al. 1981). Furthermore, fruiting bodies apparently develop abnormally and sporulation diminishes when the mushrooms are grown under filtered light using coloured cellophane papers (Tokimoto and Komatsu 1978).

2.3.4. Nutrition

Saprophytic fungi such as shiitake mushroom obtain their carbohydrates and nutrients from decay of the sapwood in log-based cultivation systems (Kaul 1997; Sabota 1998; McCoy and Bruhn 2005). The process involves decomposition of insoluble material present in the wood into simple form of sugars by secreting enzymes and subsequent absorption of these sugars (Przybylowicz and Donoghue 1988).
Sources of carbon (C) such as lignin, glucose and fructose are essential for energy supply for a range of metabolic processes of mushrooms (Zanetti and Ranal 1997 cited in Rossi et al. 2003). A study of Lentinula edodes grown on liquid medium showed that glucose was compulsory for ligninolytic activity (Leatham 1986). The rates of lignin degradation decreased markedly after 25-30 days of growth compared to initial growth. Supplying additional glucose supported degradation activity, whereas supplying the medium with an additional nitrogen (N) source (L-glutamic acid) reduced degradation activity (Leatham 1986). Doubling the concentration of glucose resulted in an increase in ligninolytic activity of as much as 30%.

Nitrogen is important for mushroom growth for construction of protoplasm and structural elements of cells. Major sources of N include organic and inorganic compounds such as ammonium, nitrate, urea and proteins released during degradation of wood. A small amount of soluble N is also absorbed from xylem and phloem in fresh wood (Chen 2005). The N-containing compounds, ammonium chloride (NH₃Cl), ammonium nitrate (NH₄NO₃) and acetamide (CH₃CONH₂) appear to promote growth of mycelia, while other N-containing compounds (i.e. sodium nitrite (NaNO₂), potassium nitrite (KNO₂), calcium nitrite (Ca(NO₂)₂), ammonium sulphate ((NH₄)₂SO₄) and ammonium phosphate ((NH₄)₃PO₄) do not (Han et al. 1981).

Nitrogen sources are not evenly distributed throughout logs. Most N-containing compounds are located in the cambium area and availability decreases towards the core or heartwood. For example, 4-5% of N is found in tree bark and only 0.4-0.5% is found in xylem (Chen 2005). This helps to explain why mycelia of shiitake grow best in sapwood compared to heartwood. The optimum level of N during the spawn run is between 0.016 and 0.064% N, while optimum nitrogen levels during fruiting is 0.02% N.
The ratio of C to N (C:N ratio) is an important aspect in the rate of decomposition of plant tissues (Flaig 1964, cited in Arya and Arya 2003). In the production phase of spawn culture, the C:N ratio can also affect mushroom productivity and quality (Tokimoto and Kawai 1975). The optimum C:N ratio for mycelial growth of shiitake is 25:1, while a ratio of 40:1 is suggested for the mushroom production stage.

2.3.5. Other chemical factors

Different species of mushrooms require a specific range of substrate pH (Kaul 1997). A large number of mushroom species show excellent vegetative growth at a pH slightly below 7.0 (e.g. pH 6.5-6.8), though, this may differ according to strain used and fruiting or vegetative state (Miles and Chang 1997). Shiitake may prefer even more acidic substrates. For example, optimum mycelium growth of *Lentinula edodes* in agar occurs in the range of 5.0-6.0 (Hiroe and Kamiyoshi 1937, cited in Tokimoto and Komatsu 1978), whereas optimum primordium formation occurs in the range of 3.5-4.5 (Tokimoto, unpublished, cited in Tokimoto and Komatsu 1978). This pH range is also suitable for fruit body development in laboratory culture on artificial media (Tokimoto and Kawai 1975). In comparison, the optimum pH for other wood decay fungi ranges between 4.5 and 5.5 (Scheffer 1973, cited in Przybylowicz and Donoghue 1988). For shiitake mushroom, the optimum pH during mycelial growth is 4.3 (Tokimoto and Komatsu 1978). The pH of logs varies from 4.5 to 5.0 and may become more acidic as mycelia decay the wood (Scheffer 1973 and Tokimoto *et al.* 1980, cited in Przybylowicz and Donoghue 1988).

The composition of atmospheric gases (including oxygen (O$_2$), carbon dioxide (CO$_2$), N$_2$ and other gases) around developing mushrooms has been shown to significantly influence the appearance of fruiting bodies (Przybylowicz and Donoghue 1988; Kaul 1997; Miles and Chang 1997). For example, O$_2$ and CO$_2$ play an important role in commercial production of button mushroom (*Agaricus brunnescens* Peck) (Donoghue and Denison 1995. At least a minute quantity of CO$_2$ is required by all fungi for growth (Deacon 2006), and it is an essential component of several important metabolic reactions (Carlile *et al.* 2001). The role of CO$_2$ has been studied in numerous species includes *Agaricus bisporus*, *Agaricus bitorquis* (Quélet) Sacc., *Coprinus comatus* (O.F. Müll.) Persoon, *Flammulina velutipes*, *Ganoderma lucidum* (Curtis) P. Karst, *Hericium*
erinaceus (Bull.) Persoon, Lentinula edodes, Pleurotus ostreatus Champ. Jura. Vosg., Polyporus brumalis Persoon and Schizophyllum commune Fries (Oei 1996; Kaul 1997; Miles and Chang 1997). Generally, the tolerance level of CO₂ during spawn run is greater than during fruiting and several species are sensitive to high CO₂ levels during fruiting (Oei 1996; Kaul 1997). For example, initiation of fruiting of Agaricus bisporus occurs at a CO₂ concentration ranging between 0.03 and 0.10% and a concentration of 0.5-1.0% is high enough to inhibit the formation of primordia. Lentinula edodes reacts in much the same way as Agaricus bisporus and the optimum levels of CO₂ range between 0.1 and 0.2% (Przybylowicz and Donoghue 1988; Stamets 2000).

Although the role of gas concentrations on mushroom growth and development is interesting it may not be relevant for those growing shiitake outside using bed logs where gas composition can not be easily measured or controlled (Donoghue and Denison 1995).

2.4. Summary
This chapter describes the classification of shiitake mushroom, and the environmental factors affecting mycelial growth and fruit development of Lentinula edodes. The information relating to physical and chemical factors outlined in this chapter is essential in order to understand the cultivation of shiitake. The studies described in the following chapters will investigate the growth, fruiting and nutrient content of mushrooms grown on alternative log-based substrates.
Chapter 3
Mushroom cultivation on logs

3.1. Introduction
Although *Lentinula edodes* (shiitake) is the most common, there are a number of edible mushroom species that are commercially grown on whole logs, including *Ganoderma lucidum* (reishi), *Grifola frondosa* (Dicks.) Gray (maitake), *Hericium erinaceus* (lion’s mane and others), *Pleurotus ostreatus* (oyster mushroom), *Tremella fuciformis* Berkeley (white jelly fungus), *Auricularia auricula-judae* (Fr.) J.Schröt. and *Auricularia polytricha* (Mont.) Sacc. (Oei 1996; Bruhn 1999). From observations and reports of traditional practices and more modern commercial experience, and a growing body of published research, much is known about the life cycle and log-based cultivation of these mushroom species and the log characteristics and environmental factors that have an influence on mushroom development and productivity. However, almost all the available information originates from the northern hemisphere and, other than some experimental work in Brazil, very little is known about the prospect for using Australian native tree species.

3.2. Historical review of cultivation
Shiitake is the second most cultivated edible mushroom in the world, following the button mushroom (*Agaricus bisporus*), and is the most common species grown commercially on logs (Przybylowicz and Donoghue 1988; Oei 1996). Log-based shiitake mushroom cultivation is believed to have originated in China during the Sung Dynasty (960-1127) (Przybylowicz and Donoghue 1988). The original technique probably involved notching the logs with a hatchet then placing a ripe mushroom against the freshly exposed wood log, where the spores would germinate and the mycelium would infect the log (Oei 1996; Royse 2001; Shen *et al.* 2004). However, over the last 75 years, techniques for shiitake cultivation on logs have improved to the point that pure cultures of mycelium from superior strains of the fungus are directly impregnated into the wood (Oei 1996; Shen *et al.* 2004).
The subsequent development of the mycelium within the log and the production of mushrooms are clearly influenced by the species of tree being used, the size and properties of the logs, the inoculation method, log storage and management, and a wide range of environmental factors.

3.3. Suitable species of trees for bed logs

Every tree species has distinctive wood characteristics which can influence shiitake mycelium growth and the development and yield of mushrooms (Leatham 1982; Przybylowicz and Donoghue 1988). The name shiitake is derived from the shii tree (Castanopsis cuspidata (Thunb.) Oerst.) that grows naturally in southern Japan and Korea. The genus Castanopsis is from the same botanical family (Fagaceae) as the oaks (Quercus spp.), chestnuts (Castanea spp.) and beeches (Fagus spp.). However, many other tree species that have proved suitable for shiitake mushroom cultivation. Most of the commercially used logs are northern hemisphere deciduous hardwood species (Royse 2001) although there has been some success with softwoods (Antonio 1981) including Pinus radiata in New Zealand (Chu-Chou 1983).

There are many studies, mostly undertaken in Japan, China, US and Taiwan, comparing the potential of a wide range of locally available tree species and using different type of fungal strains (Raaska 1992; Oei 1996). For example, in Northern Alabama in the US, Sabota (1996) tested a number of wood species including Quercus spp., Prunus serotina, Sassafras albidum and Platanus occidentalis. The logs were inoculated using eight different strains of Lentinula edodes. The results showed that logs of Quercus spp. produced significantly higher yields of shiitake mushrooms than any of the other species. Similarly, San Antonio (1981) compared a wide range of North American tree species and found that northern red oak (Q. rubra) and white oak (Q. alba L.) were the most productive over a range of shiitake strains.

Not surprisingly, the most recommended tree species for shiitake cultivation in the US belong to the beech family (Fagaceae), particularly oaks (Quercus spp.) (Leatham 1982). Bratkovich (1993) suggests that although all species of oak can be used, species
such as white oak (*Q. alba*, Linnaeus) and chestnut oak (*Q. montana* Willd.) are preferred in North America over the thinner barked species like red oak (*Q. rubra*, Du Roi), scarlet oak (*Q. coccinea* Muenchh.) and pin oak (*Q. palustris* Muenchh.). Tree species from other North American genera that have been used for shiitake production include chestnut (*Castanea* spp.); chinkapin (*Castanea pumila* (L.) P. Mill); maple (*Acer* spp.); cottonwood, poplar and aspen (*Populus* spp.); willow (*Salix* spp.); elm (*Ulmus* spp.) and alder (*Alnus* spp.) (Leatham 1982; Bratkovich 1993).

In Taiwan, tree species most suitable for shiitake cultivation are *Liquidambar formosa* Hance and members of the beech family (Fagaceae) (Shieh *et al.* 1991; Oei 1996). *Cunninghamia lanceolata* (Lamb.) Hook. is widely grown species in Taiwan but has been considered to be unfeasible for growing shiitake mushrooms on a commercial scale (Shieh *et al.* 1991). The species was found to yield mushrooms for up to 13 harvests with the largest yield obtained between 9-11 months after inoculation. However, the Taiwan Agricultural Research Institute reported that although *Cunninghamia lanceolata* is suitable for shiitake cultivation the yield is lower when compared to *Liquidambar formosa* (Oei 1996). Other species have also been reported by to provide similar or greater yields when compared to *Liquidambar formosa* (Appendix 1) (Oei 1996).

