REVIEW

Pre and post harvest management of mushrooms

ahr applied horticultural research

Horticulture Innovation Australia

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Contents

1 Introduction

The purpose of this review is to summarise recent major advances in mushroom production technology, as reported in the peer reviewed literature. The aim is to help the Australian mushroom industry identify gaps in knowledge and determine the best possible use of research levy funds. In particular, it is important to focus on areas that are not already being studied in other countries, or where overseas research has limited relevance for Australian growers.

1.1 Mushroom research

At the beginnings of mushroom growing in Australia, all you needed was "*a source of stable straw and manure for composting, a five pronged fork to hand turn the compost, a watering can for wetting the mix, a pointed stick or prongs for spawn holes, a spoon to put spawn in the holes, a shovel for applying casing, a picking/cutting knife and a strong back*"[1].

From humble beginnings, growing on outdoor ridge beds in the Hawkesbury Valley, the industry is now probably the most technologically advanced of all agricultural industries, with multi-million dollar farms and international standard facilities. Every aspect of mushroom growing has been studied and refined. As a result, since the 1950s, yield has increased from as little as 5kg.m^2 to over 50kg.m^2.

The driver for many of these improvements has been international research on mushroom science. The number of peer-reviewed papers published annually has increased from a handful in the early 60s to nearly 200 annually today (Figure 1). Leaps in knowledge are particularly evident from the early 1990s. At this time research groups in Pennsylvania State USA , the Netherlands and the UK published major advances in compost science, pest and disease management, and optimisation of growing conditions.

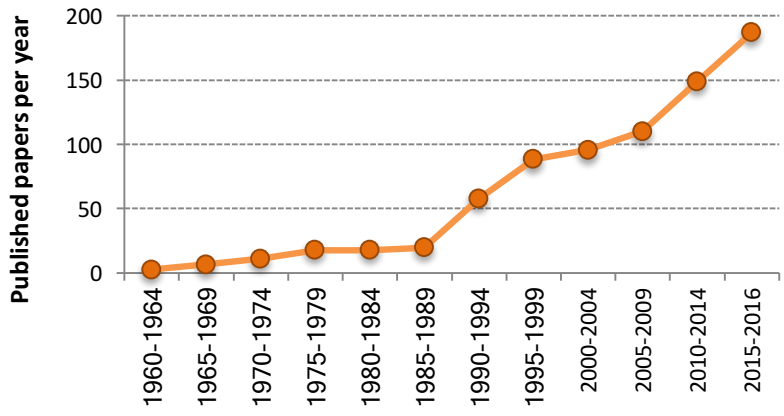

Figure 1. Number of published papers including *'Agaricus bisporus'* as a topic. Data derived from CAB abstracts

[1] Miller J. 2004. Reminiscences of a Fun'gi. The story of a community that mushroomed. Hawkesbury City Council.

The last ten years have also seen an increase in the number of published papers. Much of this resurgence is due to investment in research, and publication of results, by Chinese academics. Some of this work is of excellent quality and undoubtedly relevant to the Australian industry. However, it should be noted that a substantial number of the peer reviewed papers that include "Topic: *Agaricus bisporus"* are primarily focussed on the many other types of mushrooms grown in China and/or are written in Chinese.

At the same time, the traditional drivers of technological advances are publishing less. Whereas nearly 25% of peer reviewed papers used to be published by Dutch researchers, and around 20% by American researchers (primarily at Penn State), these hubs are now responsible for only 5% and 9% of publications respectively. Moreover, many published papers are based on research conducted by PhD and Masters students, rather than senior staff. Published research in the UK and many parts of Europe has similarly declined. The reasons for this are likely to include;

- Reduced government funding in first world countries for publicly available and, therefore, publishable agricultural research.
- Increased commercialisation of research, with results only available to the funding organisation/s.
- Internal research conducted by major mushroom supply chain companies.

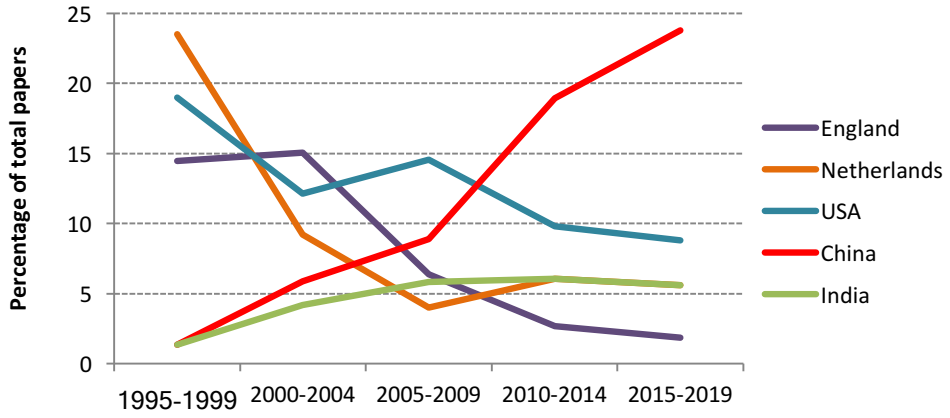

Figure 2. Origin of published papers including *'Agaricus bisporus'* as a topic, analysed by country. Data derived from CAB abstracts.

The topics of publication have similarly changed, and diversified, over time. Basic mycology has declined, as has applied biotechnology and microbiology, perhaps reflecting the increased private nature of research on these topics. The big 'winner' is food science, due to intense interest in the health benefits that mushrooms provide.

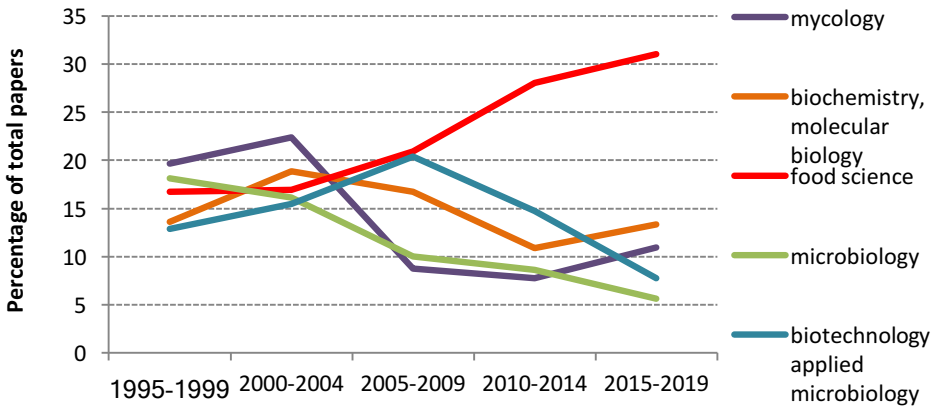

Figure 3. Subject area of published papers including *'Agaricus bisporus'* as a topic. Data derived from CAB abstracts.

1.2 This review

As a result of the changes described above, it seems likely that some, or even many, of the latest advances in mushroom science are not available through the peer-reviewed literature. To a certain extent, references to new technologies may be found in the "grey literature" – industry reports and other information not necessarily been subjected to peer review. However, much new science is likely to be the commercial property of large companies.

Despite these limitations, reviewing published literature still reveals advances in research and development, and can provide valuable directions for new research.

The focus of this review is on literature published since 2000. This is not to discredit some of the earlier research, which can still provide insights into future directions. In particular, some of the work on alternative composts, compost additives and casing materials may be worth revisiting; whereas these may not have been viable 20 years ago, increasing environmental constraints may be making such options more attractive. However, Australians generally are "early adopters" of new technologies, so mushroom producers are likely to be aware of pre-2000 research developments.

Following a roundtable discussion of R&D priorities in September 2016, industry representatives expressed interest in projects on:

- Managing pests and diseases, especially preventing development of resistance
- Waste management, including uses for spent mushroom compost (SMC)
- Sustainability - reducing energy and water use
- Food safety implementation and preparedness
- Extension of information to the industry in general
- Avoiding chemical contamination from compost (pesticides in straw, CCA treated pallets in chicken litter), and in mushrooms (mis-management of pesticides)

Further consideration suggested that these concepts could be divided into three primary research themes:

1. Productivity
    a. Pests and diseases
    b. Substrate management
    c. New technologies
2. Risk
    a. Sustainability – energy and water use, waste management
    b. Chemical contaminants
    c. Food safety
3. Knowledge
    a. Extension and best practice resources

2 Compost

Compost has been, and remains, a primary focal point of mushroom research. Compost provides the nutrients needed for mushrooms to grow, so providing the right mix is critical for good productivity. Overcoming fluctuations in the quality and availability of compost ingredients, as well as optimising the composting process, are key challenges for the Australian industry.

2.1 Compost materials

Key points

Mushroom compost is essentially composed of straw, manures and gypsum, with other materials added as needed. Substantial research has examined substitutes for wheat straw and horse manure, as alternative materials that are locally abundant eg rice straw in China, corn waste in the USA, sugarcane in India and Africa. Many such materials have been found to provide equivalent results, although ratios of ingredients, degree of aeration and additives may change in comparison to wheat straw.

In contrast, little research has examined the effect of chicken manure quality on mushroom compost outturn. Broilers and laying hens are fed very different diets, and the effects on attributes of the manure are not well understood. There is also concern whether chemicals and heavy metals in feed and bedding can transfer to mushrooms through compost.

- Mushroom compost is sometimes divided into 'natural' or 'synthetic' depending on the raw materials used. Essentially, 'natural' compost is produced using mostly stable waste ie horse manure mixed with partially decomposed straw. 'Synthetic' compost is produced using harvested straw with chicken manure alone. Gypsum and water are also added to the basic mix[2], along with optional ingredients such as dried distillers grain, seed meals, cotton hulls and other agricultural by-products[3].

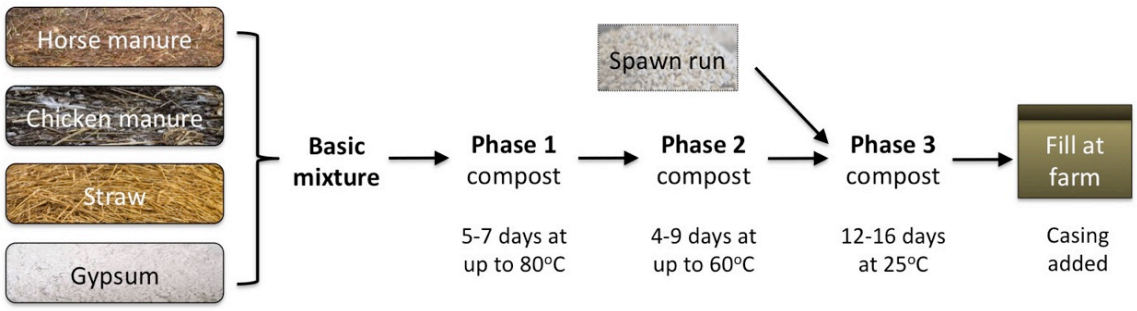

Figure 4. Basic process of mushroom compost production.

[2] Van Griensven LJLD. 1988. The cultivation of mushrooms. Darlington Mushroom Laboratories.

[3] Iiyama K, Stone BA, Macauley B. 1994. Compositional changes in compost during composting and growth of *Agaricus bisporus*. App. Environ. Microbiol. 60:1538-1546.

2.1.1 The straw

- The main ingredient in compost is wheat straw, or a similar species of dried grass. Grasses such as wheat straw are rich in carbohydrates, mainly cellulose, xylan and lignin. These combine together to form a complex, hard to degrade network[4]. The purpose of composting is to break this material down, making it easier for *Agaricus* to access the polysaccharides that fuel its growth.
- Wheat straw availability and quality is a significant issue for mushroom composters. Wheat plants are being bred to be shorter and new, efficient harvesters leave more of the stubble in the field. There is also competition for straw for use in biofuels. All are positives for wheat farmers but negatives in terms of quantity of straw available for composting[5].
- Composters may also need to consider the fungicides used on wheat straw during development; a European study found that while most fungicides were degraded during drying and composting, the fungicide flusilazole on wheat straw could potentially be present at high enough concentrations to inhibit *Agaricus* growth[6].
- As new season straw is slower to break down than aged straw, this can reduce compost yield and quality. This is partly due to chemical changes during weathering of old straw, but also due to changes in the microbial community on the straw itself. Introducing these organisms onto fresh straw could reduce this issue and accelerate the composting process[7].
- In much of Europe and the USA, horse bedding is used to make mushroom compost. As this contains a semi-composted mixture of straw and manure, it is faster to break down than the clean straw commonly used in Australia.
- In China and other parts of Asia, rice straw and cow manure are used instead of wheat straw, as these materials are more easily available[8]. Compost based on rice straw has been shown to be just as productive as that based on wheat straw, especially if kept properly aerated.
- Rice straw heats more quickly than wheat straw during all stages of composting. This is believed to be due to differences in wax and silica content of the plant cuticle, as well as both microbial diversity and abundance on rice straw. The result is more rapid degradation and lower carbon:nitrogen ratio in rice straw compost[9].
- Sugar cane waste is another product investigated as a substitute for wheat straw. For example, compost formed from a mixture of sugar cane 'straw', bagasse and chicken

[4] Jurak E, Kabel MA, Gruppen H. 2014. Carbohydrate composition of compost during composting and mycelium growth of *Agaricus bisporus*. Carbohydr. Polym. 101:281-288.