The only references to the use of eucalypt logs for shiitake production come from South America. Queiroz *et al.* (2004) report on an experience that sought to increase the yield of shiitake mushrooms from *E. saligna* logs from ‘low and variable’ by immersing them in mineral supplements. Shiomi *et al.* (2007) studied the effect of mechanical and thermal shocks on the production of shiitake from the same species. Plantation eucalypt logs of other species are available in Brazil. Andrade *et al.* (2008) tested the rate of mycelium spread of two *Lentinula edodes* strains in sawdust extracts of seven eucalypt species (*E. saligna, E. grandis, E. urophylla* S.T. Blake, *E. pellita* F.Muell., *E. paniculata* Sm., *E. citriodora* Hook. and *E. camaldulensis* Dehnh.). *E. citriodora* (now known as *Corymbia citriodora* (Hook.) K.D. Hill & L.A. Johnson) proved significantly more effective than all other species tested.
3.4. Important properties of logs

Logs of the same species can vary greatly in their suitability and productivity as a substrate for shiitake mushroom production. For example, Queiroz et al. (2004) suggest that the age of the trees, site fertility and management may explain the variability in performance of *E. saligna* logs in Brazil. The mycelium only colonise the sapwood of the log. Wood density, moisture content and nutrient concentration of the sapwood are important factors that are likely to impact on shiitake production (Przybylowicz and Donoghue 1988).

3.4.1. Sapwood/heartwood ratio

Shiitake mycelium only colonise the sapwood of a log. In the living tree, the sapwood consists of the active xylem, through which water and nutrients are transported up the stem. The xylem is also a carbohydrate bank, depending on the stage of growth, starch and sugars are either deposited or extracted from the xylem. The heartwood, on the other hand, contains no living cells and plays no role in plant growth other than providing physical support. The absence of starch and the presence of phenolic compounds often give the heartwood a darker colour and greater natural durability against fungi, insects and other decay agents (Przybylowicz and Donoghue 1988).

It is the high moisture content, open cell structure, presence of sugars and starch, and lack of inhibiting chemicals which allows saprophytic fungi, such as shiitake mushroom, to grow and spread in the sapwood zone of recently cut logs (Przybylowicz and Donoghue 1988). The width of sapwood and the ratio of sapwood to heartwood vary between tree species and amongst individuals of the same species (Przybylowicz and Donoghue 1988). Although the process of heartwood formation is not well understood, it has been shown that there is a relationship between the water needs of the canopy (leaf area) and the width of the sapwood band (Vertessy et al. 1995; Teskey and Sheriff 1996). A fast grown tree with a large healthy canopy is likely to have a wider sapwood band than a tree of slow growing tree of similar size. Most authors suggest that
the wider the sapwood band the better the log is for shiitake production (Przybylowicz and Donoghue 1988; Davis 1993; Sabota 1998; 2007).

3.4.2. Nutrient content of the sapwood

Nutrients required by shiitake are obtained by decaying the wood, particularly in the sapwood area. The process involves decomposition of insoluble materials present in wood into simple form of sugars by enzymes secreted by fungal hyphae (Przybylowicz and Donoghue 1988). The type and level of nutrient in the substrate could influence the fungal growth (Silva, Machuca and Milagres 2005). They found that the addition of nitrogen could accelerate mycelial growth.

As reported above, Queiroz et al. (2004) experimented with fertilisation, including both nitrogen and phosphorous, of E. saligna logs in an attempt to increase productivity. Although mineral supplementation did increase production, the authors suggest that the problem of poor productivity of eucalypt logs in Brazil is often due to the low vigour of the trees being used.

The nitrogen levels in wood are generally very low: ranging from between 0.03% and 0.3% (Przybylowicz and Donoghue 1988). Whilst this may be partly explained by difference between species, many authors like Queiroz et al. (2004), prefer to focus on the fact that the mineral content of the wood in a tree generally reflects the fertility of the site on which it is growing (Aerts 1995, Lambers et al. 1998 and Schlessinger 1997 cited in Meerts 2002). Thus, for the purpose of shiitake production, logs should be sourced from healthy, fast growing trees growing on high fertility sites.

For most, if not all tree species, the concentration of carbohydrates in sapwood fluctuates throughout the growing season. For example, ten species from temperate forest including six deciduous broad-leaved species (Acer campestre L., Carpinus betulus L., Fagus sylvatica L., Prunus avium (L.) L., Quercus petraea (Mattuschka) Liebl., and Tilia platyphyllos Scop., one deciduous conifer (Larix deciduas Mill.) and
three evergreen conifers (Abies alba Mill., Picea abies (L.) H.Karst, and Pinus sylvestris L.) were assessed by analysing seasonal variation of non-structural carbohydrate (NSC) (i.e. sugars and starch) concentrations in leaves, branch wood and stem sapwood (Hoch et al. 2003).

The results show that the starch and sugar concentration in the leaves decreased continuously from June through to October (summer to autumn) by as much as 15 to 20%. In stem sapwood, the seasonal variations of NSC concentrations were not as significant. However, the NSC levels in some tree species, including Acer, Prunus, Pinus and Quercus, did reach their lowest levels during mid season (June) when stem growth was strongest. By contrast, other tree species, Carpinus, Fagus, Abies, and Picea, had slightly higher NSC levels during the very same period.

The seasonal variation in the starch content of eucalypts has been studied in the context of understanding the risk of Armillaria infection following native forest logging operations in central Victoria (Tomkins et al. 1989). The starch levels in E. globulus and E. obliqua L'Hér. stumps were found to reach a maximum in late spring to early summer and a minimum in mid-winter and mid- to late summer.

### 3.4.3. Wood density

Wood density can affect the productive life and the total yield of a shiitake log with increasing density being associated with higher yields for the tree species commonly used in the northern hemisphere (Przybylowicz and Donoghue 1988; Davis 1993; World Agroforestry Centre 2007). Wood density is commonly presented as either the basic density (the weight of oven dried wood divided by its green volume) or the specific gravity (the weight of oven dried wood divided by its dry volume). Table 3.1 provides the specific gravity of a number of species used for shiitake production in North America. Along with the oaks (Quercus spp.) other higher density tree species used for shiitake production include Beech, Birch, Hickory and Hop-hornbeam. Low density species mentioned as suitable for shiitake production include Alder and Willow.
Both high and low density wood species have proved successful for shiitake production in comparative trials. San Antonio (1981) compared 16 North American tree species which found that the highest yields came from the low density black willow and high density red oak. Based on his own extensive practical and scientific experience Stamets (2000) argues that wood density is important with the denser hardwoods having a productive life of as long as six years whereas the lighter, less durable species may only last half that. This view was support by Bratkovich (1991) who set up a trial comparing the productivity of oak (*Quercus* sp.) and beech (*Fagus grandifolia* Ehrh.) and reports eliminating the beech from the experiment due to “poor fruiting, low log moisture content, and splitting/sloughing bark”. McCoy and Bruhn (2005) also suggest the higher density sugar maple and white oak as being the best species.

Higher wood density does appear to correlate with having to wait longer before fruiting. Bratkovich (1993) reports that although the thin-barked low-density species (such as the Poplars or Willows) can generate high yields relatively soon after inoculation they do require greater care and lack the longevity of the higher density species. Sabota (1998) suggests that white oak may take as long as 8 to 12 months before it begins to fruit whereas the lower density species can produce fruit within 6 to 8 months.

<table>
<thead>
<tr>
<th>Species</th>
<th>Specific Gravity *</th>
<th>Moisture content freshly cut **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alder</td>
<td>0.37</td>
<td>49</td>
</tr>
<tr>
<td>Beech</td>
<td>0.56</td>
<td>39</td>
</tr>
<tr>
<td>Birch</td>
<td>0.57</td>
<td>42</td>
</tr>
<tr>
<td>Chestnut</td>
<td>0.40</td>
<td>55</td>
</tr>
<tr>
<td>Hickory</td>
<td>0.65</td>
<td>39</td>
</tr>
<tr>
<td>Hop hornbeam</td>
<td>0.63</td>
<td>34</td>
</tr>
<tr>
<td>Maple, soft</td>
<td>0.44</td>
<td>61</td>
</tr>
<tr>
<td>Maple, hard</td>
<td>0.56</td>
<td>44</td>
</tr>
<tr>
<td>Oak, white</td>
<td>0.60</td>
<td>41</td>
</tr>
<tr>
<td>Oak, red</td>
<td>0.54</td>
<td>43</td>
</tr>
<tr>
<td>Willow, black</td>
<td>0.34</td>
<td>58</td>
</tr>
</tbody>
</table>

*Specific gravity: the ratio of the weight of wood compared to the weight of an equal volume of water
** Moisture content was calculated from an average of heartwood and sapwood, using fresh weight as the base.
Wood density can vary with age, growth rate and position in the tree. At the cellular level, wood density also varies across the growth ring with the transition from earlywood and latewood and the distribution of pores in hardwoods. The impacts of age and growth rate are therefore important considerations in selection of logs for shiitake production. In some hardwood species, such as oak (Quercus spp.), large open pores form a ring in early wood (ring-porous species) at the start of the growing season accentuating the appearance of the growth ring. Since bands of pores have the same width irrespective of growth rate, the densest timber comes from the fastest growing trees because there are fewer rings of open pores (Haygreen and Bowyer 1989). Hence, the heaviest oak timber is produced from the fastest growing trees on the most fertile sites.

For diffuse porous species, such as poplar and eucalypts, growth rate does not seem to affect wood density, however, the age of the tree and position in the stem are important due to a phenomenon called ‘juvenile’ or ‘crown’ wood. Wood produced by the cambium in close proximity to the actively growing canopy tends to have a lower density than that produced lower in the stem or trunk. As a result, the average density in a eucalypt, for example, tends to increase in successive growth rings from the centre of the log to the outside, and decreases up the stem (Dean and Baldwin 1996; Downes et al. 1997).

### 3.4.4. Log moisture content

Survival of shiitake mycelium depends on water retained in logs (Hill 2002). Opinion varies as to the ideal log moisture content (LMC) for shiitake production. According to Przybylowicz and Donoghue (1988) and Hill (2002), log moisture content should be maintained above 35%, whereas Oei (1996) suggests a range of between 40 and 45%. Low moisture contents (less than 20-25%) restricts mycelium growth and can ultimately result in death of the mycelium (Oei 1996; Hill 2002). Log moisture content above 60% is also unfavourable for mycelial growth and can allow competitive fungal species to flourish (Oei 1996).
The initial log moisture content of freshly cut logs varies both among species and individuals (Przybylowicz and Donoghue 1988, Table 3.1). To avoid excessive moisture loss, it is recommended that the ends of logs and the points of inoculation, are coated with a sealant such as beeswax (LeBauer 2004; Sabota 2007). Maintaining the log moisture content over the course of the year using sprinklers is important in maintaining a consistent level of mushroom production (Farr 1983). Over watering, high humidity and poor ventilation increase the risk of surface molds such as *Trichoderma* spp. (Bratkovich 1991).

### 3.4.5. Bark Characteristics

Bark plays an important role in providing a protective layer around the external part of the log reducing moisture loss and shielding the wood from contamination by rival fungi (Przybylowicz and Donoghue 1988). The thickness, persistence, durability and structure of the bark vary between species and with the age of the tree. With respect to shiitake production, the time of cutting may be important. Considering deciduous trees, those cut down during summer are less suitability for shiitake cultivation. Due to bark that is normally more loosely bound and likely to strip or peel off, thus increasing the possibility of contamination by competitive organisms or drying out (Royse 2001).

Tree species with long bark retention are favourable for shiitake cultivation. One study by Bratkovich (1991) aimed to compare shiitake production from two wood species: oak (*Quercus* sp.) and American beech (*Fagus grandifolia*). However, not long into the trial the beech was eliminated due to poor fruiting, low log moisture content, and splitting/sloughing bark.

Whilst it is clear that the persistence and durability of the bark is critical in conserving moisture and that thick heavy bark may restrict mushroom development there has been little research undertaken to assess and describe the ideal bark characteristics of a shiitake log.
3.5. Preparation of logs for cultivation

Logs for shiitake cultivation are preferably cut when the nutrient content of the logs is at their highest level. Mori (1986) reports that, in Japan the trees are traditionally felled in autumn when the leaves are coloured. It is believed that prior to winter, when approximately one third of the leaves begins to turn red, the nutrient content in wood begins to improve and continues to elevate prior to budding in spring (Ito 1978). Trees are generally cut into logs immediately after felling (Przybylowicz and Donoghue 1988); or up to two weeks before inoculation (Sabota 2007) and are cut into logs 1-1.5 m in length (Ito 1978). The most efficient diameters for logs are reported to be 5-15 cm (Ito 1978; Royse 2001). Logs that are approximately 1 m long and between 5 and 15 cm in diameter are easy to handle and are generally considered to be the most productive (Leatham 1982; Sabota 2007).

3.6. Inoculation procedures

Inoculation is the process of inserting the spawn of shiitake into sapwood of the log. The stages of inoculation includes: drilling, inoculating and sealing the log.

There are differing opinions on when inoculation of logs should take place. Some research suggests that logs have to be inoculated soon after cutting (Akiyama et al. 1976, Shinkosha 1981, and Przybylowicz and Donoghue 1987 cited in Przybylowicz and Donoghue 1988), whereas others recommend waiting for at least two to three weeks (Leatham 1982) or even four to eight weeks (Ito 1978). It is thought that the living tissues in freshly cut logs inhibit growth of *Lentinula edodes* (Leatham 1982). However, if logs are left too long there is an increased risk of competitive species infecting the log or excessive loss of moisture before logs are sealed. Another consideration in the timing of inoculation is the condition of bark. Generally, the ideal condition is to maintain the bark intact, clean and dry as wet bark may attract moulds which could unintentionally get into the inoculation holes (Przybylowicz and Donoghue 1988).

Different techniques of inoculation have been used in log-based mushroom cultivation including wood plugs (dowels), wood wedges and sawdust (Oei 1996). Of these, the wood plugs and sawdust inoculation are used most frequently in modern shiitake
cultivation (Przybylowicz and Donoghue 1988; Davis 1993; Sabota 1996; Royse 2001). Wood plugs have a number of advantages compared to sawdust spawn (Royse 2001). For example, the chamfered end of the plug or dowel makes it easy to insert and overcomes the need for special equipment required for sawdust inoculation. In addition, wood plugs are more resistant to drying once inserted because small diameter holes results in less surface exposure. Other advantages and disadvantages of different techniques used for inoculation are detailed in Table 3.2. Wood wedges have not been used extensively for shiitake inoculation due to the lack of availability and the need for special equipment to make them (Oei 1996).

Table 3.2 Advantages and disadvantages of different inoculation techniques (from Oei 1996; Royse 2001).

<table>
<thead>
<tr>
<th>Inoculation techniques</th>
<th>Advantage</th>
<th>Disadvantage</th>
<th>Equipment needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood plugs or dowels</td>
<td>Fast and efficient, easy and does not require special tools to insert plugs</td>
<td>Electricity should be available to drill the holes, spawn relatively expensive</td>
<td>High speed drills with special drill bit</td>
</tr>
<tr>
<td>Wood wedges</td>
<td>No need for electricity, low investment</td>
<td>This type of spawn is not available everywhere, special equipment is necessary to produce it</td>
<td>Hammer with sharp end</td>
</tr>
<tr>
<td>Sawdust</td>
<td>Spawn is readily available in the region which also produces mushrooms in plastic bags, cheap waste materials can be used to produced spawn</td>
<td>Inoculation is slightly more laborious (the spawn has to be pressed into the holes, which should be sealed with wax)</td>
<td>High speed drill with special drill bit, spawn plungers or a simple stick; wax-applicators</td>
</tr>
</tbody>
</table>

The accepted practice of inoculation with plugs or dowels involves drilling holes at regular intervals along and around the log and inserting a wooden dowel that has been previously inoculated with the appropriate fungal strain. The spacing of inoculation holes is thought to influence the rate of colonisation and the cost of production. Oei (1996) provides a table to assist producers in Taiwan and Japan determine the number of holes and rows required for a log of certain size (Table 3.3). The recommendations correspond to an inoculation rate of approximately one inoculant for every 190 square centimetres of surface area of the log. Bratkovich (1991) adopted an inoculation pattern
that involved having one row of holes per 2.5 cm of log diameter and spacing the holes ever 15 cm along each row. For a 15 cm diameter log this represents one inoculant for every 117 cm$^2$. There is no published research that compares different rates of inoculation.

The pattern of inoculation is also thought to influence the colonisation of the log by the fungi. Przybylowicz and Donoghue (1988) propose several inoculation patterns which include diamond, modified diamond and ring patterns (Figure 3.1) but suggest that the most common is the diamond pattern with spacing between rows of approximately 5-10 cm with the holes drilled at 15-25 cm spacing along the row. The shiitake mycelium grows more rapidly along the grain than across the grain so to facilitate colonisation the holes in each new row are offset from the previous row to form the diamond pattern as shown in Figure 3.2 (Leatham 1982; Przybylowicz and Donoghue 1988; Royse 2001).

Table 3.3 Number of inoculation holes per log (from Oei 1996)

<table>
<thead>
<tr>
<th>Diameter of Logs (cm)</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>15</th>
<th>18</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rows (rows run parallel to the length of the log)</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Number of holes per log of 1.2 m</td>
<td>12</td>
<td>16</td>
<td>20</td>
<td>24</td>
<td>28</td>
<td>36</td>
<td>44</td>
</tr>
<tr>
<td>Surface area of log (cm$^2$)</td>
<td>2262</td>
<td>3016</td>
<td>3770</td>
<td>4524</td>
<td>5655</td>
<td>6787</td>
<td>7918</td>
</tr>
<tr>
<td>Concentration of inoculants (surface area per inoculant)</td>
<td>188</td>
<td>188</td>
<td>188</td>
<td>188</td>
<td>201</td>
<td>188</td>
<td>180</td>
</tr>
</tbody>
</table>

Figure 3.1 Inoculation patterns on logs used for shiitake mushroom cultivation (from Przybylowicz and Donoghue 1998)
Figure 3.2 The diamond pattern for inoculation of logs for shiitake cultivation (from Przybylowicz and Donoghue 1998)

The holes for wood plugs are drilled to a diameter of 8.5 mm (Przybylowicz and Donoghue 1988) or 10-15 mm (Oei 1996), depending on the type and availability of wood plugs spawn, and to a depth of approximately 1.5 to two times the length of the wood plugs (Przybylowicz and Donoghue 1988). When wood plugs are inserted into the drilled holes and levelled with the surface of the log, an empty space is created underneath the plugs (Figure 3.3) and is rapidly colonised by mycelium (Przybylowicz and Donoghue 1988). For the sawdust technique, holes drilled are usually larger in diameter (12 mm) to allow a spawn transfer tool to be used to insert the sawdust (Figure 3.3). Oei (1996) underlines the importance of levelling the surface of the sawdust in the hole, although no clear explanation of why this has to be done is provided (Figure 3.4). For the wood wedge technique, holes need to accommodate the whole wedge thus ensuring that none of the inoculants is exposed (Oei 1996). To do this, a special hammer to make holes and insert the wedge is required (Oei 1996, Figure 3.5).

Figure 3.3 Appropriate depth of spawn hole for wood plugs and sawdust (from Przybylowicz and Donoghue 1988)
In order to maintain moisture levels in logs, especially in drier areas, sealing inoculation holes is encouraged. This process is particularly important when employing sawdust spawn to maintain moisture and to keep sawdust in drilled holes (Przybylowicz and Donoghue 1988). Materials that have been used to seal inoculation holes include paraffin and cheese wax, foam plugs, paint, cork and grafting seals (Sabota 1996; Przybylowicz and Donoghue 1988; Davis 1993; Mc Coy and Bruhn 2005; Anderson and Marcouiller 1990; and Jenkins et al. n.d). The most effective and commonly used method is wax (Przybylowicz and Donoghue 1988).
The choice of wax and temperature of application are important. Molten paraffin may dry to form a thin, tough, transparent covering over the spawn when applied at 127 °C but forms a thick opaque covering which can be knocked off easily when paraffin is applied at lower temperatures (Przybylowicz and Donoghue 1988). Softer, more pliable waxes including cheese wax and bees wax can also be used for sealing holes and are popular in areas with cold winters. However, bees wax often attracts bees when heated (Przybylowicz and Donoghue 1988; Sabota 2007).

3.7. Stacking
After inoculation, logs enter an incubation period where they are managed to encourage the spawn run (Davis 1993). Several methods have been used to stack logs, including crib stacks or criss-cross, lean-to stacks, A or X-frame stacks and bulk stacks (Przybylowicz and Donoghue 1988; Anderson and Marcouiller 1990; Bratkovich and Gilbert 1993). The arrangement of logs is important to ensure adequate ventilation whilst reducing the risk of drying.

3.7.1. Crib stacks
Crib stacks, also known as criss-cross, square or cabin style stacks, consist of horizontal layers of logs where each layer of log is perpendicular to the layer beneath. The number of logs in each layer may vary depending on the size of the logs (4 to 8 logs per layer) and heights of stacks range from 60-120 cm (Figure 3.6, Przybylowicz and Donoghue 1988).

Figure 3.6 Crib stacks for shiitake cultivation (from Przybylowicz and Donoghue 1988)
The crib stack method provides good air circulation around logs which can help reduce the risk of surface moulds (Bratkovich 1991). This arrangement is also space-efficient and keeps logs well off the ground. This can be an advantage in areas of poor drainage and, when termites are a problem (Przybyłowicz and Donoghue 1988). However, the crib stack method requires near-level ground and environmental conditions, particularly temperature and relative humidity, may not be uniform throughout the stack (Bratkovich and Gilbert 1993; Davis 1993). To ensure even mycelium growth logs can be carefully watered to ensure all the logs are evenly wet (Przybyłowicz and Donoghue 1988).