[5] Jones N. 2013. Raw materials review: wheat straw. The Spawn Run December 2013 pp7-8.

[6] Potocnik I. et al. 2012. Impact of fungicides used for wheat treatment on button mushroom cultivation. Pesticidi I Fitomedicina 27:9-14.

[7] Kertesz MA, Bell TL, Safianowicz K. 2015. Improving consistency of mushroom compost through control of basic biotic and abiotic parameters. Final Report MU10021 Hort Innovation Aust.

[8] Song T-t et al. 2014. Comparison of microbial communities and histological changes in Phase I rice straw based *Agaricus bisporus* compost prepared using two composting methods. Sci. Hortic. 174:96-104.

[9] Wang L. et al. 2016. Comparison of characterisation and microbial communities in rice straw and wheat straw based compost for *Agaricus bisporus* production. J. Indust. Micro. Biotech. 43:1249-1260.

manure was more suitable for growing *Agaricus* than composts based on soybean bran[10]. Another research team reported that compost made from bagasse, sulfitation cake (a by-product of sugar extraction), straw, peanut fertiliser, urea, ammonium bicarbonate, gypsum and lime produced even higher yields than compost based on wheat straw[11].

- Corn stover, which is the residue left after growing corn, is another potential base for mushroom compost. Chemical analysis shows that corn stover and wheat have similar pH, moisture content and nitrogen levels after composting. Trials at Penn State have demonstrated that composts based on corn stover yield similarly to traditional wheat straw based formulations[12].

Table 1. Yield comparisons from compost based on wheat straw, 50% wheat + 50% corn stover or 100% corn stover. Numbers in columns followed by the same letter are not significantly different (p, 0.05). From Pecchia et al., 2016.

TREATMENT	kg/m²				% Bio-Efficiency
	Break 1	Break 2	Break 3	Total	
Control	10.68 b	8.83 B	4.04 b	23.56 b	78.00
50% Corn Stover	11.82 a	11.53 A	5.25 a	28.60 a	89.00
100% Corn Stover	12.26 a	11.36 A	4.15 b	27.78 a	91.00

2.1.2 Chicken manure

- The quality of chicken manure and materials used in bedding for chickens are also critical to compost quality. In Australia, chicken manure is sourced from broiler sheds or barn based / cage egg production. The mixture can contain bedding material, manure, feathers, blood, eggs etc.. Manure attributes are influenced by the hens diet, and potentially contain antibiotics or other compounds.
- For example, chicken litter can contain ammonia suppressants, added through the diet of the hens or directly to the bedding material. These materials work by lowering litter pH, reducing microbe activity or binding nitrogen. Limited studies in the USA[13] and Canada[14] have examined the effect of ammonia suppressants used at recommended rates, and generally found no adverse effects on compost quality or mushroom yield.
- However, it has been shown that mushrooms bio-accumulate heavy metals if compost is contaminated with these materials. A trial in Africa found that chromium, zinc and cobalt

[10] Jesus JPF et al. 2013. Yield of different white button strains in sugar cane by-product based composts. African J. Ag. Res. 8:824-831.

[11] HaiQin L et al. 2015. Cultivation of *Agaricus bisporus* using bagasse and sulfitation cake. Acta Ed Fungi. 22:31-36.

[12] Pecchia JA, Beyer DM, Xiao L. 2016. The use of corn stover to replace straw in compost formulations for the production of *Agaricus bisporus*. The Spawn Run Sept. 2016:9-11.

[13] Beyer DM et al. 2000. Influence of poultry manure treated with ammonia suppressants on the substrate for the commercial mushroom. In "Proc. Int. Composting Symp., Nova Scotia 1999" pp 942-957.

[14] Gonzalez-Matute R, Rinker DL. 2006. Compatibility of ammonia suppressants used in poultry litter with mushroom compost preparation and production. Biores. Tech. 97:1679-1686.

in mushrooms grown on contaminated substrate exceeded recommended limits for human consumption[15].

- Little research has been conducted on the effect of chicken manure attributes on compost quality and mushroom production, or the extent to which contaminants in chicken bedding can transfer into mushrooms.

2.2 Phase I

Key points

The processes occurring during Phase I composting are biochemically complex, but relatively well understood. Recent research has focused on the microbial community within compost, and how this changes during different stages of the process. Optimising the microbes in compost may help improve the process and make more nutrients available to the *Agaricus* fungi.

- During Phase l composting, microbes degrade the carbohydrates and proteins present in the base materials, reducing dry matter, releasing heat and forming ammonia. This is the first stage in making the nutrients in the compost available to the *Agaricus* fungus.
- Large numbers of different organisms are involved in the composting process including mesophilic (20 to 45°C), thermotolerant (35 to 50°C) and thermophilic (>50°C) bacteria. The appearance of specific microbes during composting can indicate its maturity[16].
- Janssen[17] describes the changes in microbial biota and structure of the basic materials during Phase l composting:
    - At up to 50°C ammonia helps to destroy the cuticle of the straw, a process which can be accelerated by adding urea or sulfate of ammonia. This is the most odoriferous stage, due to the presence of anaerobic bacteria. Odours can be reduced by turning the compost to keep it well aerated and not adding excessive water. *NB. A microbial based product developed by NSW DPI researchers – 'Actizyme' – was previously sold commercially to reduce odours and enhance compost quality. However the cost of this product has seen it disappear from the market.*
    - Between 50-60°C microbes in the mix degrade carbohydrates into their components, forming a firm biomass. Ensuring that the material does not heat too fast at this stage helps to ensure maximum nutrients will be available in the end product.
    - At around 65°C thermophilic bacteria dominate and less oxygen is needed.
    - Browning due to the Maillard reaction (caramelisation) occurs at over 70°C. This can help protect remaining carbohydrates such as cellulose and hemi-

[15] Sithole SC et al. 2017. Pattern and concentrations of trace metals in mushrooms harvested from trace metal-polluted soils in Pretoria, South Africa. South Af. J. Bot. 108:315-320.

[16] Ishii K, Fukui M, Takii S. 2000. Microbial succession during a composting process as evaluated by denaturing gradient gel electrophoresis analysis. J. Appl. Microbiol. 89:768-777

[17] Janssen J. 2016. Aerated composting. A silent practical breakthrough. ISMS Proc. 19:175-179.

cellulose from other fungi, but is readily broken down by *Agaricus* mycelium. Remaining starch is degraded and the thermophilic fungi are killed, releasing polyphenols and nitrogen into the mix.

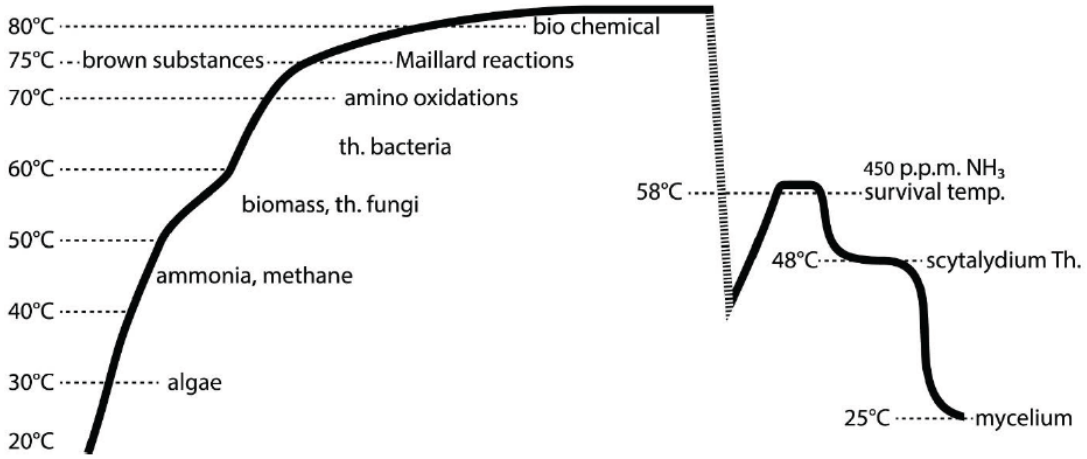

Figure 5. Reactions during Phase 1 composting. From Janssen, 2016.

- Much recent work has focussed on examining the microbial communities that develop during composting. This has become possible due to the advent of new techniques including:
    - Phospholipid fatty acid analysis (PLFA)[18]
    - Amplified DNA based methods such as polymerase chain reaction denaturing gel electrophoresis (PCR-DDGE)[9]
    - "Next-generation" Illumina Miseq sequencing[7].
- These methods allow identification of microbes to genus and even species level, as well as estimates of population within the substrate.
- Recent Australian research has identified over 30,000 different microbes in high-diversity compost at the end of Phase 1. This bacterial community was dominated successively by *Acinetobacter* followed by *Bacillus*, an unidentified *Proteobacterium* and *Thermus*[7]. This project also identified fungal successions in the compost. Strains of *Lewia* and *Myceliophora* were followed by an identified species, then *Penicillium* and *Scytalidium thermophilum*.

[18] Vos AM et al. 2017. Microbial biomass in compost during colonization of *Agaricus bisporus*. AMB Express. 7:12.

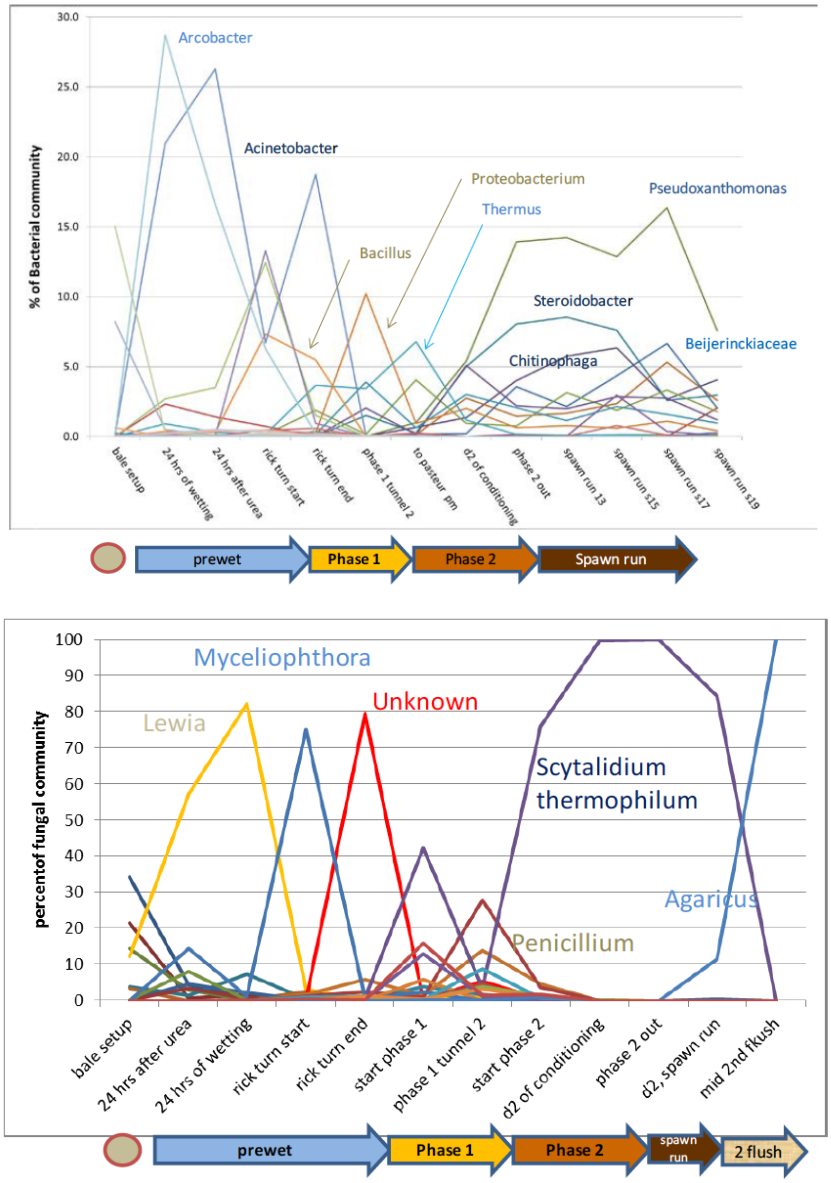

Figure 6. Changes in bacterial (top) and fungal (base) populations in Australian mushroom compost. From Kertesz et al, 2015.

2.3 Phase II

Key points

As with Phase I, much of the recent research on Phase II composting has focused on microbial communities. In particular, the role of the thermophilic fungus *Scytalidium thermophilum*, and whether inoculating compost with this organism can speed the process. Australian research has confirmed this is the dominant fungal species during Phase II composting. However, supplementing compost with this organism is only likely to be beneficial if populations are low. This may occur if materials have not been subjected to Phase I composting, or if inputs are not good quality.

Both commercial amendments and natural products such as oil seeds and protein-rich meals have been shown to improve mushroom yield. As with *S. thermophilum*, benefits are more likely to be achieved if the compost batch is low in that substance, but otherwise of good quality; "*Adding amendments cannot make bad compost good*".

- Phase II of composting ideally begins with a pasteurization period of 8 hours at 56-60°C and continues with conditioning at 45-50°C. This lasts until ammonia has cleared. Ammonia disappears most rapidly at 40-45°C. It is considerably slower to dissipate if initial levels are high, oxygen is low (eg 14%) or CO_2 is high[19]. The optimal ammonia level is 400-500ppm, this provides protection against weed moulds without affecting growth of Agaricus[17].
- If Phase II composting temperatures fall below 40°C or rise above 55°C the resulting compost is not selective for *Agaricus* and will support growth of other, unwanted, fungal species.
- However, if the compost is fully sterilised (all microbes killed), then growth of *Agaricus* is greatly reduced. It appears that while compost meets nutritional requirements, successful colonisation depends on a range of other factors[20].
- At the end of Phase II, 50-60% of celluloses and xylans in the starting material have been degraded, while lignin largely remains intact. This makes it suitable for *Agaricus*, as the mycelia produce enzymes that break down and digest both xylans and celluloses.[21].

2.3.1 Phase II amendments

- There are numerous commercial amendments that can be added to Phase II compost, or later at casing. Commercial supplements can increase protein (eg isoleucine and other amino acids), lipids (fats and oils) and micronutrients (phosphorus, selenium, boron). They can also include microbial amendments. Some supplements are designed to have controlled release over time while others are more immediately available for growth. Adding meals and oils can have an additive effect, while protein generally enhances

[19] Ross RC, Harris PJ. 1982. Some factors involved in Phase II of mushroom compost preparation. Scientia Hort. 17:223-229.

[20] Fermor TR, Grant WD. 1985. Degradation of fungal and actinomycete mycelia by *Agaricus bisporus*. J. Gen. Appl. Microbiol. 126:1729-1734.

[21] Jurak E. 2015. How mushrooms feed on compost: conversion of carbohydrates and lignin in industrial wheat straw based compost enabling the growth of *Agaricus bisporus*. Dissertation, Wageningen Uni.

yield. However, supplements need to be suited to the compost mixture. According to Beyer[22], supplements can make good compost better, but have little benefit if compost quality is poor.

- Current private research at MLMRU is investigating a liquid, fermented product which can be added to Phase II / Phase III compost. While the exact nature of the amendment is commercial in-confidence, initial results suggest that both yield and quality may be improved with this supplement.
- Numerous researchers have reported strong stimulation of growth of *Agaricus* in compost that has been pre-inoculated with the thermophilic fungus *Scytalidium thermophilum.* This organism is commonly found in Phase II compost and helps to reduce the ammonia concentration[23]. For example, a study in India found that inoculation of this organism during Phase II composting increased the rate of degradation of cellulose, hemicellulose and carbon, reducing the time required to complete Phase II to 13 days and increasing total yield[24].
- Mexican researchers have investigated growing mushrooms on semi-composted substrates. Mechanically ground substrates of grain, corncobs and wheat straw were not subjected to Phase I composting, but artificially colonised with *S. thermophilum* (*St*) during Phase II composting[25]. While *St* addition increased mushroom pro9duction, total yields remained much lower than those achieved with standard compost. Adding a protein-rich supplement (soybean, black bean or cow peas) further enhanced *Agaricus* growth in the semi-composted materials[26]. However, in this second study, yield was not compared to normal practice.
- Australian research has also found that *S. thermophilum* dominates the fungal population during Phase II composting. However, commercial products based on this species have had limited success at accelerating this process, so are not generally used[7].

[22] Beyer DM. 2014. Spawning and casing supplements. Presentation for Hooymans compost. Accessed online http://hooymanscompost.nl/media/2014/11/3-David-Beyer-CW-Supplements_Final-2.pdf

[23] Straatsma G et al. 1989. Population dynamics of *Scytalidium thermophilum* in mushroom compost and stimulatory effects on growth rate and yield of *Agaricus bisporus*. J. Gen. Microbiol. 135:751-759.

[24] Vijay B, Pathak A. 2014. Exploitation of thermophilic fungi in compost production for white button mushroom (*Agaricus bisporus*) cultivation – a review. Proc. 8th Int. Conf. Mushroom Biol. Mushroom Products, New Delhi India Nov. 2014. pp292-308.

[25] Sanchez JE, Royse DJ. 2009. *Scytalidium thermophilum* colonised grain, corncobs and chopped wheat straw substrates for the production of *Agaricus bisporus*. Bioresource Tech. 100:1670-1674.

[26] Coello-Castillo MM, Sanchez JE, Royse DJ. 2009. Production of *Agaricus bisporus* on substrates pre-colonised by *Scytalidium thermophilum* and supplemented at casing with protein-rich supplements. Bioresource Tech. 100:4488-4492.

2.4 Re-using compost

Key points

After 2-3 cropping cycles, spent mushroom compost (SMC) remains. The reasons for decreasing yields with cropping cycles is still not clear. Possible mechanisms include accumulation of toxins within the compost and/or casing, decreases in nitrogen, lack of readily available nutrients and increases in nitrates.

While SMC is a valuable material that retains many nutrients, disposal remains a significant issue for Australian mushroom growers. Re-using this material could reduce raw material costs and waste. Penn State University researchers have investigated mixing a percentage of SMC into new Phase II compost. Another option is to re-cycle compost after 2 breaks, fragmenting and mixing with supplement before re-casing the material. Excellent results are reported for both methods. However, the issue of disease potential in recycled materials is not fully addressed.

Combining the Penn State research with disease control measures (cookout and pesticides) could possibly provide a way to increase productivity and reduce waste.

- The productivity of mushroom compost declines rapidly after 2 cropping cycles, with the 3rd crop not always economically viable. After cropping, spent mushroom compost (SMC) remains:
    - SMC typically contains 1–2% nitrogen, mushroom mycelium and unused supplements and raw substrate.
    - It is generally believed that yields decline, especially after the second flush, due to depletion of specific mushroom nutrients[27].
    - Other researchers have suggested that decline is due to accumulation of toxins within the substrate[28]. These could include soluble salts in the casing layer, or metabolites produced by microflora within the compost[29].
    - Australian research recently pointed to accumulation of nitrates within compost as a possible reason for falling yields[7]. A University of Sydney honours student is investigating this further.
    - However, it is important to differentiate nitrates from total nitrogen; Royse and Chalupa[30] suggested that it was the reduction in nitrogen levels from 3% to 2.1% that was responsible for lower yields in research center trials.

[27] Royse DJ. 2008. Re-supplementing and re-casing mushroom (*Agaricus bisporus*) compost for a second crop. World J. Microbiol. Biotechnol. 24:319-325.

[28] Schisler LC. 1990. Why mushroom production declines with each successive break, and the production of a second crop of Agaricus mushrooms on 'spent' compost. Appl. Agric. Res. 5:44-47.

[29] Nair NG. 1976. Studies on recycling spent compost for mushroom cultivation. Aust. J. Agric. Res. 27:857-865.

[30] Royse DJ, Chalupa W. 2009. Effects of spawn, supplement and Phase II compost additions and time of re-casing second break compost on mushroom (*Agaricus bisporus*) yield and biological efficiency.

- Even though SMC is a valuable addition to horticultural soils and potting mixes, issues with disposal remain. Many Australian mushroom growers currently pay to have SMC removed, so finding ways to re-use this material would provide a significant cost saving.
- As many useful organic materials remain in SMC, there is considerable research interest in recycling it as a compost ingredient[31].
    - Murphy[32] suggested that 25% SMC could be re-mixed into Phase I compost without adversely affecting yield.
    - Producing more than one crop of mushrooms from a batch of compost has been a particular focus of Penn State researchers. Bishop et al[33] recently demonstrated that including 20% SMC during Phase 1 composting reduced yield of the subsequent crop. However, this drop in productivity could be reversed by increasing the percentage of materials rich in lignin. In this case corn stover, corncobs, cottonseed hulls and straw were used.
    - Yield from compost prepared with 20% SMC plus additional lignin matched or exceeded yields from normal compost in three separate trials.
- Another option is re-mix and re-case second break mushroom compost. After two breaks mushroom production declines, so it is possible to obtain the majority of production then remove the casing and recycle the material:
    - To maintain yield, commercial supplements, amino acids and hydrolyzed proteins are added[34] during fragmentation of the compost and re-mixing, or by using a spawning machine to spread on top of compost beds.
    - Fragmentation can be achieved by passing the second break compost through a turner fitted with a rotating drum and bars to break up the material. Simple fragmentation can increase yield by 30% compared to non-fragmented material, while combining fragmentation with a commercial supplement increased yield by 53-56%[35].
    - Additional spawn can also increase yield, but costs are likely to exceed benefits[30].
    - Penn state researchers[30] have found that second break compost re-mixed with supplement plus 15% fresh Phase II compost OR re-mixed with supplement then cased after 10 days, yielded as well or better than fresh Phase II compost (Figure 7).

[31] Fidanza MA et al. 2010. Analysis of fresh mushroom compost. HortTech 20:449-453.

[32] Murphy WS. 1972. Development of a mushroom production medium without Phase I composting. Mush. News 20:4-22.

[33] Bishop EL et al. 2016. Effects of spent mushroom compost (SMC) as an ingredient in Phase I compost on production of *Agaricus bisporus*. Compost Sci. Util. 24:246-258.

[34] Royse DJ et al. 2008. Re-supplementing and re-casing mushroom (*Agaricus bisporus*) compost for a second crop. World J. Microbiol. Biotechnol. 24:319-325.

[35] Royse DJ. 2010. Effects of fragmentation, supplementation and the addition of Phase II compost to 2nd break compost on mushroom (*Agaricus bisporus*) yield. Biores. Technol. 101:188-192.

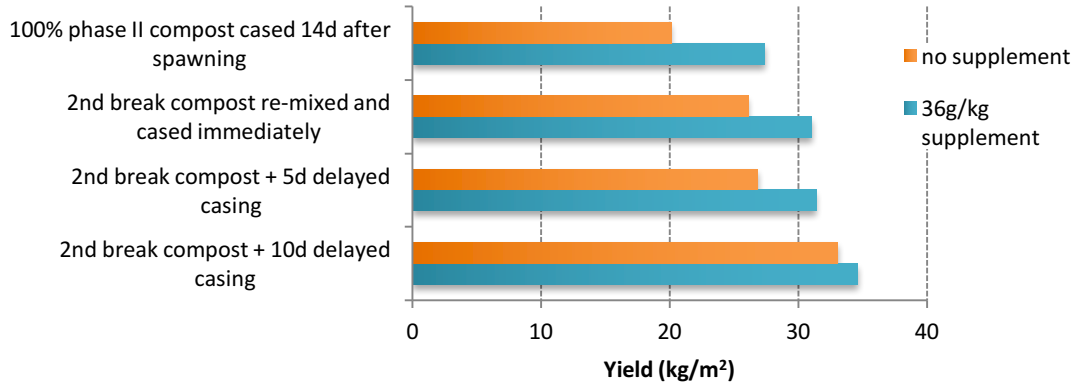

Figure 7. Yield from second break compost that has been re-mixed and re-cased after 0, 5 or 10 days, compared to yield from fresh Phase II compost. Derived from Royse and Chalupa, 2009.

- The Penn State results appear extremely promising. It is noted that removing the casing prevents buildup of growth limiting materials (salts etc). However, there is only minimal discussion of increased pest and disease risk from re-cycling composts. While one trial tested addition of fungicide with supplement, it appears that these tests were not affected by significant disease pressure. If extended on a commercial scale, disease could limit use of this method. However, this remains to be investigated.

2.5 Non-composted substrates

Key points

Non-composted substrates include grain, sawdust and even hydroponic systems. While there appeared to have been keen interest in this option ten years ago, no references could be found from 2008 onwards. Although such mixtures can fulfill the nutritional needs of mushrooms, they presumably do not contain the complex microbial populations found in good quality compost.

- A number of researchers have investigated whether the nutritional needs of mushrooms could be met without the need to compost materials at all.
- Bechara et al[36] grew mushrooms hydroponically. However yields were very low (for both the test units and the control).
- The same group also tested non-composted grain-based substrates consisting of various proportions of millet grain, soybean, rye grain spawn and perlite bulking agent[37]. The substrates were cased with a sterilised mixture of peat and calcium carbonate including 25% activated charcoal. The 100% millet mix provided similar yield to normal compost although, again, yields were low at only 7.7 to 8.7kg/m^2. Yields were improved in a subsequent trial, which included sterilised millet, oilseeds and a commercial delayed release supplement. This increased production to 16.9kg/m^2[38].
- Mamiro and Royse[39] also tested non-composted substrates of oak sawdust, millet, rye, peat, ground alfalfa, ground soybean, wheat bran and lime. The best yields were obtained using a 50/50 mixture of SMC and non-composted materials.
- Despite this early work on non-composted substrates, little further research appears to have been conducted. It is possible that some of the larger European companies are progressing this technology, but that this information is not publicly available (Seymour, pers. com.).

[36] Bechara MA et al. 2006. *Agaricus bisporus* mushroom cultivation in hydroponic systems. Trans. ASABE 49:825-832.