3.7.2. Lean-to stacks
Lean-to stacks are also known as centipede stacking (Przybyłowicz and Donoghue 1988). The stack is constructed by leaning vertical rows of logs onto a horizontal rail or wire (Figure 3.7A, Przybyłowicz and Donoghue 1988). Lean-to stacks work effectively when applied on a hill slope or uneven terrain (Figure 3.7B, Anderson and Marcouiller 1990; Sabota 2007). This stacking arrangement is the most broadly applicable method for shiitake mushroom cultivation due to its flexibility and can be applied in artificial and natural spawn run areas (Przybyłowicz and Donoghue 1988; Bratkovich and Gilbert 1993).
Lean-to stacks make it possible to keep logs moist due to the relatively small distance between the logs and the ground. Mycelium spreads from the logs to the ground which acts as a moisture reservoir sustaining survival and growth of fungi, particularly during dry weather (Przybylowicz and Donoghue 1988). Exposure to rainfall can be varied by altering the angle of logs to be nearer to vertical or horizontal, resulting in less or greater exposure to rain, and a steeper angle can increase water runoff log surfaces (Bratkovich and Gilbert 1993).

Compared to the crib stack method, the lean-to stack method requires more space, needs greater handling of logs and has the disadvantage of dissimilarity in temperature and humidity between both ends of the logs (Przybylowicz and Donoghue 1988; Davis
1993). To prevent this, Przybylowicz and Donoghue (1988) recommend turning logs over end-for-end halfway through incubation to promote uniform mycelial growth.

**3.7.3. A-frame and X-frame stacks**

A-frame stacks, also known as X-frame stacks, are similar to lean-to stacks. The initial log in the A-frame stack leans on a support, while subsequent logs rest at an angle on the lower end of the log on the ground and the upper end on the previous log (Figure 3.8A, Przybylowicz and Donoghue 1988). The upper end of logs can also lean on a rail or wire at an angle of 45 to 60˚ to the ground (Figure 3.8B and C).

These stacking methods are suitable for all areas, particularly more humid locations where other stacking methods may cause excess surface moisture and encourage growth of mould (Przybylowicz and Donoghue 1988; Bratkovich and Gilbert 1993). Mushrooms can be picked without difficulty and restacking (Przybylowicz and Donoghue 1988), however, this arrangement is not favourable during the spawn run (Bratkovich and Gilbert 1993).
Figure 3.8  A-frame and X-frame stacking methods leaning on (A) a single supporting block to form an A-frame, (B) and a rail or (C) threaded with a wire to form an X-frame (A from Przybylowicz and Donoghue 1988; B from Anderson and Marcouiller 1993; C from Sabota 2007)
3.7.4. Bulk stacks

Bulk stacks are formed by aligning logs in the same direction either vertically or horizontally (Figure 3.9, Przybyłowicz and Donoghue 1988). By being tightly stacked, this method is regarded as the most space efficient stacking system. Water loss from the logs inside the stack is low and changes in temperature and humidity are limited by the thermal mass of the logs and the metabolic heat emitted by the growing mycelium (Przybyłowicz and Donoghue 1988). However, the interior logs are not well ventilated and are at increased risk of mould growth from competitive fungi and other diseases (Sabota 2007; Przybyłowicz and Donoghue 1988). Close connection between logs allow the mycelium to bind them together which increases the likelihood of bark damage when logs are moved (Sabota 2007).

3.8. Spawn run

Following inoculation, the mycelia will start to spread throughout the sapwood drawing on available nutrients and carbohydrates (Sabota 1998; Davis 1993). The spawn run depends on tree species, log size, spawn cultivar, moisture content and temperature of the environment, the amount of spawn in each log and other variables (Royse 2001; Davis 1993). The fungus requires between 4 and 10 months to fully colonise logs (Bates 1994), while this period could extend to as long as 18 months in cool or dry areas (Royse 2001; Davis 1993; and Jenkins et al. n.d.). During the spawn run period, the environmental conditions need to be monitored to avoid excessive moisture loss from the logs and prevent, control pests and avoid moulds (Thomas and Schumann 2003). In most cases, this can be achieved by shading the logs from direct sunlight, supplementing rainfall and allowing adequate air movement (Przybyłowicz and Donoghue 1988; Sabota 2007).
3.9. Fruiting cycle

Once logs are considered to be fully colonised by shiitake mycelium, initiation of fruiting can occur. Oei (1996) suggests that logs will have a pH of around 5.5-6 at the time of inoculation and that they are ready to fruit when the pH decreases to around 3.8-4. Another technique for determining whether the logs are fully colonised involves staining a cross section of wood with ferric chloride (FeCl₃). Due to tannin degrading
activity of the shiitake mycelium the infected area will turn white and unoccupied wood turns brown (Oei 1996). Once the cross section turns 75% white, fruiting can be initiated. Alternatively, simply observe the presence of white markings on cut ends of the log in the sapwood area (Leatham 1982) or wait until the logs begin to fruit in the stack.

This development of mushrooms progresses through several stages: induction, pinning, and fruiting.

3.9.1. Induction
Sudden changes in environmental factors, such as temperature, moisture, and nutrient levels, trigger mushroom development in well-colonised logs (Przybylowicz and Donoghue 1988). In natural situations, shiitake fruiting occurs under prolonged cool, moist conditions with fruit development generally occurring within two weeks of a heavy natural rainfall event (Anderson and Marcouiller 1990). This can be mimicked by immersing logs in cold water. The period of soaking depends on the dryness of the logs. Suggestions include soaking the logs overnight (12 hours) (Hill 2003), 48 hours (Royse 2001) or even up to 72 hours (Sabota 2007). The variation in the appropriate soaking time depends on the difference between water and air temperatures, where the greater the temperature difference, the less soaking time is needed (Anderson and Marcouiller 1990).

Soaking may need to be longer when denser wood species such as oak, large logs, and young logs are used (Sabota 2007). The optimal time for soaking of oak logs was found to be 5 hours within 24 hour period of testing (Tokimoto et al. 1998). Once soaked, logs are removed from tanks, and stood up in a well ventilated area. Sabota (2007) suggests it is the process of drying after soaking that initiates pinning.
3.9.2. Pinning

Once placed in the fruiting area, mycelium will start to form reproductive nodules under the bark as the logs dries. The emerging primordial fruit bodies are also known as pins (Koske 1998, McCoy and Bruhn 2005, Oei 1996). During formation of primordial fruit bodies, the environment is managed to ensure the right number of pins form and begin to grow out. Recommended moisture content of logs (LMC) varies at this stage of development. Komatsu and Tokimoto (1982 cited in Przybylowicz and Donoghue 1988) and Tokimoto et al. (1980) recommend a range of LMC from 35 to 65% with optimum LMC between 55 and 65%. Koske (1998) suggests maintaining the LMC within a much narrower range of 55 to 60%. Humidity around the logs of between 60 to 85% is necessary to avoid premature drying of the primordial (ibid).

3.9.3. Fruiting

Under natural condition, fruiting occurs primarily in spring and autumn due to seasonal rains and temperature changes (Leatham 1982). While, in forced fruiting, mushrooms can be produced more frequently and even during winter and summer by carefully managing temperature and humidity conditions (Anderson and Marcouiller 1990).

Different strains respond differently to forcing and produce mushrooms with different characteristics (McCoy and Bruhn 2005). Different strains are often categorized by fruiting temperature requirements. Shiitake from strains of cool season, wide range and warm season will generally fruit at log temperatures between 5 and 20 °C, 10 and 27 °C, and 10 and 30 °C, respectively. Productivity, appearance, mushroom size and length of time it takes to fruit will also differ as a result of different strain employed (Sabota 1998). Whilst, requirement of LMC for fruiting is over 40%, however, higher LMC is favourable (Leatham 1982).

Once fruiting has commenced, logs that are allowed to fruit naturally may continue to repeat fruiting during spring and autumn for another 3 to 5 years (Davis 1993) or longer up to 7 years (Leatham 1982). Logs that are grown indoors can fruit more frequently by
using a forced fruiting method and requires logs to be immersed in water every 10 to 12 weeks (Sabota 1998). Forcing reduces the productive life of the logs to 2 or 3 years (Davis 1993).

The development of the fruiting body ends when the mushroom is harvested. Mature fruit bodies are harvested when the caps are 50 to 80% open (Figure 3.10) the veil has broken (Sistani et al. 2007), gills are exposed (Koske 1998) and the margin of the pileus is still slightly convex (Pire et al. 2001). The best grade of shiitakes has caps that still have a slight curl at the edge (Beetz and Kustudia 2004). Picking is done by cutting or twisting the stem off the log and the mushrooms are cleaned, trimmed and made ready for market (Koske 1998).

![Figure 3.10 Stages of maturing fruit body (from Koske 1998)](image)

3.9.4. Resting Period

When fruiting is complete, logs are returned to the incubation area and allowed to rest for varying amounts of time from at least one month (Campbell and Racjan 1999) to 10-12 weeks (Sabota 1998; Sistani et al. 2007). Resting is necessary for the fungus to replenish the nutrient supplies to ensure that the mycelium is still actively growing before fruiting again. To support recovery of mycelium, logs are maintained at 30-45% moisture content (Oei 1996; Sabota 2007) and at temperature between 15 and 25 °C without compromising their productive potential (Przybylowicz and Donoghue 1988; Sabota 2007).
Chapter 4
Methods

4.1. Introduction
The logs employed in this trial were drawn from a large batch of logs that were harvested and inoculated prior to the involvement of the author. This work was undertaken by the project supervisor (Mr Rowan Reid, Senior Lecturer) and staff of CERES Community Environment Park including Mr Parsuram Sharma-Luital. During the winter of 2006, 605 fresh logs (each approximately 1 m in length) of six tree species were harvested from three farms located in Bambra and Ellinbank in Victoria. The vast majority of the logs were of either shining gum (*Eucalyptus nitens* (H. Deane & Maiden) Maiden) or English oak (*Quercus robur* L.). A small number of other tree species were inoculated for comparison including poplar (*Populus* L. hybrid), alder (*Alnus glutinosa* (L.) Gaertn.), sugar gum (*Eucalyptus cladocalyx* F. Muell) and blackwood (*Acacia melanoxylon* R.Br.). For the purpose of this research ten logs of each of the predominant species were selected as representative and combined with all the logs of these minor species to make up the research lot of 48 logs.

Because the logs were already inoculated it was not possible for the author to control the selection of logs prior to harvest, the post harvest care, the timing of inoculation or the intensity of inoculation. This chapter provides a description of the process and methods adopted from the time of harvest and inoculation (has described to the author), through the period of the author’s personal involvement (covering 4 fruiting cycles) and onto the post-trial fruitings that were undertaken by a private grower in Colac, in Western Victoria.
4.2. Harvest and inoculation

Logs were stored for a short time prior to inoculation with shiitake (*Lentinula edodes* (Berk.) Pegler) spawn imported from the United States. The inoculation involved drilling many small holes along the length of each log and hammering in a short wooden dowel of alder (*Alnus* spp) which carried the fungal hyphae. Bees wax was used to seal the holes and ends of each log to preserve moisture. The inoculated logs were then stacked in a shade house.

The logs were watered regularly for more than 4 months prior to being soaked to stimulate fruiting and stacked in fruiting racks for observation and harvesting. The mushrooms produced on each log were harvested and weighed. Each mushroom was harvested just as the lip of the mushroom cap began to unfold. When fruiting ceased, logs were stacked and rested for about 2 months before the process was repeated. A selection of mushrooms produced on each tree species during the second harvest were assessed for quality including moisture content and total solids.