[37] Bechara MA et al. 2006. Non-composted grain-based substrates for mushroom production (*Agaricus bisporus*). Trans. ASABE 49:819-824.

[38] Bechara MA et al. 2008. Cultivation of *Agaricus bisporus* and Agaricus blazei on substrates composed of cereal grains and oilseeds. Bio. Eng. 1:65-78.

[39] Mamiro DP, Royse DJ. 2008. The influence of spawn type and strain on yield, size and mushroom solids content of *Agaricus bisporus* produced on non-composted and spent mushroom compost. Biores. Tech. 99:3205-3212.

2.6 Phase III, spawn run

Key points

As with Phase I and II, shifts in microbial populations following the addition of spawn have been of key research interest in recent years. The process by which *Agaricus* mycelia absorb nutrients from compost has also been a favored topic, mainly due to a high quality PhD by Edita Jurak at Wageningen University. Multiple papers and spinoff research has resulted from her discovery that *Agaricus* cannot degrade certain forms of xylan, one of the key components in compost. Finding a way to degrade this material could significantly increase nutrient availability and prolong the productivity of compost.

- In the last 10 years there has been a move away from using Phase II compost in cropping rooms to filling trays or beds with compost already inoculated with spawn (Phase III compost).
- The rate at which mycelium is able to colonise compost has a positive relationship with subsequent yield. Colonisation cannot be reliably measured by changes in compost temperature. However, falls in pH (due to production of oxalate by mycelium) and increases in laccase are good indicators of the spread of mycelia through compost and, therefore, potential yield[40].

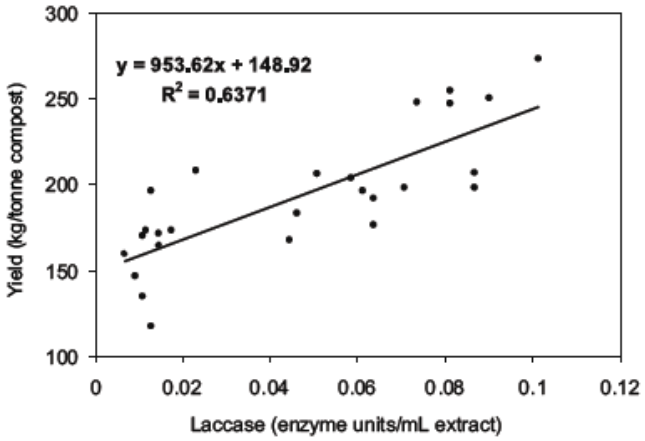

Figure 8. Relationship between production of laccase enzyme by Agaricus mycelia and yield from compost. From Noble et al. 2008.

- Increasing the rate of spawn addition from 0.5% to 0.8% w/w increased yield by approximately 15kg.tonne^{-1} compost and reduced time taken for Phase III by one day[40].
- During mycelium growth, *Agaricus* consumes some of the other microbes present in the compost. Vos[18] demonstrated that bacterial biomass decreased from 17.7 to 4.7mg.g^{-1} of compost during mycelial growth. Naturally, living fungal biomass increased. Not all of this increase was due to *Agaricus*, however, with a portion due to other fungal species in the compost. In the absence of *Agaricus*, other fungal species declined by 50%. This demonstrates that whole fungal communities are important for maximal growth of *Agaricus*.

[40] Noble R et al. 2008. Measuring and improving the rate of spawn-running in compost. Proc. 17th ISMS pp207-219.

- At the start of Phase III, only 'difficult to degrade' carbohydrates are left in the compost. In the case of wheat straw, these are the cell walls, which are mainly composed of cellulose (25–40%), xylan (30–45%) and lignin (~10%). The cell walls are broken down by the *Agaricus* mycelia as they grow. As a result, 40% of lignin in the compost is metabolised, as well as 10% of the celluloses[41]. The remaining lignin is modified, so could be considered partially consumed.
- However, the fungus is unable to degrade some forms of xylan, with the result that less than 6% of the xylan in compost is metabolised during Phase III[42]. The lack of enzymes to break down xylan reduces *Agaricus* ability to use all of the energy contained in compost[21]. Finding a way to make these xylan fragments available to *Agaricus* would increase food availability and thereby potentially extend the productive life of compost.

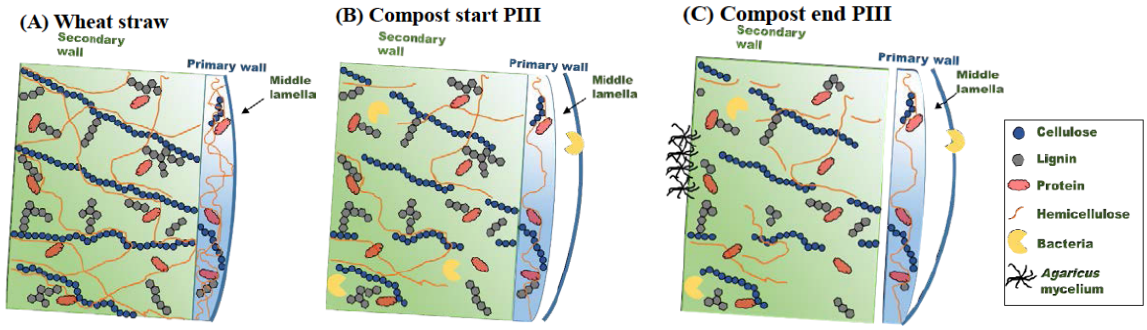

Figure 9. Proposed cell wall model of wheat straw. During Phase I and II 50% of the compost carbohydrates are metabolised, and the middle lamella separates, reducing rigidity of the cells. During Phase III, lignin is metabolised, xylan becomes more water-soluble and the bacterial population declines. From Kabel et al, 2016.

[41] Jurak E et al. 2015. Fate of carbohydrates and lignin during composting and mycelium growth of *Agaricus bisporus* on wheat straw based compost. PLoS ONE. 10:e0138909.

[42] Kabel M. et al. 2016. Fate of lignin and substituted xylan during commercial cultivation of *Agaricus bisporus*. Proc. ISMS 19:350-353.

3 Casing and mushroom initiation

3.1 How casing works

Key points

The addition of casing to compost stimulates formation of fruiting bodies ie mushrooms. The mechanism by which this occurs is believed to involve bacteria in the casing, in combination with low temperatures and reduced CO_2 concentrations. It appears likely that the eight carbon volatile 1-octen-3-ol – which is produced by *Agaricus* – is degraded by Pseudomonads and other bacteria in the casing. This allows formation of primordia. Activated charcoal can also absorb volatile compounds and may have a similar effect. It is surprising that substitutes for peat casing that include activated charcoal have not been more widely investigated.

- While *Agaricus* mycelia grow very well through compost, the formation of the fruiting bodies – mushrooms (sporophores) – is only stimulated when a casing layer is added. In Australia peat is used, but casing can be soil, sand or various mixtures. The mechanism by which *Agaricus* is stimulated to change from vegetative to reproductive growth is complex and still not entirely understood. Proposed mechanisms[43] include:
    - A reduction in temperature from 25 to 18°C
    - Ventilation of the growth room gases, reducing CO_2 below 1,000ppm
    - Gradients in CO_2 concentration between the compost and the casing
    - Volatile compounds produced by *Agaricus* which are trapped / destroyed by the casing
    - Pseudomonad bacteria present in the casing
- There is good support for a role for bacteria in stimulating mushroom formation[44], possibly by metabolizing substances that otherwise inhibit this process[45]. Casing contains a large bacterial population, which includes Pseudomonads, *Arthrobacter*, *Sphingobacterium*, *Bacillus* and other species. Bacterial populations have been shown to increase in the interval between application of casing and formation of primordia[46]. Moreover, both Pseudomonads and *Arthrobacter* isolated from casing have been shown to stimulate growth of *Agaricus*[47].

[43] Hayes WA, Randle PE, Last FT. 1967. The nature of the microbial stimulus affecting sporophore formation in *Agaricus bisporus*. Ann. Appl. Biol. 64:177-187.

[44] Hayes WA. 1979. Progress in the development of an alternative casing medium. Mushroom J. 78:266-271.

[45] Miller N, Gillespie JB, Doyle OPE. 1995. The involvement of microbiological components of peat based casing materials in fructification of *Agaricus bisporus*. In Elliot TJ "Science and Cultivation of Edible Fungi" pp 313-321

[46] Cai W. et al. 2012. Microbial community diversity and structure analysis of casing soil during mushroom (*Agaricus bisporus*) growth. Proc. ISMS 18:784-793.

[47] Siyoum NA. 2013. Microbial dynamics of different casing materials in the production of white button mushrooms (*Agaricus bisporus*). PhD dissertation, University of Pretoria.

- Conversely, sterilising the casing material has been reported to inhibit, or even prevent the development of mushrooms[48].
- The *Agaricus* mycelia produce a range of volatile organic substances, mainly eight-carbon (C8) compounds. One of these, 1-octen-3-ol, was shown to be highly inhibitory of primordia formation. However, Pseudomonad bacteria found in the casing metabolise C8 compounds. It is this process that allows mushrooms to form.
- Activated charcoal also absorbs volatile organic compounds, and has been shown to have a similar effect[49]. Bechara et al.[50] claimed that adding activated carbon to peat-based casing increased yield, especially if used with a non-composted substrate consisting only of grain spawn and perlite.
- Heat treating the casing material also increased yield, the authors hypothesising that this reduced microbial competition within the casing layer[50]. However, in this study mushrooms were only grown in small containers, so results may not be transferable to commercial situations.

[48] Eger G. 1972. Experiments and comments on the action of bacteria on sporophore initiation in *Agaricus bisporus*. Mush. Sci. 8:719-726.

[49] Noble R et al. 2009. Volatile C8 compounds and pseudomonads influence primordium formation of *Agaricus bisporus*. Mycologia 101:583-591.

[50] Bechara MA. 2009. Evaluating the additioon of activated carbon to heat-treated mushroom casing for grain-based and compost-based substrates. Biores. Technol. 100:4441-4446.

3.2 Initiation and development of mushrooms

Key points

Changes in volatiles, cool temperatures and low CO_2 initiate the formation of mushrooms. If too many pins form, then mushrooms will be small and low quality and picking costs are increased. Environmental conditions need to be managed closely to ensure optimum mushroom density. Salt may potentially be used in the future to reduce density, but this technique requires more work and is likely to reduce recycling opportunities.

- A model has been proposed by Eastwood et al[51] which involves three separate environmental factors at different stages of mushroom development:
    1. The C8 volatile 1-octen-3-ol regulates the change from vegetative hyphae to the multicellular knots that give rise to mushrooms. Levels of 350ppm are inhibitory. Once levels drop, the fruiting process starts.
    2. Low temperatures allow formation of the primordia. Only primordia that form below the surface of the casing eventually turn into mushrooms. Smooth, undifferentiated primordia that appear on the casing surface – as occurs at 25°C – fail to develop further.
    3. CO_2 levels determine the number of primordia that develop into mushrooms (generally 5-10%). High CO_2 levels (>3,000ppm) reduce the number of primordia that develop into mushrooms.

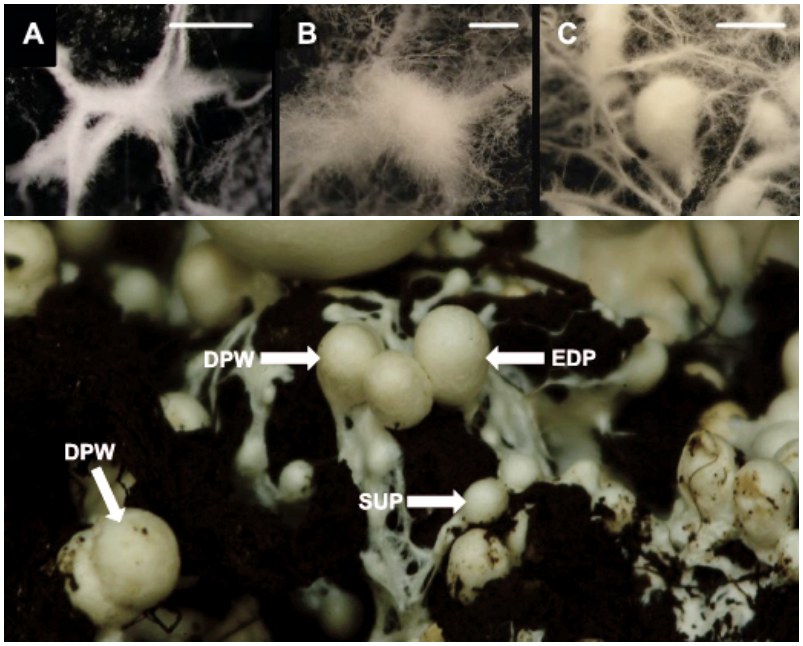

Figure 10. Mushroom development. A) fluffy mycelia; B) hyphal knots; C) fluffy, undifferentiated primordia; D) pinning with smooth, undifferentiated primordia (SUP), elongated differentiated primordia (EDP) and differentiated primordia with waist (DPW). From Eastwood et al, 2013.

[51] Eastwood DC et al. 2013. Environmental regulation of reproductive phase change in *Agaricus bisporus* by 1-octen-3-ol, temperature and CO2. Fungal Genetics Biol. 55:54-66.

- If a very large number of primordia, or 'pins', form then mushrooms will be small and low quality, increasing picking costs and reducing profitability. Strain, compost, supplements and environmental conditions have all been shown to influence the number of mushrooms forming during a flush[52]. However, even strict control of conditions does not guarantee a good result. If too many pins form, then shutting down the room ventilation to allow CO_2 to rise, or increasing watering, can provide some control.
- Alternatively, Desrumaux et al[52] investigated applications of salt to developing primordia to reduce mushroom formation. Application of 14g.m^2 salt reduced the number of pins by 24%, with increasing effects up to 56g.m^2 (54% reduction). However, the data was extremely variable, with variety and the size of primordia at the time of application both significantly affecting results.
- Termination of pins only occurs while they are small; once primordia reach 10mm they will continue to develop into mushrooms. Initial development is extremely rapid, slowing to an exponential rate with diameter doubling every 1.7 days until the mushrooms reach maturity, indicated by veil breaking (Figure 11). Research by Straatsma et al[53] indicates that all of the mushrooms that develop in the first and second flushes are present as primordia within the first week of casing. Effectively, some of the pins go into a resting stage. This is broken by the start of harvesting, with the re-activated pins forming the second flush.

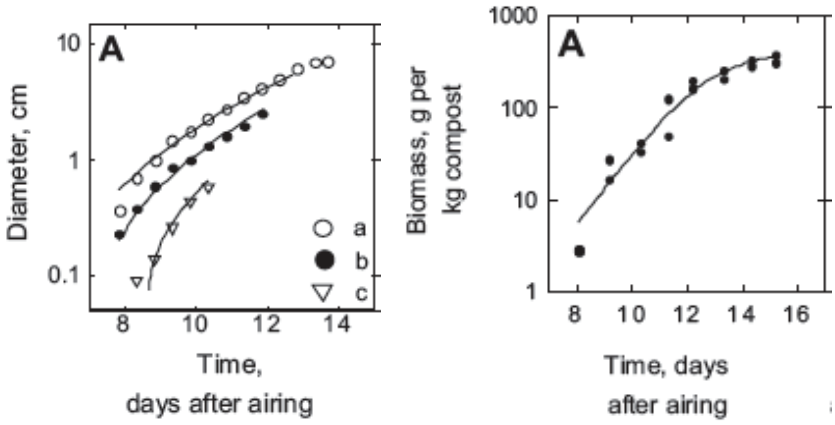

Figure 11. Increases in cap development (left) and biomass (right) of developing mushrooms. From Straatsma et al, 2013.

[52] Desrumaux B et al. 2006. Reducing the pin number in an Agaricus culture with sodium chloride. Proc. ISMS 16:PP?

[53] Straatsma G, Sonnenberg ASM, Van Griensven LJLD. 2013. Development and growth of fruit bodies and crops of the button mushroom, *Agaricus bisporus*. Fungal Biol. 117:697-707

3.3 Alternative casing materials

Key points

Peat has qualities that make it ideal to use as casing material. However, use is likely to be increasingly restricted and expensive in the long term. Many other materials have been investigated for use in casing, whether alone or in combination with peat. Spent mushroom compost has had the most attention. However, issues with salinity, density and variability generally have limited its use.

- The qualities of peat make it ideal to use as casing material. As well as stimulating the formation of mushrooms, casing materials must also provide physical support, have a high capacity to absorb and release water, withstand frequent irrigation without disintegrating, are permeable to gases and have a low salt content. Casing should also have low nutritional value and be free of pests and diseases[54].
- Peat is used as casing by all Australian mushroom farms; only the type of peat varies. However, peat is a limited resource. Australia used to import peat from Ireland, but now sources material mainly from Poland. The cost of peat is likely to increase as availability declines. Pressure to reduce or eliminate use of peat can also come from other sources. For example, 80% of mushrooms produced in Ireland are exported to the UK. However, the industry has been under pressure from Government to reduce peat use due to concerns about conservation of peat bogs and climate change[55].
- Alternative casing materials have been widely researched since the 1980s. South Africa have been leaders in this field; the only available peat within South Africa is reed-sedge peat, which has a high clay content and dirties the mushrooms, and is now protected from further exploitation. Purchasing peat from Europe was initially impossible and later prohibitively expensive. As a result, a huge number and variety of materials have been investigated in South Africa (and other countries), including:
    - Carpet wool
    - Coffee grounds
    - Composted mushroom stalks
    - Composted vine shoots
    - Composted water weeds
    - Cotton husks
    - Fine particle tailings from coal mining
    - Floculated rock wool
    - Gum sawdust
    - Lignite
    - Loamy top-soil
    - Mineral soil
    - Palm fibre
    - Paper pulp

[54] Pardo A, de Juan JA, Pardo JE. 2003. Characterisation of different substrates for possible use as casing in mushroom cultivation. Food Ag. Environ. 1:107-114.

[55] Barry J. et al. 2012. Supplementation of spent mushroom substrate to improve its structure and productivity as a casing material. Proc. ISMS 18:735-742.

- Pine sawdust

- South African company Mabu Casing soils has developed a commercial casing material made from sugarcane pith. This is a waste product from sugarcane bagasse that has been processed to make paper. Launched in 2015, the product was developed by Dr Linda Meyer and Ane va Heerden, both researchers from the University of Pretoria. Tests in the Netherlands with Dutch company BVB Euroveen found that a 50:50 blend of Mabu and peat resulted in a good mix of large and small mushrooms while maintaining yields of approximately 35kg.m^2. Small adjustments needed to be made to irrigation, as Mabu has a high ash content and does not retain moisture as well as peat.
- The material that has received the most attention is spent mushroom compost (SMC). This material is attractive because using it could reduce both the cost of casing and issues with waste disposal.
- Numerous published papers detail methods for using SMC alone, or in combination with other products, as a casing material. The main drawbacks of SMC are its variable composition, relatively poor water holding capacity and high salinity[56]. Weathering SMC can help to reduce salinity, especially in high rainfall areas[57].
- For example, Irish studies have found that combining 80% peat with 20% SMC was comparable to commercial peat based casing. However, the SMC was first leached to an EC of 4mS then heat-treated for 2 hours at 60°C before use[58]. Adjusting irrigation so as to increase the volumetric water content of peat blended with SMC, or SMC alone, can also help restore yields to those achieved with peat[59].
- Other papers have examined ways to eliminate peat altogether, basing the casing material on SMC plus additives. However, even 'optimised' mixes – such as SMC plus 30% vermiculite[55] – fail to yield as well as peat.

[56] Riahi H, Zamani H. 2008. Use of spent mushroom compost and composted azolla as an alternative for casing soil. Proc. ISMS. 17:333-339.

[57] Eicker A, van Greuning M. 1989. Economical alternatives for topogenous peat as casing material in the cultivation of *Agaricus bisporus* in South Africa. South Af. J. Plant Soil. 6:129-135.

[58] Barry J et al. 2008. Partial substitution of peat with spent mushroom substrate in peat-based casing blends. Proc. ISMS 17:288-309.

[59] Barry J et al. 2016. Influence of irrigation management on the quantity and quality of Agaricus bisporus produced on spent mushroom substrate (SMS) based casings. ActaHort. 1112:299-306.

3.4 Recycling casing

Key points

Another way to reduce use of peat is to re-use it. Netherlands researchers have developed equipment and method to separate casing from compost and recycle it for further use. Suggested rates are 20-30% recycled casing material added to fresh peat.

- Another potential way to increase sustainability of the industry would be to recycle and re-use casing soil. Researchers in the Netherlands[60] have developed a method to separate casing from the underlying compost.
    - To ensure good separation between casing and the relatively saline compost, mycelium is allowed to thoroughly colonise the compost under high CO_2 conditions (~10,000ppm), before the casing layer is added.
    - At the end of cropping the casing material is removed and ground, chopping up *Agaricus* mycelia and stipes.
    - It is then heat treated with steam to kill any pests or diseases (12 hours at 70^{o}C)
    - Finally, a mixture of bacteria (mainly *Bacillus subtilis*) is added to aid decomposition.
    - A 50/50 mixture of new and recycled casing material produced around 96% of normal yield. It is therefore recommended to add no more than 20–30% recycled material to fresh casing, at least initially.

[60] Oei P, Albert G. 2012. Recycling casing soil. Proc ISMS 18:757-765.

4 Pest and Disease Management

4.1 Insects

Key points

Fungus gnats, or 'mushroom flies', fall into two groups; sciarids and phorids. These families differ in how and when they attack mushroom crops, and also in control methods. Insecticides, particularly diazinon, have been effective at controlling fungus gnats, although some researchers have reported impacts on yield. Moreover, diazinon is an organophosphate with restricted use and that could potentially leave residues in crops. Incorporating cyromazine in compost and triflumuron in casing can be an effective control method but may also impact yield.

Predatory mites and nematodes could offer an alternative to insecticides for control of fungus gnats. However, it is not clear if these are a cost effective option. Despite a significant research effort from 1990 to 2009, there appears to have been little further advance in the use of biocontrol agents in mushroom crops.

- Fungus gnats, otherwise known as mushroom flies, are a pest in growing rooms worldwide. The two major classes are flies in the family Sciaridae, primarily *Lycoriella* sp. and *Bradysia* sp.[61] and the Phorid fly *Megaselia* sp.. The sciarids are most frequently a problem in production systems where Phase II compost is transferred to mushroom growing houses before it is colonised by mycelium. In contrast, Phorids more commonly invade compost that is already colonised by *Agaricus* mycelia[62]. Infestations of fungus gnat can reduce yield by 15-22kg.m^2 [63].
- The larvae of fungus gnats feed on compost, damage developing spawn, and burrow through mushrooms leaving holes that directly damage the product[64]. Tunneling inside mushrooms is due to infestation at pinning. Larvae prefer to eat young pins, with the result that only six larvae per 30g casing can cause the loss of one-third of developing mushrooms[65].
- Adult fungus gnats, particularly phorids, are also an important disease vector within growing rooms[62]. Clift et al[66] showed that fungus gnats were strongly associated with infection by dry bubble disease in mushroom farms around Sydney. This is because the fungus has sticky spores, which easily adhere to visiting insects. Subsequent trials at

[61] Castilho RC et al. 2009. The predatory mite *Stratiolaelaps scimitus* as a control agent of the fungus gnat *Bradysia matogrossensis* in commercial production of the mushroom *Agaricus bisporus*. Int. J. Pest Mgmnt. 55:181-185.

[62] Jess S, Schweizer H. 2009. Biological control of *Lycoriella ingenua* in commercial mushroom (*Agaricus bisporus*) cultivation: a comparison between *Hypoaspis miles* and *Steinernema feltiae*. Pest Mgmnt. Sci. 65:1195-1200.

[63] Nair NG, Clift AD. Integrated pest and disease management. Horticulture Australia Final Report MU002.

[64] White PF. 1985. The effect of sciarid larvae (*Lycoriella auripila*) on cropping of the cultivated mushroom (*Agaricus bisporus*). Ann. Appl. Biol. 109:11-17.

[65] Shamshad A, Clift AD, Mansfield S. 2009. Effect of compost and casing treatments of insecticides against the sciarid *Bradysia ocellaris* and on the total yield of cultivated mushrooms *Agaricus bisporus*. Pest Mgmnt. Sci. 65:375-380.

[66] Clift A, Shamshad A, Terras MA. 2004. Flies and dry bubble on cultivated mushrooms. Proc ISMS 16:459-464.

MLMRU established a clear link between spread of disease and introduction of the phorid fly *Megaselia halterata*.

- Female fungus gnats have been shown to be attracted to green mould (*Trichoderma aggressivum*), preferring infected compost to compost with *Agaricus mycelia* alone. This is consistent with the observation that fungus gnats also spread this disease around mushroom houses[67].
- Each mature female fungus gnat can lay around 10-15 separate sets of eggs, rapidly increasing populations of flies and leading to further spread of disease[68].
- Mushrooms are most at risk from fungus gnats between the end of Phase II composting and final harvest. There is some evidence that adult mushroom flies are attracted by the volatiles given off by compost in the cool down period after pasteurisation[69].
- To protect crops, growers may incorporate insecticides into compost and/or peat applied at casing. In many countries, diazinon is (or was) incorporated at compost manufacture to control fungus gnats. A dose of 200-500ppm in compost is sufficient to control phorids, while 1,000 to 1,500ppm may be needed to control sciarids[70]. However, as an organophosphate, diazinon is increasingly controlled. According to Jess[72], diazinon has been withdrawn from use in parts of Europe. He argues that it has in any case become ineffective due to development of resistance.
- A new (2017) report found that residues exceeded the MRL for diazinon when the product was applied at 1,000ppm (to control sciarids). Moreover, if insecticides are applied at casing, this can allow insect populations to develop, and damage mycelia, during the 14–19 days following pasteurisation. Residue levels declined slightly in second and third flushes[70].
- Organophosphates such as diazinon can potentially delay flushing and reduce yield by 4 to 14%[71], [72]. However research results on this effect are mixed, with some researchers finding no effect[70].
- The insecticides diflubenzuron and chlorpyrifos can also provide control, but may reduce mushroom yield by 20 to 69%[73].
- A 2009 study at the MLMRU by Shamshad et al[65] tested a number of different insecticides against fungus gnats. In this trial diazinon and fipronil both failed to protect against mushroom fly. Cyromazine incorporated into the compost provided some

[67] Cloonan KR, Andreadis SS, Baker TC. 2016. Attraction of female fungus gnats, Lycoriella ingenua, to mushroom-growing substrates and the green mold Trichoderma aggrassivum. Ent. Exp. Applicata. 159:298-304.