4.3. Experimental location

The experiment was conducted at the CERES Community Environment Park which is located in Brunswick East, Melbourne in Victoria (37° 46’ S, 144° 58’ E). Logs used in the experiment were stacked and placed inside a shade house with good drainage. The structure of the bedding of the shade house is shown in Fig. 4.1 and Fig. 4.2. The logs were protected from direct sunlight and wind by two layers (i.e. inner layer and outer layer) of 80% green polypropylene shade cloth.

The six different species of logs were inoculated between the end of September and the third week of October 2006. Fruiting was first initiated in April 2007 and repeated for the purpose of this research in August and December 2007 (Table 4.1).
Figure 4.1 The structure and layout of the shade house used for shiitake cultivation in this study. Note that the diagram shows all logs in a fruiting position. In practice the logs were initially crib-stacked.

Legend:  
A: Inner layer of green polypropylene shade cloth  
B: Outer layer of green polypropylene shade cloth  
C: Automatic water sprinkler/spray  
D: Bed logs  
E: Wire rope  
F: Support post
Figure 4.2  The shade house which the logs stacked during the initial spawn run (April 2007).

Table 4.1  Dates of production process of shiitake during 2006-2008

<table>
<thead>
<tr>
<th>Inoculation</th>
<th>Stacked</th>
<th>Soaking</th>
<th>Standing/Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>June-August 2007</td>
<td>20 August 2007</td>
<td>August-September 2007</td>
<td></td>
</tr>
</tbody>
</table>
4.4. Log selection and preparation

Logs of six tree species were harvested from small stands on three properties in southern Victoria: two in the Bambra area of south western Victoria (38° 22’ S, 143° 55’ E) and the third near Ellinbank in West Gippsland (38° 13’ S, 145° 55’ E, Table 4.2).

Table 4.2 Sources and characteristics of logs involved in the trial

<table>
<thead>
<tr>
<th>Common name</th>
<th>Species</th>
<th>Number of logs in trial</th>
<th>Source</th>
<th>Stand age and type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackwood</td>
<td><em>Acacia melanoxylon</em></td>
<td>7</td>
<td>Bambra Agroforestry Farm</td>
<td>Directed seeded and planted seedlings, less than 20 years old</td>
</tr>
<tr>
<td>Shining gum</td>
<td><em>Eucalyptus nitens</em></td>
<td>10</td>
<td>Bambra Agroforestry Farm</td>
<td>11 to 18 year old seedlings</td>
</tr>
<tr>
<td>Alder</td>
<td><em>Alnus glutinosa</em></td>
<td>7</td>
<td>Bambra Agroforestry Farm</td>
<td>11 year old seedlings</td>
</tr>
<tr>
<td>Poplar</td>
<td><em>Populus hybrid</em></td>
<td>7</td>
<td>Bambra Agroforestry Farm</td>
<td>19 year old cuttings</td>
</tr>
<tr>
<td>Sugar gum</td>
<td><em>Eucalyptus cladocalyx</em></td>
<td>7</td>
<td>Brickmakers Road, Bambra</td>
<td>Young (about 3 years) coppice regrowth</td>
</tr>
<tr>
<td>Oak</td>
<td><em>Quercus robur</em></td>
<td>10</td>
<td>Alexandra farm, Ellinbank</td>
<td>Dense grown seedlings approximately 16-18 years old</td>
</tr>
</tbody>
</table>

Each log was numbered and measured prior to inoculation. The diameter at each end was measured with a diameter tape. A measure of the width of the heartwood area was taken at the end of each log to determine the percentage area of sapwood. Samples cut from the end of a sample of logs from each species were weighed and volume assessed by immersion prior to being dried in an oven (100°C for 48 hours).

During the initial log preparation those involved cut discs from the freshly sawn ends of a number of logs of each species. Each disc (including bark) was weighed and its volume calculated by water displacement before being dried in a oven at 100°C for at least 2 days. Table 4.3 presents the results as provided. Whilst the logs that were later selected for the research trial were selected from the same batch not all the logs sampled for wood density were included in the trial. The dried samples used for the moisture
content and density study were later used to assess the percentage sapwood area of the three more promising species.

Table 4.3 Fresh moisture content and basic density (oven dried) of logs prior to inoculation and measured sapwood percentage of oven dried samples. Confidence intervals based on alpha = 0.05

<table>
<thead>
<tr>
<th>Tree species</th>
<th>No. of logs sampled</th>
<th>Mean fresh moisture content (%).</th>
<th>Mean basic density (Kg/m^3)</th>
<th>Percentage sapwood (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oak</td>
<td>20</td>
<td>39 ±2.1</td>
<td>606 ±20.6</td>
<td>70 ±3.4</td>
</tr>
<tr>
<td>Blackwood</td>
<td>5</td>
<td>34 ±1.6</td>
<td>671 ±38.5</td>
<td></td>
</tr>
<tr>
<td>Shining gum</td>
<td>15</td>
<td>41 ±2.6</td>
<td>587 ±28.6</td>
<td>47 ±5.3</td>
</tr>
<tr>
<td>Alder</td>
<td>5</td>
<td>44 ±0.7</td>
<td>426 ±12.3</td>
<td></td>
</tr>
<tr>
<td>Poplar</td>
<td>5</td>
<td>51 ±1.4</td>
<td>361 ±7.2</td>
<td></td>
</tr>
<tr>
<td>Sugar gum</td>
<td>6</td>
<td>42 ±2.4</td>
<td>646 ±39.2</td>
<td>83 ±12.2</td>
</tr>
</tbody>
</table>

4.5. Shiitake cultivation

4.5.1. Inoculation

Shiitake spawn was imported from Fungi Perfectii, a private company located in Washington State, United States. Inoculation of logs involved drilling many shallow holes (approximately 1.5 to two times the length of the wood plugs) through the bark and into the sapwood of each log (at approximately 15 cm spacing). Wooden (alder) plugs (0.8 cm diameter, 2.5 cm depth) were hammered into the holes and melted bees wax was used to seal the wounds. Wax was also applied to the ends of the logs to reduce drying.

Based on experience of the CERES project leader and published practical advice, holes were drilled approximately 15 cm apart in rows along each log. The number of rows varied with the diameter of the logs such that they were about 5 to 7 cm apart around the circumference of the log. The aim was to use 25 to 35 plugs per log although some of
the larger logs had more than this and the very small logs received less. The dimensions and number of drill holes for each log involved in the trial are given in Table 4.4.

Calculation of the volume of each log was needed to determine the intensity of inoculation. Intensity of inoculation ranged from ‘high’ with one inoculation plug per 171 cm$^3$ of log volume to ‘low’ with one inoculation plug for each 435 cm$^3$ of log volume (Table 4.4). Once inoculated, logs were stacked in the shade house and watered regularly during the summer months.

### 4.5.2. Incubation of logs during mushroom cultivation

Watering logs during the cultivation period depended on daytime temperature. An automatic timing device installed inside the shade house was set to water logs twice a day for approximately 5 minutes when the temperature remained between 15 to 25 °C. When the temperature in the shade house was between 25 and 35 °C, the automatic watering system was set for watering three times a day with one or two additional periods of watering by spray nozzles. Four automatic watering periods per day along with one or two periods using spray nozzles were necessary when the temperature was over 40 °C. Information of weather conditions during the time of mushroom incubation were obtained from the Bureau of Meteorology.

As the fungal mycelium spread under the bark, logs were turned occasionally within the stack to prevent them sticking together. The extent of mycelium spread was monitored visually by viewing the end of the logs. Samples cut from the logs suggested the fungi spread rapidly in the sapwood along the length of the log from each point of inoculation and under the bark (Fig 4.3). Spread of fungal mycelium around the log was slower. The mycelium did not spread into the heartwood. By April 2007, the mycelium appeared to be well developed in most species and volunteer mushrooms were beginning to appear indicating it was time to soak the logs to initiate fruiting.
Table 4.4 Measurements of individual logs used to determine inoculation intensity with plugs containing shiitake hyphae

<table>
<thead>
<tr>
<th>No</th>
<th>Sample No</th>
<th>Species</th>
<th>Diameter (cm)</th>
<th>Length (cm)</th>
<th>Number of inoculations</th>
<th>Log volume (cm³)</th>
<th>Inoculation intensity (cm³/inoc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tagged end</td>
<td>Plain end</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>311</td>
<td>Blackwood</td>
<td>14.6</td>
<td>13.8</td>
<td>90</td>
<td>33</td>
<td>14266</td>
</tr>
<tr>
<td>2</td>
<td>305</td>
<td>Blackwood</td>
<td>12.5</td>
<td>11.0</td>
<td>98</td>
<td>39</td>
<td>10671</td>
</tr>
<tr>
<td>3</td>
<td>298</td>
<td>Blackwood</td>
<td>14.0</td>
<td>12.3</td>
<td>91</td>
<td>31</td>
<td>12412</td>
</tr>
<tr>
<td>4</td>
<td>304</td>
<td>Blackwood</td>
<td>16.0</td>
<td>13.0</td>
<td>85</td>
<td>41</td>
<td>14188</td>
</tr>
<tr>
<td>5</td>
<td>310</td>
<td>Blackwood</td>
<td>13.6</td>
<td>11.7</td>
<td>92</td>
<td>42</td>
<td>11629</td>
</tr>
<tr>
<td>6</td>
<td>308</td>
<td>Blackwood</td>
<td>12.2</td>
<td>11.1</td>
<td>96</td>
<td>40</td>
<td>10257</td>
</tr>
<tr>
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Figure 4.3 Cross section cut through one of the *E. nitens* logs in late January 2007, showing the restricted spread of mycelium around the sapwood and the absence of mycelium from the heartwood zone.

The first soaking of the trial logs took place during autumn (April 2007). Logs were unstacked and submerged in a large tank (1.5 m diameter and 1 m depth) of water for approximately 24 hours (Figure 4.4). Subsequent to soaking, logs were removed from the soak tank and placed inside the shade house. Logs were inclined at approximately 70° from the ground in the shape of a fork and wooden rails were utilised as a support (Figure 4.5).
Figure 4.4 The drum used to soak the sample logs to initial fruiting. Heavy weights were used to submerge the logs.

Figure 4.5 After soaking the logs were stood up against a timber rack for fruiting and harvesting.
4.6. **Fruit development and harvesting**

Fruiting was evident within 3-5 days of soaking logs. The first harvest of shiitake mushrooms occurred soon after in May 2007. Mushrooms were harvested when the lip of the cap began to unfurl (Figure 4.6). The mushrooms were carefully removed from the surface of the logs by twisting the stem of the mushroom and were counted and weighed for each log. Total mushroom production for each log and for each species of log was determined using these values. When production ceased, logs were allowed to rest for a period of about two months before repeating the process.

![Figure 4.6 Each mushroom was harvested when the lip of the cap began to unfurl.](image)

4.7. **Post trial results**

Following the fourth fruiting cycle the trial site was abandoned. Most of the logs involved in the trial were moved to another site at Colac in Western Victoria where member of the Otway Agroforestry Network undertook a further two fruiting cycles (August and December 2008). The poplar logs were discarded because the bark was peeling off and they appeared to have dried out. Whilst is it not appropriate to
incorporate the data from this fruiting cycle it was collected by the group in order to provide some indication of the ongoing performance.

4.8. Chemical composition of shiitake mushrooms
During the second production cycle, samples of approximately 100 g of shiitake mushrooms from each of the log species was collected for determination of nitrogen concentration, total solids, moisture content and total dietary fibre. Samples were stored in resealable plastic bags to reduce moisture loss and were analysed as soon as possible on return to the laboratory.