[68] Pyck N. 2015. Fungal diseases of mushrooms and their control. MushTV Factsheet 04/15. Ag Hort Dev Board. www.mushtv.eu

[69] O'Connor L, Keil CB. 2005. Mushroom host influence on *Lycoriella mali* life cycle. J. Econ. Entomol. 98:342-349.

[70] Navarro MJ, Merino L, Gea FJ. 2017. Evaluation of residue risk and toxicity of different treatments with diazinon insecticide applied to vegetable crops. J. Environ. Sci. Health. 52:218-221.

[71] Clift AD, Terras MA. 1991. Effects of pesticides on the yield and production patterns of three standard and six hybrid strains of cultivated mushrooms in New South Wales. Aust J. Exp. Agric. 31:427-430.

[72] Jess S, Kilpatrick M. 2000. An integrated approach to the control of *Lycoriella solani* during production of the cultivated mushroom (*Agaricus bisporus*). Pest Mgmnt Sci. 56:477-485.

[73] Brar DS, Sandhu GS. 1991. Effect of insecticidal incorporations on the growth and yield of white button mushroom. Proc. ISMS 13:477-486.

protection against flies until the first flush, but lost effectiveness in later flushes and gave no control in the casing layer. Triflumuron incorporated into the casing was most effective at reducing fly emergence from both compost and casing. The authors suggest incorporating 10mg.kg^{-1} cyromazine into compost and 250g.kg^{-1} triflumuron into casing material to provide full control of fungus gnats. It should be noted that although the insecticides reduced yield by approximately 10-15%, this difference was not statistically significant.

- The development of biological control agents for fungus gnats has been investigated over many years. Candidates include the predatory nematode *Steinernema feltiae*, predatory mites such as *Hypoaspsis miles* or *Stratiolaelaps scimitus* and the bacteria, *Bacillus thuringiensis* (Bt).
- A Chinese study found that Bt could reduce sciarid fly populations by 74 to 99%. However this work was done on a very small scale[74]. In contrast, Jess et al[72] found that Bt was ineffective against sciarids.
- The predatory mite *H. miles* was most effective when added at the end of Phase II composting (87% reduction in fly numbers) whereas nematodes (*S. feltiae*) were only effective when released after casing (82% reduction in fly numbers)[72]. Commercial scale trials confirmed that *H. miles* was the more efficient biocontrol agent. However, in a commercial situation it took 3 hours to disperse the mites compared to 15 minutes releasing nematodes, suggesting nematodes could be a more cost effective solution[62].
- Unlike the reported results with *H. miles*, *S. scimitus* was best at reducing numbers of *Bradysia matogrossensis* when released immediately after casing. This resulted in improved yield, with little benefit from a second release[75].

[74] YingChun S et al. 2014. Effectiveness of Bacillus thuringiensis microbial agents in controlling sciarid fly infestation in *Agaricus bisporus* cultivation rooms. Acta Edul. Fungi. 21:76-80.

[75] Castilho RC et al. 2009. The predatory mite *Stratiolaelaps scimitus* as a control agent of the fungus gnat *Bradysia matogrossensis* in commercial production of the mushoom *Agaricus bisporus*. Int. J. Pest Mgmnt. 55:181-185.

4.2 Fungal diseases

Key points

Dry bubble

Dry bubble disease can cause major losses, especially as it is resistant to a number of common fungicides. Newer products such as Vivando® may still be effective, and research has identified a strain of *Streptomyces bacteria* that may help manage this disease. Brewed 'teas' prepared from SMC have been shown to be effective against dry bubble, likely due to the microbial populations they contain. Results would seem likely to vary according to the substrate used. It is no longer recommended to remove infected material and/or treat with salt. Instead, infected mushrooms should be covered with sheets of thick, damp paper.

Wet bubble

Less common than dry bubble, this disease has not developed resistance to fungicides. It may also be controllable with essential oils.

Cobweb disease

Infections of cobweb can spread rapidly. Disturbing diseased areas, even simply through irrigation, releases masses of spores. These can travel through air-conditioning units and infect new areas. Cobweb is resistant to a number of fungicides, but can still be controlled with a combination of hygiene, fast response to infection, and selection of appropriate fungicides.

Green mould

Infection by green mould is initially difficult to detect but can cause total crop loss. Severity of loss is strongly affected by the amount of inoculum present. Recent work has shown that green mould spreads only small distances during Phase III composting. However, the process of moving bulk Phase III compost into growing rooms can spread the disease widely. Moreover, equipment used to handle infected Phase III compost readily cross-contaminates clean batches of material. Cleaning, disinfection and isolation of equipment and workers handling Phase III compost from other farm tasks are essential to prevent outbreaks. Green mould is susceptible to common fungicides. Artificial non-grain spawn may also help control this disease, as grain is very suitable nutrient source for *Trichoderma*.

Detection

New PCR methods can provide early warning of outbreaks as well as verify whether hygiene measures have been effective. Australia is a leader in this field, with a commercial product based on industry-funded research soon to be released. While the method currently indicates only presence or absence, in the future PCR may also be able to provide quantitative information.

4.2.1 Dry bubble disease – *Verticillium fungicola*

- Dry bubble disease can cause significant losses even at a relatively minor level of infection. Losses are strongly affected by the virulence of the strain as well as the rate and time of infection, ranging from negligible to 30%[76].
- Dry bubble can attack crops at any stage. Infection during early mushroom development results in deformed, puffball-like masses – bubbles – that consist of mycelia of *Agaricus* and *V. fungicola* growing together. Infection at later stages of cropping results in mushrooms with brown lesions or spots and/or blown out stipes, rendering them unmarketable[77].

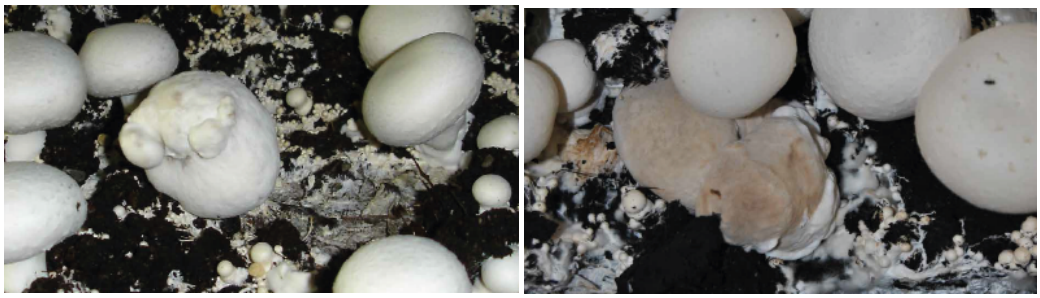

Figure 12. Dry bubble symptoms. From Pyck, 2015.

- Mushroom flies are attracted to infected mushrooms, and can spread the disease further as they move around the farm and between growing rooms[78].
- Sanitation and chemical fungicides can generally control the disease. In many countries, prochloraz has been the only effective fungicide to control dry bubble[79]. However, sensitivity to fungicides is highly isolate-dependent due to development of resistance. Resistance to prochloraz has been reported by several researchers[80], as has a lack of persistence in compost during later flushes[81].
- Shamshad[76] found that while one Australian isolate was effectively controlled by carbendazim and prochloraz Mn (Sporgon), another two isolates were resistant to carbendazim and poorly controlled by Sporgon. Allan et al[102] tested eight isolates of dry bubble for fungicide sensitivity in-vitro, and also found widespread resistance to carbendazim, with half of the isolates additionally resistant to prochloraz Mn. Imazilil remained effective against Australian isolates of dry bubble[82].

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[76] Shamshad A, Clift A, Butler R. 2008. Studies on dry bubble disease caused by *Verticillium fungicola*. Proc. ISMS 17:570-579.

[77] Grogan DG, Keeling C, Jukes AA. 2000. In vivo response of the mushroom pathogen *Verticillium fungicola* to prochloraz manganese. Proc. Brighton Crop Prot. Council. pp273-278.

[78] Pyck N. 2015. Fungal diseases of mushrooms and their control. MushTV Factsheet 04/15. Ag Hort Dev Board. www.mushtv.eu

[79] Largeteau ML, Savoie J-M. 2010. Microbially induced diseases of Agaricus bisporus: biochemical mechanisms and impact on commercial mushroom production. Ann. Appl. Biotechnol. 86:63-73.

[80] Gea FJ. Et al. 2005. Reduced sensitivity of the mushroom pathogen Verticillium fungicola to prochloraz-manganese in vitro. Mycol. Res. 109:741-745.

[81] Grogan HM, Jukes AA. 2003. Persistence of the fungicides thiabendazole carbendazim and prochloraz-Mn in mushroom casing soil. Pest Mgmnt. Sci. 59:1225-1231.

[82] Shamshad A, Clift AD, Mansfield S. 2009. Imazilil, manganese prochloraz and carbendazim treatments do not affect yield of *Agaricus bisporus*, hybrid strain Sylvan A15 in New South Wales. Plant Prot. Q. 24:50-54.

- Use of fungicides while cultivating Agaricus can reduce yield and result in undesirable residues in the harvested product. For example, Navarro et al[83] found that iprodione significantly reduced yield (approx. 15%). The same study also found that treatment with 0.1% iprodione, carbendazim and thiophanate-methyl all resulted in residues exceeding the MRL in first flush mushrooms, especially when fungicides were applied at the second irrigation time.
- Several papers have been published on using 'tea' made with spent mushroom compost to manage dry bubble disease. Gea et al[84] found the best results using a 'tea' brewed using a mixture of SMC and Topterra® peat casing. The SMC was heat treated at 70°C for 12 hours then composted for 57 days before preparation. It was then mixed 1:4 with water and left for 24 hours at 20°C. The liquid was strained and watered onto the mushroom beds. In this case, the 'tea' was more effective than prochloraz in reducing the number of infected mushrooms.
- This result was confirmed in a subsequent publication. It was also shown that aeration of the 'tea' (or not) made no difference to its effectiveness. Large microbial populations were found in the liquid, including bacteria (1.1 x10^7 cfu.ml^{-1}), Pseudomonads (9.8 x10^6 cfu.ml^{-1}), fungi (6.8 to 9.3 x10^6 cfu.ml^{-1}) and Actinomycetes (2.2 to 6 x10^6 cfu.ml^{-1})[85].

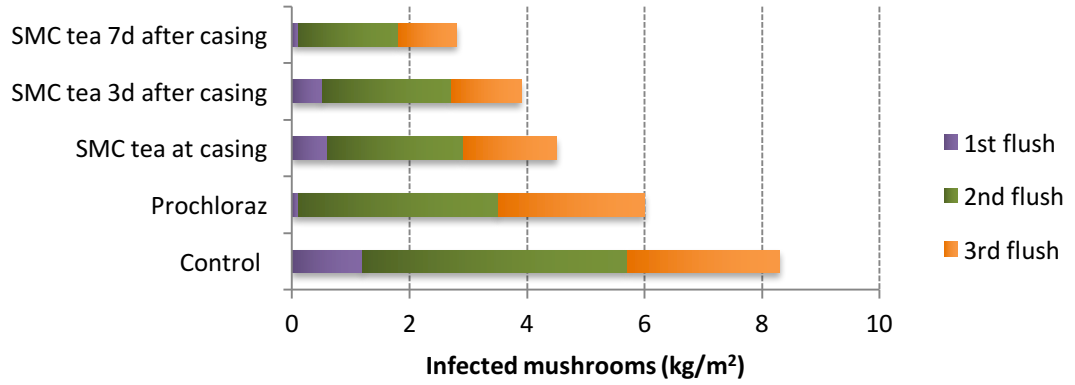

Figure 13. Effect of prochloraz or a 'tea' prepared from SMC on infection levels in mushrooms artificially infected with dry bubble disease. The tea was applied 0, 3 or 7 days after casing. Derived from Gea et al. 2011.

- The new fungicide metrafenone (Vivando®) was recently tested for efficacy against dry bubble. Metrofenone is one of the pyrimidine group of fungicides, which are based on naturally occurring plant compounds such as Vitamin B. Metrofenone was as effective as prochloraz Mn (Sporgon). Perhaps surprisingly, application also resulted in a statistically significant increase in yield[86].

[83] Navarro MJ et al. 2011. Toxicity of compost tea from spent mushroom substrate and several fungicides towards *Agaricus bisporus*.

[84] Gea FJ et al. 2011. Effectiveness of compost tea from spent mushroom substrate on dry bubble (*Lecanicillium fungicola*). Proc. 7th Int. Conf. Mushroom Biol. Mushroom Prod.

[85] Gea FJ et al. 2014. Control of dry bubble disease (*Lecanicillium fungicola*) in button mushroom (*Agaricus bisporus*) by spent mushroom substrate tea. Eur. J. Plant Pathol. 138:711-720.

[86] Pyck N. et al. 2016. Evaluation of metrafenone against *Verticillium* and *Cladobotryum* spp. – causal agents of dry bubble and cobweb disease. Proc. ISMS. 17:82-85

- Beyer et al[87] tested a number of bio-fungicides and a bio-control agent for control of dry bubble, comparing the percentage of infected mushrooms against those protected using a commercial fungicide treatment of thiabendazole (7 days after casing) plus chlorothalonil (9 days after casing). None of the plant-based bio-fungicides were effective, however the bacteria *Streptomyces griseoviridis* controlled dry bubble as well as the chemical control. The bacterial strain was most effective when it was inoculated repeatedly 7, 9 and 15 days after casing.
- If localised outbreaks occur, Pyck[78] advises not to try to remove infected material, as this risks spreading the spores further. In the past salt and inverted plastic containers were used to contain spread. It is now recommended to cover the area with layers of pre-dampened, strong paper tissue. This should extend at least 5cm past the infected zone. If there are developing mushrooms within this area then the stipes should be broken to stop them growing.

4.2.2 Wet bubble – *Mycogone perniciosa*

- Symptoms of wet bubble include swollen stipes, deformed caps, and development of irregular masses of white tissue. Droplets of light brown fluid can appear on the growths, with the tissue becoming soft and rotten[78].
- Fewer research papers focus on wet bubble than dry bubble, perhaps reflecting that this disease is less frequently a major problem. However, sporadic outbreaks do occur, and it is also possible that symptoms of wet bubble can be masked by, or misidentified as, dry bubble[88].
- Wet bubble produces two types of spores; tough walled chlamydospores and small, airborne spores. Infection is usually associated with contaminated compost or spawn, as the chlamydospores can survive in soil or debris[78]. The smaller spores can spread through air conditioning systems, creating secondary infections[89].
- The disease is highly fungicide sensitive, so can be effectively controlled by carbendazim or prochloraz-Mn. It is considered unlikely that the disease will develop resistance to either of these fungicides[88].
- Regnier and Combrinck[89] screened a number of essential oils, and pure compounds derived from those oils, for activity against wet bubble. While many of the oils tested were toxic to both *Agaricus* and *Mycogone*, thymol and nerol (from thyme and lemon verbena) applied at 40µl.L^{-1} inhibited wet bubble without affecting mushroom yield. Potocnik et al[90] also found that thyme oil was highly inhibitory against the disease. While these are promising results, it should be noted that much of this work has been on a small scale and needs further replication.

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[87] Beyer DM, Pecchia JA, Paley K. 2016. Evaluation of bio-fungicides for the control of fungal diseases on *Agaricus bisporus*. Proc. ISMS. 17:86-90.

[88] Gea FJ, Tello JC, Navarro M-J. 2010. Efficacy and effects on yield of different fungicides for control of wet buble disease of mushroom caused by the mycoparasite *Mycogone perniciosa*. Crop Prot. 29:1021-1025.

[89] Regnier T, Combrinck S. 2010. In vitro and in vivo screening of essential oils for the control of wet bubble disease of *Agaricus bisporus*. S. African J. Bot. 76:681-685.

[90] Potocnik I et al. 2010. Sensitivity of Mycogone perniciosa, pathogen of culinary-medicinal button mushroom *Agaricus bisporus* to selected fungicides and essential oils. Int. J. Medicinal Mushrooms. 12:91-98.

4.2.3 Cobweb disease – *Cladobotrium dendroides, C. mycophilum*

- Cobweb is one of the most serious diseases of *Agaricus* worldwide. Fluffy white mycelia grow over the casing layer, rotting the underlying pins and causing spotting on developed mushrooms. It grows at least 2cm.day^{-1}, so a small patch can rapidly engulf the mushrooms around it[78].
- Losses from cobweb are highest if infection occurs at pinning, with infection between spawning and casing having less effect[91]. If colonies are disturbed (eg by watering) they can release masses of dry, powdery spores, which quickly spread through air-conditioning systems and form secondary colonies[92].
- Symptoms usually appear at or after second flush, with the majority of loss occurring in second flush mushrooms. Symptoms can occur earlier if infection is widespread, in which case losses in second and third flush will be particularly severe.

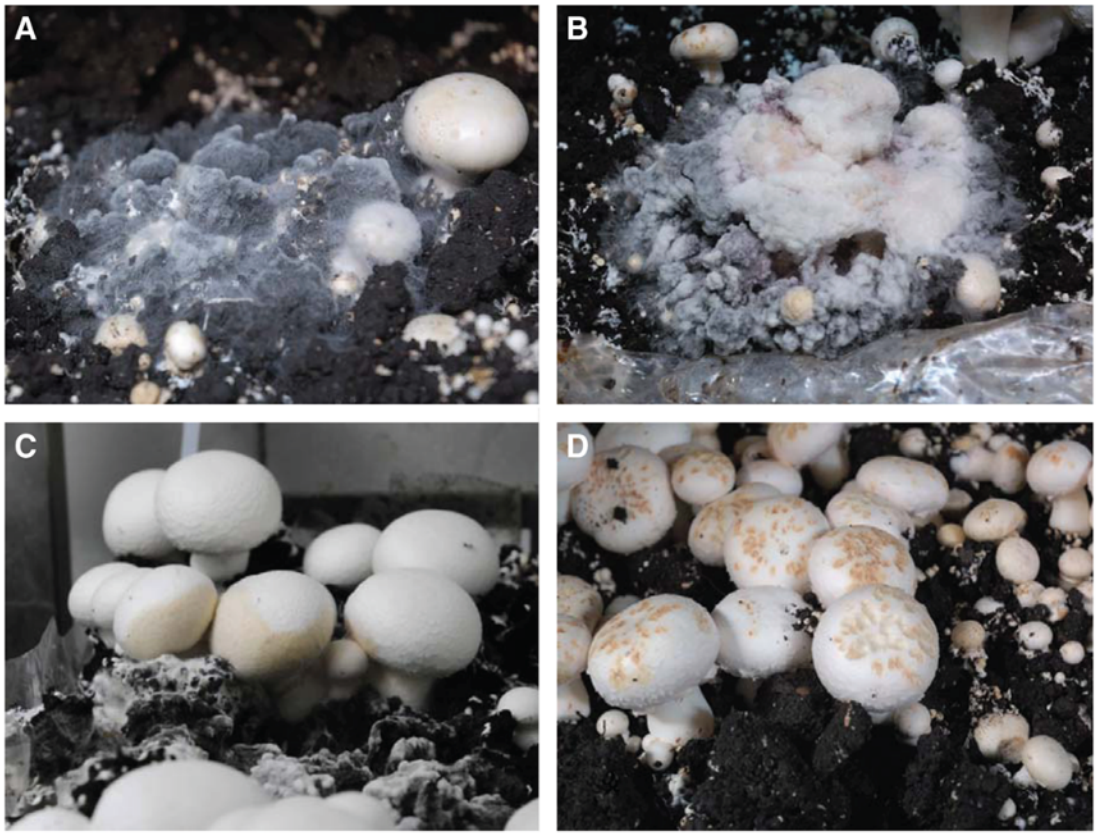

Figure 14. Cobweb mycelia on the casing surface and attacking mushrooms (A, B). The disease discolours mushrooms as it colonises their surfaces (C) and can also cause cap spotting (D). From Carrasco et al, 2015.

- Although Largeteau and Savoie[79] state that cobweb disease is rarely epidemic although common, serious infections can occur. For example, in the mid 1990's widespread

[91] Jandaik S, Guleria DS, Parmar YS. 2004. Effect of *Cladobotryum dendroides* on the yield of *Agaricus bisporus*: Inoculum factors and timing of infection. Proc. ISMS. WHAT??

cobweb infections caused up to 40% losses in UK and Ireland[92]. Similarly, although cobweb was always present, it only began causing major problems in Spain in 2010. Decreases in yield can reach 36%, with around 32% of all crops affected in some way[93].

- Cobweb disease became a problem in Australia in 1999-2000. At this time thiabendizole, benomyl and prochloraz manganese were all registered for use on mushrooms. Development of fungicide resistance, especially to TBZ, was one reason for the sudden epidemic. Poor hygiene and slow responses to outbreaks of disease were also key reasons cobweb developed into a serious problem[94].
- Pyck et al[86] found that prochloraz Mn (Sporgon) applied at 3g.m^2 casing and metrofenone (Vivando) applied at either 150ml.m^2 or 1L.m^2 at aeration all gave equally good control of cobweb disease. It is interesting to note that there was no difference between the two rates of application of Vivando, even though the more dilute rate was well below the manufacturers recommendation.
- Australian work with commercial producers found that cobweb could be controlled adequately by a combination of hygiene, fast response to infection, and selection of appropriate fungicides[94].
- As with dry bubble, it is no longer recommended to use salt on infected areas. Instead, they should be well covered with pre-wetted sheets of strong tissue paper to at least 5cm past the edge of the infected area[78].

4.2.4 Green mould – *Trichoderma sp.*

- Green mould has been a major disease affecting mushroom production since the mid 1980's. Yield loss is closely related to the amount of inoculum present and the degree to which it is mixed into the compost[95].
- Initially, green mould affected Phase II compost used in-situ in mushroom growing houses. However, in 2006 it reached the technologically advanced bulk spawn run systems in the Dutch industry, resulting in multi-million-Euro losses[96]. The strain responsible was identified as *T. aggressivum f. europaeum*.
- *Trichoderma* species produce whitish mycelia that are indistinguishable from *Agaricus* during early growth. As it develops, and often in response to light, the fungus starts to

[92] Adie B. et al. 2006. Temporal and spatial dispersal of Cladobotryum conidia in the controlled environment of a mushroom growing room. Appl. Environ. Microbiol. 72:7212-7217.

[93] Carrasco J et al. 2015. Incidence, identification and pathogenicity of *Cladobotryum mycophilum*, causal agent of cobweb disease on *Agaricus bisporus* mushroom crops in Spain. Ann. Appl. Biol. 168:214-224.

[94] Fletcher JT, Allan J, Seymour GK. 2004. Managing cobweb disease in Australia. Proc. ISMS 16:711-716.

[95] O'Brien M, Kavanagh K, Grogan H. 2017. Detection of *Trichoderma aggressivum* in bulk Phase III substrate and the effect of T. aggressivum inoculum supplementation and substrate mixing on *Agaricus bisporus* yields. Euro. J. Plant Path. 147:199-209.

[96] Hermans C. 2006. Triggers of *Trichoderma* and smokey mould. Mushroom Bus. 19:8-9.

produce spores. These are also initially white, but turn green on maturity. Over a few days masses of dark green spores can appear in the compost or casing layer[97].

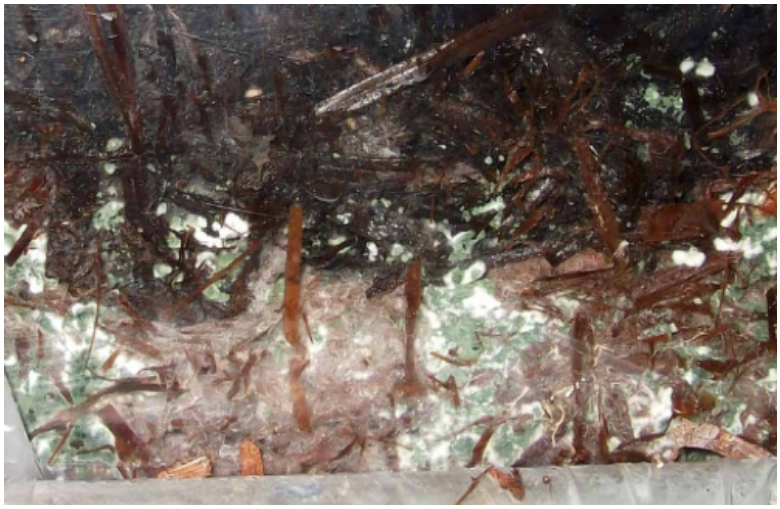

Figure 15. Early symptoms of green mould infection - white strappy growth. From Kilpatrick, 2015.

- Once compost is fully colonised by Agaricus mycelia it is considered to be less susceptible to green mould infection. Also, as there is only limited access to the substrate after spawning, infections tend to be limited to the edges of the compost block or bag[98].
- In bulk spawn run systems, large volumes of air are re-circulated through compost, suggesting that a single source of green mould could potentially infect the entire batch. However, this does not occur. Different growers getting compost from the same tunnel have reported widely different infection levels, ranging from zero to total crop loss[99].
- Recent work by Kilpatrick et al[100] has shown that Trichoderma spreads only 0.5-1.0m through compost during a 17 day bulk spawn run, despite aeration. This is because it does not produce spores under these conditions. The results also indicate the infection moves upwards through the stack, rather than along. At this stage the fungus is not readily visible and growth of Agaricus mycelia is only slightly retarded at close to the original infection point.
- Reductions in yield in the subsequent crop depend on where the compost was in the stack during spawn run. Compost closest to the inoculation point suffered a complete loss of yield, whereas that only 0.6m along the stack had negligible loss. Compost above the inoculation point had either 24% or 42% yield loss, depending on proximity to the original infection.