4.8.1. Nitrogen concentration and protein content
Dried samples of finely chopped shiitake mushroom were ground to powdered form for 3 minutes using a plant tissue grinder (TissueLyser, Qiagen/Retsch). During this process, samples were pulverising and homogenised into a fine powder with constant particle size. Using a micro-analytical balance, duplicate powdered samples of approximately 5 to 10 mg of mushroom collected from each log species were weighed into individual aluminium cups or tin capsules (9 mm) and weighs were recorded to two decimal places.

In addition, 10 samples of a standard (*Eucalyptus yarraensis* bulk leaf #45 for C:N standards) were also weighed (approximately 5 mg). Once the correct amount of sample had been placed in the capsule, it was folded into a small square by softly applying force using forceps. Samples were then stored into an alpha-numeric 96-well cell (polystyrene) plate (with cover) for easy storage and transporting. The samples were analysed for % total N through Dumas Oxidation conducted on a Carlo Erba NA 1500 NCS using NA 1500 NCS Fisons instruments.
In order to determine protein content, the following formula was used:

\[
\text{% protein content} = \text{% nitrogen} \times 4.38^* 
\]

*Protein measured as a percent of dry weight using a crude protein correction factor of 4.38 (from Crisan and Sands 1978)

The following instructions (section 4.6.2 and 4.6.3) are taken from Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) International (1995) and are added for the completeness of this section.

4.8.2. Determination of total solids and moisture content

Approximately 5 g of fresh shiitake mushrooms collected from each of the log species were sliced into small pieces and placed in a dish that had been heated at 100 °C for approximately 1 h and allowed to cool to room temperature inside a desiccator. The process of drying, cooling and weighing of the dish was repeated until constant mass was achieved. The mass \(m_1\) of the dried and cooled dish was recorded. The fresh weight of the sample was recorded \(m_2\) then dried for 2.5 h at 100 °C. The sample was removed from the oven, cooled to room temperature inside a desiccator and reweighed. Drying for 1 h followed by cooling was repeated until a constant mass was achieved. The final dry mass \(m_3\) was recorded.

Total solids and moisture content calculated using the following formula:

\[
\begin{align*}
\text{% Total solids} &= \frac{m_3 - m_1}{m_2 - m_1} \times 100 \\
\text{% Moisture} &= \frac{m_2 - m_3}{m_2 - m_1} \times 100 \\
\end{align*}
\]

\(m_1\) = constant weight (g) of empty dish

\(m_2\) = weight (g) of the dish plus test sample before drying

\(m_3\) = weight (g) of the dish plus dried sample
4.8.3. Total dietary fibre in shiitake mushroom

a. Sample preparation and enzyme digestion

Duplicate portions of dried shiitake mushroom collected from each log species were ground to powdered form for 5 min using the plant tissue grinder (TissueLyser, Qiagen/Retsch) and two samples of 3000.0 ± 5.0 mg (M1 and M2) accurately weighed to 0.1 mg into 400 mL (or 600 mL) tall-form beakers. Each sample had 40 mL of MES-TRIS (0.05 M MES (2-(N-morpholino) ethanesulfonic acid) and 0.05 M TRIS (Tris (hydroxymethyl) aminomethane)) buffer solution (pH 8.2) added and stirred using a magnetic stirrer until the sample was dispersed completely.

To allow hydrolysis and depolymerisation of starch, 50 µL of heat-stable α-amylase solution was added and stirred at low speed. The beaker was covered with aluminium foil and incubated at 95-100 ºC in a water bath for 15 min with continuous agitation. Once the bath temperature had reached 95 ºC the samples were heated for approximately 35 min longer.

The samples were removed from the water bath and cooled to 60 ºC. If a gelatinous ring had formed inside the beaker it was scraped off and any gelatinised material that had collected at the bottom of beaker was dispersed using a clean spatula. The beaker walls and spatula were rinsed with 10 mL H₂O. To solubilise and depolymerise proteins, 100 µL of protease solution was added to each beaker, covered with aluminium foil and incubated for 30 min at 60 ºC with continuous agitation. Once the bath temperature reached 60 ºC, timing was initiated.

After the second incubation the samples were removed and 5 mL of 0.561N HCl was added with continuous stirring. The pH of the solution was adjusted to 4.0-4.7 at 60 ºC by adding 1N NaOH or 1N HCl. To hydrolyse starch fragments to glucose, 300µL of amyloglucosidase solution was added while stirring. The samples were covered with
aluminium foil and incubated for 30 min at 60 °C with constant agitation. Timing was initiated once the bath temperature reached 60 °C.

b. Determination of total dietary fibre

While samples were still in the waterbath, 225 mL of 95% ethanol warmed to 60 °C was added to each. The ratio of ethanol to sample volume was 4:1. Alcohol-treated samples were removed from the waterbath, covered and allowed to stand at room temperature for approximately 1 h to allow a precipitate of soluble fibres to form.

A bed of Celite held within a pre-weighed crucible was wet and redistributed with 15 mL of 78% ethanol. Suction was applied to the crucible to draw Celite onto fritted glass as an even mat. The alcohol-treated enzyme digest samples were filtered through the crucible taking care to quantitatively transfer all remaining particles to the crucible using 78% ethanol and rubber spatula. Using a vacuum, each residue was washed twice using 15 mL portions of an alcohol solution (78% ethanol, 95% ethanol and acetone). The crucible containing the washed residue was dried overnight at 105 °C and cooled inside a desiccator for approximately 1 h. The crucible containing the dietary fibre residue and Celite was weighed to nearest 0.1 mg and the residue weight was calculated by subtracting the weight of the dried crucible and Celite.

The duplicate sample of each residue derived by the above method was incinerated for 5 h at 525 °C in a muffle furnace, cooled in a desiccator and weighed to the nearest 0.1 mg. The crucible and Celite weight was subtracted from this weight to determine ash weight.

c. Calculations

Blank determination:

\[ B = \frac{(BR_1 + BR_2)}{2} - P_B - A_B \]
BR_1 and BR_2 = residue weights (mg for duplicate blank determinations)

P_B and A_B = weights (mg) of protein and ash, respectively, determined on the first and second blank residues

Dietary fibre (DF) determination:

\[
DF = \left\{\frac{(R_1 + R_2)/2 - P - A - B}{(M_1 + M_2)/2}\right\} \times 100
\]

R_1 and R_2 = residue weights (mg) for duplicates samples

P and A = weights (mg) of protein and ash, respectively, determined on the first and second residues

B = blank weight (mg)

M_1 and M_2 = weights (mg) of samples

Total dietary fibre determination was calculated by summing insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) as determined in procedures outlined above.
Chapter 5

Results

5.1 Environmental conditions during the trial
Melbourne has a Mediterranean climate with mild, wet winters (June – August) and hot, dry summers (December – February). The weather conditions over the course of the trial are shown in Figure 5.1. The average daily temperature in winter ranged from a minimum of 6.8°C to a maximum of 13.8°C. In summer, the average daily temperature ranged from a minimum of 18.3°C to a maximum of 29°C. Total monthly precipitation ranged from 9.2 to 71.2 mm. The 2007/8 summer was unusual in that rainfall was high in late spring and early summer. The daily average relative humidity ranged from between 43 and 71% peaking in winter.

Figure 5.1  Mean monthly maximum and minimum temperature, rainfall and humidity at the CERES trial site during shiitake cultivation periods. (Source: Australian Government-Bureau of Meteorology)
5.2 Shiitake Production

5.2.1 Log viability

Fruiting was initiated four times during the course of the trial. The percentages of logs of each species that produced mushrooms at each fruiting cycle are shown in Figure 5.2. Although the oak logs were significantly more viable than the logs of any other species in the first fruiting cycle, at least 80% of the logs of each species produced mushrooms in the third fruiting cycle. The results from the forth fruiting cycle were low across all the species.

![Figure 5.2 The percentages of logs of each species that produced mushrooms during each fruiting cycle.](image)

5.2.2 Mushrooms yields

The fresh mushrooms produced from each log during each fruiting period were collected and weighed. Although the oak performed well (70 % viability and a total yield of almost 2 kg per log) during the first fruiting period the poor performance of the other species (less than 30 %) made it impossible to perform any meaningful statistical analysis. Interestingly, an alder log had the highest individual production (600 g). As viability improved over the course of the trial differences in yield between the species became more evident.

Oak again performed well in the second fruiting (90 % viability and an average yield of over 500 g/log). One oak log produced 1 kg of fresh shiitake mushroom in a single
fruiting cycle. Their superior early viability and production meant the oak logs had produced, on average, 686 gram of mushrooms in two fruiting cycles compared to just 298 g per log for alder, 255 g per log for sugar gum, 188 g per log for shining gum, 32 g per log for blackwood and 31 g per log for poplar. It was not until the third fruiting cycle that the viability and production from the other species approached that of oak.

Although the average yields from the oak, alder and sugar gum actually declined the third fruiting cycle was, overall, the most successful with log viability across the trial being over 90% and almost 50 per cent of the logs producing more than 200 g of shiitake mushrooms. Table 5.1 and Figure 5.3 summarise the results and show that, in terms of shiitake production per log, there was no significant difference between the performance of the sugar gum and oak. Shining gum produced significantly less than the oak and sugar gum but more than either the blackwood or poplar. The alder logs were highly variable.

The total production from each species over the course of the four fruitings is presented in Table 5.1 and Figure 5.4. Oak proved to be significantly better than any other species in the trial producing almost 1 kilogram of fresh produce per log. The eucalypt species and alder were not significantly different producing around 400 to 500 g per log on average for each species. Blackwood and poplar were very poor shiitake producers yielding less than 150 g per log.
Table 5.1 The yield of fresh mushrooms per log (including unviable logs) for each fruiting cycle and over the course of the trial.

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<th>Total yield (g)</th>
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<th>Viability (%)</th>
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<td>7</td>
<td>100%</td>
<td>1885</td>
<td>269^e</td>
<td>29%</td>
<td>17</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wood Species</th>
<th>No. logs</th>
<th>Viability (%)</th>
<th>Total yield (g)</th>
<th>Log Yield (g/log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oak</td>
<td>10</td>
<td>100%</td>
<td>9914</td>
<td>991^a</td>
</tr>
<tr>
<td>Blackwood</td>
<td>7</td>
<td>100%</td>
<td>687</td>
<td>98^c</td>
</tr>
<tr>
<td>Shining gum</td>
<td>10</td>
<td>80%</td>
<td>3894</td>
<td>389^b</td>
</tr>
<tr>
<td>Alder</td>
<td>7</td>
<td>100%</td>
<td>3257</td>
<td>465^bc</td>
</tr>
<tr>
<td>Poplar</td>
<td>7</td>
<td>86%</td>
<td>977</td>
<td>140^d</td>
</tr>
<tr>
<td>Sugar gum</td>
<td>7</td>
<td>100%</td>
<td>3686</td>
<td>527^b</td>
</tr>
</tbody>
</table>

Figure 5.3 The average yield of fresh mushrooms per log (including unviable logs) of each species over the course of trial.
5.2.3 Mushrooms yields at post-trial site

Following the 4th fruiting, the trial was abandoned. Many of the logs were moved to another site at Colac 150 km west of Melbourne where the Otway Agroforestry Network continued to measure yields. The poplar logs were discarded due to bark shedding and associated loss of moisture. A few of the other logs were misplaced.
Figure 5.6 shows the performance of every log in the trial and the yields obtained from a fifth and sixth fruiting cycle.

Of interest is the fact that the two shining gum logs and one alder log that did not produce any mushrooms over the course of the trial did produce mushrooms at Colac. Shiitake production from blackwood, which performed very poorly during the trial (averaging less that 100 g/log), increased dramatically. The production from sugar gum at Colac was very poor and may have been due to the small size of the logs. The oak and shining gum continued to perform well suggesting that the poor yield in the 4th fruiting was not an indication that the logs were exhausted.

Figure 5.6  The yield of fresh mushrooms for each log. The first four harvests constitute the research trial. The fifth and sixth harvest were managed and measured by members of the Otway Agroforestry Network.
5.3 Examination of factors that may have influenced yields

5.3.1 Number of inoculations per log
An ANOVA analysing the total mushroom production against the species and the number of inoculations per log suggests that some of the variance in yield may be due to the number of inoculations (Table 5.2). The productivity of logs of alder, oak and shining gum were generally higher in those logs that had more inoculation points (Figure 5.8).
Table 5.2 Analysis of Variance for Total Yield (g/log) against wood species and the number of inoculations per log.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood Species Code</td>
<td>5</td>
<td>4203508</td>
<td>3804848</td>
<td>760970</td>
<td>5.95</td>
<td>0.000</td>
</tr>
<tr>
<td>Number of inoculation</td>
<td>1</td>
<td>948764</td>
<td>948764</td>
<td>948764</td>
<td>7.42</td>
<td>0.010</td>
</tr>
<tr>
<td>Error</td>
<td>37</td>
<td>4730108</td>
<td>4730108</td>
<td>127841</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>9882380</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 5.8 Scatter plot of total yield (g/log) versus number of inoculation

5.3.2 Log Volume

An ANOVA analysing the total mushroom production against the species and log volume showed that log volume was a significant factor (Table 5.3). The productivity of logs of alder, oak, shining gum and sugar gum was generally higher in those logs that had a greater volume (Figure 5.9).

Table 5.3 Analysis of Variance for Total Yield (g/log), against wood species and volume

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood Species Code</td>
<td>5</td>
<td>4203508</td>
<td>3804848</td>
<td>760970</td>
<td>5.95</td>
<td>0.000</td>
</tr>
<tr>
<td>Volume (dm³)</td>
<td>1</td>
<td>1019848</td>
<td>1019848</td>
<td>1019848</td>
<td>8.10</td>
<td>0.007</td>
</tr>
<tr>
<td>Error</td>
<td>37</td>
<td>4659025</td>
<td>4659025</td>
<td>125920</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>9882380</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.3.3 Concentration of inoculation

A test was then conducted to assess whether the concentration of inoculations was a significant factor affecting productivity (Table 5.4). The result was inconclusive (Figure 5.10) and may relate to the fact that the large logs received a greater number of inoculations (Figure 5.11) making it difficult to distinguish the number of inoculations from the size of the logs.

Table 5.4 Analysis of Variance for Total Yield (g/log) against log species and the concentration of inoculations per log (Inoculations/dm$^3$).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood Species Code</td>
<td>5</td>
<td>4203508</td>
<td>4333330</td>
<td>866666</td>
<td>5.82</td>
<td>0.000</td>
</tr>
<tr>
<td>Inoc/Vol (dm$^3$)</td>
<td>1</td>
<td>173047</td>
<td>173047</td>
<td>173047</td>
<td>1.16</td>
<td>0.288</td>
</tr>
<tr>
<td>Error</td>
<td>37</td>
<td>5505825</td>
<td>5505825</td>
<td>148806</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>9882380</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.10  Scatter plot of total yield (g/log) versus inoculation per volume (dm$^3$)

Figure 5.11  The number of inoculations in each log against its volume shows that the larger logs commonly received more inoculations.

5.4 Chemical composition of shiitake mushroom

The results of a series of laboratory analyses undertaken to allow a comparison of the nitrogen (hence protein), total solids, moisture content and dietary fibre content of mushrooms harvested from each log species are presented in Tables 5.5 to 5.8. The mushrooms used in the analyses were collected during the second and third fruiting.

A Completely Randomised Design (CRD) Test was conducted analysing the protein content against the wood species. The results show that wood species was a significant
factor and suggests that some of the variance in protein content may be due to the difference in wood species (Table 5.6). Mushrooms harvested from blackwood showed the highest protein content whereas mushrooms collected from sugar gum were the lowest. While, protein content of mushrooms from oak, shining gum and poplar were in mid range.

In order to determined which wood species has the most significance influence on protein content, an advanced test was performed using the Duncan’s Multiple Range Test 5% (DMRT5%) (Table 5.7). The result shows that Blackwood has the highest protein content and is significantly different from other wood species.

Table 5.5 Protein content of shiitake mushroom grown on various wood species

<table>
<thead>
<tr>
<th>Log Species</th>
<th>%N</th>
<th>Protein*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackwood</td>
<td>4.12</td>
<td>18.0</td>
</tr>
<tr>
<td>Oak</td>
<td>3.59</td>
<td>15.7</td>
</tr>
<tr>
<td>Alder</td>
<td>2.93</td>
<td>12.8</td>
</tr>
<tr>
<td>Shining gum</td>
<td>3.27</td>
<td>14.3</td>
</tr>
<tr>
<td>Poplar</td>
<td>3.36</td>
<td>14.7</td>
</tr>
<tr>
<td>Sugar gum</td>
<td>2.65</td>
<td>11.6</td>
</tr>
</tbody>
</table>

*Protein measured as a percent of dry weight using a crude protein correction factor of 4.38

Table 5.6 CRD Test (5%) for protein content against log species

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Fcrit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood Species</td>
<td>5</td>
<td>101.38</td>
<td>20.28</td>
<td>17.51</td>
<td>2.77*</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>20.84</td>
<td>1.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>122.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.7 DMRT 5% for Protein content against log species

<table>
<thead>
<tr>
<th>Wood Species</th>
<th>Protein Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oak</td>
<td>15.7 c</td>
</tr>
<tr>
<td>Blackwood</td>
<td>18.0 d</td>
</tr>
<tr>
<td>Sugar gum</td>
<td>11.6 a</td>
</tr>
<tr>
<td>Shining gum</td>
<td>14.3 bc</td>
</tr>
<tr>
<td>Alder</td>
<td>12.8 ab</td>
</tr>
<tr>
<td>Poplar</td>
<td>14.7 c</td>
</tr>
</tbody>
</table>

Data with similar letter are not significantly different.
A Completely Randomised Design (CRD) Test was conducted analysing the moisture content against the wood species. The results show that wood species was a significant factor and suggests that some of the variance in moisture content may be due to the difference in wood species (Table 5.9). Moisture content of fresh shiitake produced from different species varied between species. Mushroom produced from Poplar has the highest moisture content followed closely by blackwood, with value of 88.9% and 87.9%, respectively. The lowest moisture content mushroom was produced by oak (80.8%). While, moisture content of mushrooms from alder, shining gum and sugar gum were in mid range.

In order to determined which wood species has the most significance influence on moisture content, an advanced test was performed using the Duncan’s Multiple Range Test 5% (DMRT5%) (Table 5.10). The result shows that poplar has the highest moisture content, however, poplar moisture content are not significantly different compare to that of oak, blackwood and shining gum.

Results from total fibre determination were not statistically analysed due to shortage of sample material, thus will be explained descriptively. The results show that mushroom collected from oak was the highest (10.2) whereas blackwood and poplar were the lowest both having total fibre of 5.9. The eucalypts (shining gum and sugar gum) and alder possessed close values from that of oak with total fibre of 9.1, 8.1 and 8.3, respectively.

Table 5.8  Total solids and fibre content of shiitake mushroom grown on various wood species

<table>
<thead>
<tr>
<th>Log Species</th>
<th>Total Solids</th>
<th>Moisture content</th>
<th>Total Fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oak</td>
<td>19.2</td>
<td>80.8</td>
<td>10.2</td>
</tr>
<tr>
<td>Blackwood</td>
<td>12.1</td>
<td>87.9</td>
<td>5.9</td>
</tr>
<tr>
<td>Alder</td>
<td>15.7</td>
<td>84.3</td>
<td>8.3</td>
</tr>
<tr>
<td>Sugar gum</td>
<td>15.1</td>
<td>84.9</td>
<td>8.1</td>
</tr>
<tr>
<td>Shining gum</td>
<td>17.1</td>
<td>82.9</td>
<td>9.1</td>
</tr>
<tr>
<td>Poplar</td>
<td>11.1</td>
<td>88.9</td>
<td>5.9</td>
</tr>
</tbody>
</table>

All data presented as percentage of dry weight except moisture content (percentage of fresh weight).

Table 5.9 CRD Test (5%) for moisture content against log species

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Fcrit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood Species</td>
<td>5</td>
<td>137.34</td>
<td>27.47</td>
<td>150.54</td>
<td>3.11*</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>2.19</td>
<td>0.18</td>
<td>150.54</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>139.54</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5.10  DMRT 5% for moisture content against log species

<table>
<thead>
<tr>
<th>Wood Species</th>
<th>Protein Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oak</td>
<td>80.81 b</td>
</tr>
<tr>
<td>Blackwood</td>
<td>87.90 b</td>
</tr>
<tr>
<td>Sugar gum</td>
<td>84.85 a</td>
</tr>
<tr>
<td>Shining gum</td>
<td>82.92 b</td>
</tr>
<tr>
<td>Alder</td>
<td>84.26 a</td>
</tr>
<tr>
<td>Poplar</td>
<td>88.86 b</td>
</tr>
</tbody>
</table>

Data with similar letter are not significantly different.
Chapter 6
Discussions

6.1 Introduction
This research trial represents the first of its kind in Australia and the first direct comparison of eucalypt logs with deciduous hardwoods of genotypes more commonly used for shiitake production in the northern hemisphere. The results suggest that shining gum and sugar gum may be viable alternative species for log-based shiitake production in Australia. This chapter reviews the results of the trial with a particular focus on exploring the factors that appear to affect the viability of log-based shiitake production in Australia.

6.2 Climatic conditions
The research clearly demonstrates that shiitake mushrooms can be grown in Victoria on logs of a number of native and introduced species. As outlined by Przybylowicz and Donoghue (1988), temperature is one of the factors which affects mushroom cultivation and has a strong influence on survival, growth rate, time of fruiting, yield and the shape of the mushrooms produced. Reports in the literature vary as to the optimum temperature for Lentinula edodes (shiitake) mycelial development. Tokimoto and Komatsu (1982) suggest a range of between 24 and 28 °C whereas Sabota (2007) reports a range of between 15 and 24 °C. Sabota (2007) goes on to suggest that the temperature required for the fruiting phase falls within the range from 5 to 30 °C (41-86 °F) whilst Przybylowicz and Donoghue (1988) are more restrictive suggesting a range between 10 and 25 °C. Temperatures above 45 °C can result in mycelium death (Tokimoto and Komatsu 1978).

The climate in Melbourne over the course of the trial was mild relative to the winters experienced in North America and north-east Asia where much of the strain selection and production research has been done. However, summers in Melbourne are likely to be hotter and drier than those experienced by northern hemisphere shiitake growers and researchers. Although log temperatures were not measured during the trial it is likely
that, given the protection afforded by the shade house the logs were at a temperature within the range suitable for both mycelium growth and mushroom production across all seasons.

The poor production achieved in the fourth fruiting, which occurred following the hottest and driest months, is thought to be due inadequate moisture in the logs, rather than high temperatures. As suggested by the success of the subsequent fruitings undertaken at Colac most of the logs were able to recover.

6.3 Mushroom yields from the different species

Care should be taken when interpreting the yield data. Whilst every attempt was made to harvest each mushroom at the same stage of development, the fresh weight of the produce is clearly affected by the stage of maturity, the time since watering and the humidity. Harvesting and weight measurement was done in such a way as to be appropriate to a typical commercial enterprise. However, there was no grading of produce.

There were four harvests during the trial period which began in September 2006 and ran until March 2008. The first fruiting occurred in May 2007 and subsequent fruiting was induced every 3 months. Out of the six logs species, oak produced significantly more mushrooms by total weight per log than any other species. The production from oak also peaked earlier than for the other species which would be an important commercial consideration. Shining gum, alder and sugar gum produced reasonable yields of more than 150 g/log in the second and third fruiting cycles. Shiitake production from poplar peaked in the third fruiting at just over 100 g/log. Although blackwood was the poorest performing species in the trial the production from four of the same logs in the post-trial fruiting undertaken at Colac exceeded 200 g/log.

Over the course of the four fruitings, oak (*Quercus* sp.) produced an average of just under 1 kg of fresh shiitake mushrooms per log. This is more than reported in many other studies: For example, Bratkovich (1999) tested a number of shiitake strains on oak logs over a period of three years in Ohio, USA. Yields were highest in the first year with the best strains producing 460 g/log. Over the course of 3 years the best performing strain yielded 594 g/log. Whilst a direct comparison is difficult due to the
lack of knowledge of the size of the logs used in the Ohio trials it is worth noting that the average yield achieved for oak over the course of a single year (991 g/log) in this research was well in excess of the best performing strain as reported by Bratkovich over the course of three years.

San Antonio (1981) reported shiitake production from a trial in Maryland, USA involving a number of trees species. Over a period of up to 40 months the average across 62 logs of 16 species was 42 grams of fresh mushrooms per kilogram of fresh log (at the time of inoculation). The best performing logs produced in the order of 100 – 190 g/kg. Converting the results of this trial to allow a comparison shows that oak produced an average of 101 g/kg (best 176 g/kg), alder 74 g/kg (best 223 g/kg), sugar gum 85 g/kg (best 136 g/kg), shining gum 37 g/kg (best 81 g/kg), poplar 16 g/kg (best 38 g/kg) and blackwood 11 g/kg (best 37 g/kg).

Queiroz et al. (2004) report the yield of shiitake mushrooms grown on *E. saligna* logs as yield of wet biomass (g) per kg of dry log. Without nutrient supplementation the best performing strains produced and average of 51 g/kg over the course of four fruitings. With supplementation of nitrogen and phosphate this was increased to 93 g/kg. Converting the results of this trial to allow a direct comparison over the same number of fruitings shows that oak produced 166 g/kg, alder 151 g/kg, sugar gum 141 g/kg, shining gum 63 g/kg, poplar 40 g/kg and blackwood 16 g/kg.

Bratkovich (1991) first soaked logs for fruiting a full year after inoculation. He then reports that production from oak generally peaked in the first year and fell over the course of the three-year trial. San Antonio (1981) reports that ‘oak logs required an average of 20 months after inoculation to produce the first fruit’. Using eucalypt logs in Brazil, Queiroz et al. (2004) found that there was ‘negligible’ fruiting production at the first fruiting (after 6 months of incubation) and that production peaked in the forth fruiting (12 months after inoculation). In this trial, oak and alder shiitake production peaked in the second fruiting (almost 12 months after inoculation) whereas all the
Australian native species peaked in the third fruiting cycle (15 months after inoculation).

The expansion of mycelium during the spawn run and subsequent fruiting are thought to be slower in the more dense woods (Przybylowicz and Donoghue 1990). Blackwood (oven dry wood density of 671 kg/m$^3$) was denser than the sugar gum, oak, shining gum, alder and poplar which had wood densities of 646, 606, 587, 426 and 361 kg/m$^3$ respectively (Table 4.3). The greater density may be the reason that blackwood took longer to produce in this trial.

Another important factor that might explain the differences in species performance is the bark type and persistence. It was noted that the bark of oak remains tightly held and resists damage during handling. The shining gum bark became soft and spongy following watering and was easily torn during handling and harvesting. Both alder and sugar gum have a tough, bark that is prone to peeling or lifting off the wood in sheets if the logs dried. The poplar bark was very thin did not persist well. The blackwood bark was similar in appearance to that of the oak although a lot thicker.

### 6.4 Other factors that may affect production

An important consideration in the selection of logs for shiitake production, other than species, is the size of the log. In this trial log size was a significant factor linked to increased production although this may have been confounded by the fact that many of the larger logs were of oak and that the large logs invariably received a greater number of inoculations. The major concern with the use of small diameter logs is the greater risk of drying out.

In a study involving nine year-old *Cunninghamia lanceolata* logs, Shieh et al. (1991) found that the closer was the inoculation spacing the greater the shiitake yield. In the current investigation, assessment on the concentration of inoculation was inconclusive.
(Fig 5.6). This may relate to the fact that the large logs received a greater number of inoculations.

Moisture content of different wood species varied considerably. Log moisture content should be maintained above 35% (Przybylowicz and Donoghue 1988; Hill 2002), although Oei (1996) suggests a range of between 40 and 45%. Low moisture contents (less than 20-25%) restricts mycelium growth and can ultimately result in death of the mycelium (Oei 1996; Hill 2002). Log moisture contents above 60% are also unfavourable for mycelial growth and can allow competitive fungal species to flourish (Oei 1996).

In the current study, the moisture content range between 34 and 51% (Table 4.3). This suggests that these logs were still within range as suggested above. Poplar had the highest moisture content followed by alder, sugar gum, shining gum, oak and blackwood. In data compiled by Przybylowicz and Donoghue (1988), almost similar moisture levels were obtained for alder (49%), while a study by Gilbert (1992 cited in Sabota 1996) showed moisture levels for red (42.5%) and white (41.5%) oak. Although poplar had the highest moisture content, the yield produced in the first flush was low. On the contrary, blackwood had the lowest moisture level and still produced low yield in the first flush.

**6.5 Chemical composition of shiitake mushroom**

The results of these experiments indicate that the chemical composition of mushrooms produced by all wood log species was within the range thought to be more than acceptable for commercial production. However, there were slight differences between mushrooms produced from different log species. Blackwood was the only species to produce mushrooms with protein content consistently higher than that of mushrooms arising from the oak logs. This may relate to the lower fibre content of the blackwood mushrooms. All the other species produced mushrooms that were similar in their protein, moisture content and fibre content to that of the oak.
Crisan and Sands (1978) report that *Lentinula edodes* mushrooms have been found to have crude protein range from 10.3% to 13.1% (dried base with conversion factors of 4.38), total solid range from 15.8% to 18.4%, and total fibre range from 6.5% to 14.7%. In the present trial, chemical composition of shiitake grown on six different wood species seem to be within range specified above, however there are some wood species that tend to give higher or lower values. The mushroom protein content found in this study ranged from between 12% and 16.8% (in the 2nd flush) and between 11.2% and 19.3% (in the 3rd flush) with oak showing the highest protein content (Table 5.5). Total solids range from 11.1% to 19.2% and the total fibre in the current analysis range between 5.9% and 10.2% with oak showing the highest for both (Tables 5.6 and 5.7).

The analyses show that some of the log-grown shiitake mushrooms have a higher protein content than previous research would suggest (Crisan and Sands 1978). It is not clear whether that research examined log-grown mushrooms and if so the species involved. Further research is needed to test the whether the variance in these quality measures has any bearing on the marketability or value of the mushrooms and whether or not factors other than log-species affect the chemical composition of the mushrooms produced.
Chapter 7
Conclusions

This research compared yield of shiitake mushroom grown on six tree species of oak (*Quercus robur*), sugar gum (*Eucalyptus cladocalyx*), alder (*Alnus glutinosa*), shining gum (*Eucalyptus nitens*), poplar (*Populus sp.*) and blackwood (*Acacia melanoxylon*). Although the yields from oak were the highest and blackwood the lowest, the research clearly demonstrates that shiitake mushrooms can be grown in Victoria on logs of native and introduced species, and that native eucalypt species, such as shining gum and sugar gum, may be viable alternative species for log-based shiitake production in Australia.

Laboratory analyses on chemical compositions of the mushroom produced by the six species of logs showed that although blackwood had lowest yield it was the only species to produce mushrooms with a protein content consistently higher than those from the oak logs. The protein content of the mushrooms harvested from the eucalypt logs varied: mushrooms from sugar gum were found to have the lowest protein content of all species whereas those from shining gum were closer to those from oak. Oak mushrooms showed the highest fibre content whilst the blackwood and poplar mushrooms had the highest moisture content. Mushrooms produced from both the eucalypt species had a higher, or similar, fibre content to those from alder. The significance of these results with respect to the marketability of the shiitake mushrooms is unknown.

This study was the first serious examination of log-based shiitake mushroom cultivation in Australia. It has demonstrated that shiitake mushrooms can be grown in the Australian environment and using native species of logs. In order to achieve commercial production environmental parameters will need further investigation. Research on the use of different strains could also help identify more appropriate strains for eucalypts. Comprehensive analyses on chemical composition and any impact that differences in the protein or fibre content of mushrooms produced from Australian tree species might have on taste and market value is also needed.
References


Oei, P 1996. Mushroom cultivation: with special emphasis on appropriate techniques for developing countries. Tool. The Netherlands.


Appendices

Appendix 1

The following trees have reported by the Taiwan Agricultural Research Institute to give similar or higher yields than *Liquidambar formosana*:

- **Acacia mangium**
- **Aleuritis Montana**
- **Alnus formosana**
- **Bridelia balansae**
- **Carpinus minutiserrata/sekii**
- **Castanea crenata**
- **Castanopsis carlesii**
- **Castanopsis hystrix**
- **Castanopsis indica**
- **Castanopsis kawakamii**
- **Castanopsis stipitata**
- **Cunninghamia lanceolata* **
- **Cyclobalanopsis gilva**
- **Cyclobalanopsis glauca**
- **Elaeocarpus decipiens**
- **Elaeocarpus japonicus**
- **Elaeocarpus sylvestris**
- **Engelhardtia roxburghiana**
- **Lagerstroemia subcostata**
- **Liquidambar formosanum**
- **Lithocarpus amygdalifolius**
- **Mallotus paniculatus**
- **Mangifera indica**
- **Pasania brevicaudata**
- **Prunus phaeosticta**
- **Quercus acutissima**
- **Quercus variabilis**

* Suitable but gives lower yield
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Title:
Development and production of Lentinula edodes (Shiitake mushrooms) on inoculated logs of a range of tree species

Date:
2009

Citation:

Persistent Link:
http://hdl.handle.net/11343/35307

File Description:
Development and production of Lentinula edodes (Shiitake mushrooms) on inoculated logs of a range of tree species

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