[97] Kilpatrick M. 2015. Understanding *Trichoderma aggressivum* in bulk Phase III compost. MushTV Factsheet 03/15. Ag. Hort. Dev. Board. www.mushtv.eu

[98] Fletcher JT. 1997. Mushroom spawn and the development of *Trichoderma harzianum* compost mould. Mushroom News 45:6-8.

[99] Lemmers G. 2010. Trichoderma in bulk Phase 3. Mushroom Bus. 40:10-13.

[100] Kilpatrick M et al. 2016. Growth, dispersal and impact on yield of *Agaricus bisporus* by *Trichoderma aggressivum* during spawn run. Proc. ISMS 17:70-74.

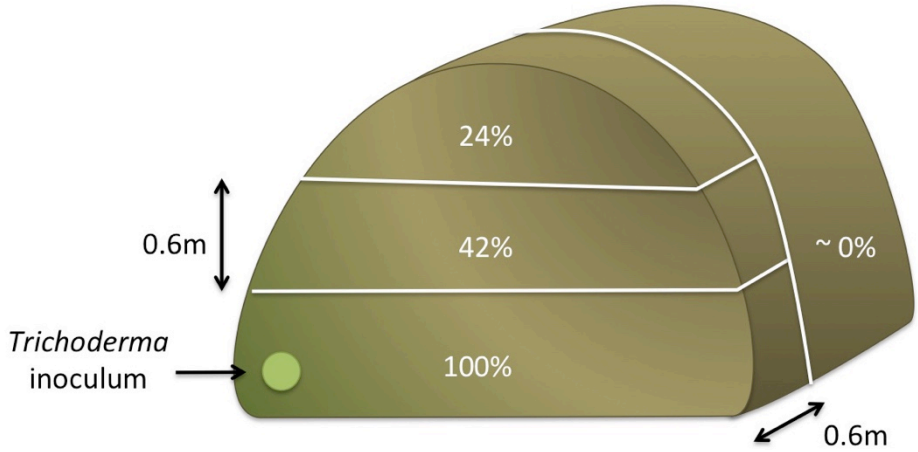

Figure 16. Crop yield loss in relation to the composts's proximity to a *Trichoderma* infection point during spawn run. Compost adjacent to the infection point yielded no mushrooms, whereas compost taken from 0.6m along the tunnel had negligible yield loss. Yield from compost above the inoculation point depended on proximity to the original infection. Derived from Kilpatrick et al. 2016.

- The same work[100] also demonstrated that equipment used to move infected compost can transfer infection to clean compost. Yield reductions of 26% to 100% occurred when clean compost was moved with contaminated conveyor equipment. The authors suggest that serious outbreaks of green mould are not due to growth within the compost during spawn run, but rather the result of bulk handling operations that spread small infections through much larger masses of compost.
- This result was confirmed by O'Brien et al[95], who showed that thorough mixing – as occurs when bulk Phase III compost is installed in mushroom growing rooms – results in heavier infection than if material is only lightly mixed – as would occur if spawn run is done within the growing room.

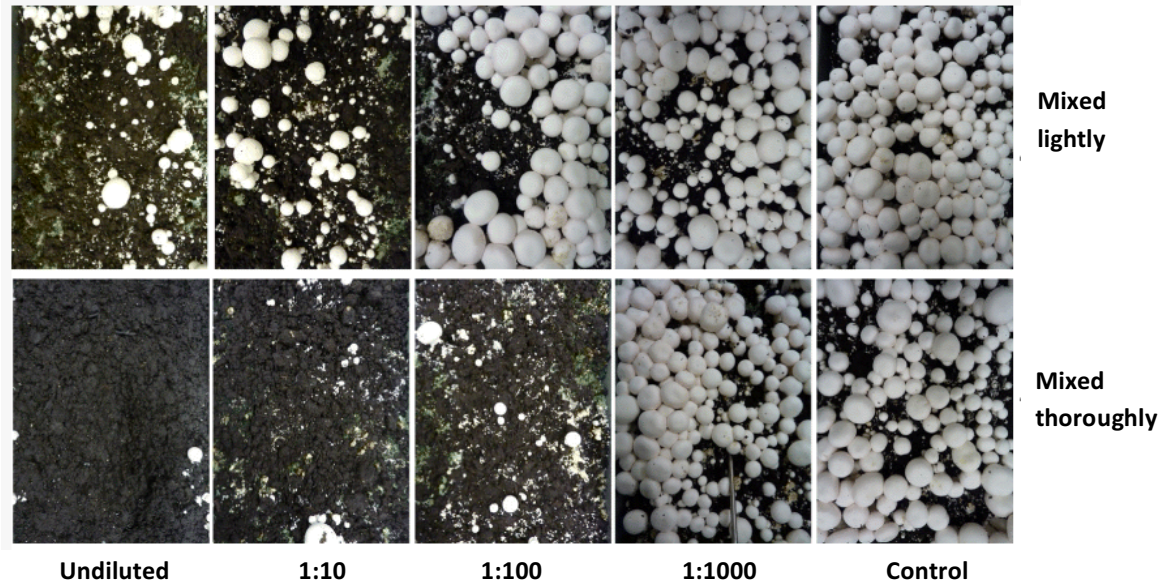

Figure 17. Effect of *Trichoderma* inoculation on mushroom growth. The inoculum was applied undiluted, or diluted 10x, 100x or 1000x then either lightly mixed or thoroughly mixed into the substrate. From O'Brien et al. 2017.

- The risk of infection with *Trichoderma* can be reduced by strict hygiene, particularly in relation to handling equipment. It is suggested that Phase III composting tunnels should be emptied and filled from different ends, using dedicated equipment for each activity. Compost debris must be removed and tunnels pasteurised between uses. The same requirements apply to haulage companies and growing facilities, with strict hygiene, thorough cleaning and use of dedicated equipment used for filling Phase III compost essential elements for control[97].
- Detection of green mould infection during spawn run could prevent infected material being moved or used. Baars et al[101] found that volatiles produced by infected Phase III compost differ from those produced during normal colonisation by *Agaricus*. It is suggested that testing the airspace inside composting facilities could provide early detection. However, it is not clear whether this method is sufficiently sensitive to detect small infections inside large chambers.
- Allan et al[102] conducted in-vitro testing of nine Australian *Trichoderma* isolates for fungicide sensitivity. All were controlled by 20ppm carbendazim, and only one showed slight resistance to prochloraz-Mn.
- A number of plant essential oils have been evaluated for activity against green mould. Oil from thyme and oregano as well as menthol have been shown to be active[103]. However, this testing was conduced in-vitro, and results are likely to be different in the complex microbial environment of compost[79].
- Serbian research has focussed on identifying biocontrol agents. A number of bacterial strains of *Bacillus subtilis, B amyloliquefaciens, B. licheniformis* and *B. pumilis* have been identified which inhibit the growth of *Trichoderma* species, particularly *T. aggressivum* f. *europaeum*[104].
- *B. subtilis* has been of particular interest. The commercial product Serenade® which consists of *B. subtilis* and its lipopeptides, has been approved for use against green mould in Europe[79]. However, although Serenade® reduces Trichoderma, it is not as effective as prochloraz-Mn[105].
- Beyer et al[87] also tested *B. subtilis*, as well as *Streptomyces griseoviridis*. Both bacteria were ineffective at controlling green mould.
- The degree to which *Trichoderma* develops on mushroom grain spawn has been reported as positively correlated with disease severity. The study by Beyer et al[87] showed that green mould failed to develop synthetic, non-grain spawn was used prior to

[101] Baars J, Rutjens J, Mumm R. 2011. Can volatiles emitted by compost during spawn run be used to detect green mould infection early? Proc. 7th Int Conf. on Mushroom Biol. Mushroom Prod. Vol 1. pp469-478.

[102] Allan J, Shah FA, Khan I. 2008. Establishing a baseline for fungicide sensitivity of three major mushroom pathogens in Australia. Proc. ISMS 17:565-569.

[103] Sokovic M, Van Griensven LJLD. 2006. Antimicrobial activity of essential oils and the components against three major pathogens of the cultivated mushroom *Agaricus bisporus*. Eur. J. Plant Path. 116:211-224.

[104] Stanojevic O. et al. 2016. Isolation and identification of Bacillus spp. from compost material, compost and mushroom casing soil active against *Trichoderma* spp. Archives of Biol. Sci. 68:845-852.

[105] Kosanovic D. et al. 2013. Trichoderma species on *Agaricus bisporus* farms in Serbia and their biocontrol. Ann. Appl. Biol. 163:218-230.

inoculation of green mould. This suggests that the grain used in mushroom spawn may be a particularly good host for *Trichoderma*, possibly giving it a 'boost' in the early stages of growth.

- Disease surveys are a useful way for growers to examine their processes and detect where infections may come from. Surveys in Ireland have shown that even farms with good hygiene can fail to eliminate green mould, this pathogen being particularly hard to kill. However, targeted hygiene and effective cook-outs can eliminate this disease[106].

4.2.5 Detecting fungal pathogens

- Early detection is key to controlling microbial pests of mushrooms. Only through early detection can outbreaks be contained, preventing them from spreading to other parts of a growing room, or even the whole farm.
- Much recent international research has examined the use of polymerase chain reaction (PCR) techniques to detect pathogens. PCR is fast, sensitive and pest specific. It has been demonstrated to provide more reliable results than plating, weed mould analysis and most probable number analysis[95].
- Rossouw et al[107] describe a quantitative PCR method in the form of direct droplet PCR for accurate detection of pathogens. The method can currently confirm presence or absence of green mould, dry bubble, wet bubble and cobweb. The aim is to further refine the method to provide quantitative information.
- In Australia, current research funded by the industry has developed multiplex tandem PCR-based methods to detect cobweb, dry bubble and green mould. Swabs taken from areas most likely to come into contact with spores (ladders, gloves, door handles etc) can be tested simultaneously for a range of pathogens. The method is being commercialised through AusDiagnostics, which will develop a fully commercial system for up to eight different pathogens[108]. As well as providing early warning of outbreaks, the method can also be used to verify that clean-up procedures have been correctly conducted.

[106] Fleming-Archibald C et al. 2016. Identifying *Trichoderma aggressivum* within the European mushroom industry. Proc. ISMS 19:65-69.

[107] Rossouw W, Duvenage S, Korsten L. 2016. Mushroom disease detection, surveillance and farm health. Proc. ISMS 19:91-95.

[108] Smith L, Stanley K. 2016. Multiplexed detection and relative quantitation of bacterial and fungal pathogens of mushrooms. Proc. ISMS 19:100-103.

4.3 Bacterial pathogens

Key points

Bacterial blotch is the main bacterial disease affecting mushrooms, with *Pseudomonas tolaasii* the usual cause. While washing mushrooms can increase the disease, antimicrobial washes can have the opposite effect and reduce bacterial blotch. Bacteriophages and antagonistic bacteria have been investigated in small trials with some promising results. Vacuum cooling can help reduce bacterial blotch postharvest.

- *Agaricus* can be affected by a number of different bacterial pathogens, including soft rot (*Burkholderia gladioli* pv. *agaricicola* and *Janthinobacterium agaricidamnosum* sp) and cavity disease (*B*. *gladioli*). The main bacterial disease affecting mushrooms is bacterial blotch. The disease can be caused by several bacterial pathogens, including *Pseudomonas tolaasii*, *P. reactans* and *P. costantinii*[79].
- Of these, *P. tolaasii* is the most common cause of bacterial blotch symptoms. The bacteria is endemic in compost and casing soil, switching between pathogenic and non-pathogenic forms in response to environmental stimuli that include temperature and humidity[109].
- While bacterial blotch can occasionally devastate crops pre-harvest, it is most commonly becomes a major problem during postharvest storage[110]. Toxins released by the bacteria (tolaasin by *P. tolaasii*) disrupt the structure of the mushroom cell membranes, triggering the appearance of yellow or brown lesions on the mushroom cap[111].
- Chlorination of irrigation water with sodium hypochlorite (bleach) was once widely practiced, but generally ineffective[112]. A wide variety of other methods of protecting mushrooms from bacterial blotch have been proposed, for both pre-harvest and postharvest application. Examples include;
    - In-vitro tests were conducted using strains of *Streptomyces* bacteria, known for their antibiotic effects. A bioactive compound was extracted which was structurally related to Penicillins[112]. Given the importance of *Streptomyces* and current concern about antibiotic resistance, this approach would seem unlikely to gain support.
    - Twenty two essential oils were evaluated for control of *P. tolaasii*, as well as other bacterial pathogens. *P. tolaasii* was the hardest to control, with only wintergreen oil (mostly methyl salicylate) providing any in-vitro effect[113].

[109] Soler-Rivas C. et al. 1999. Biochemical and physiological aspects of brown blotch disease of *Agaricus bisporus*. FEMS Microbiol. Rev. 23:591-614.

[110] Wells JM. Et al. 1996. Postharvest discolouration of the cultivated mushroom *Agaricus bisporus* caused by *Pseudomonas tolaasii*, *P. reactans* and *P. gingeri*. Phytopath. 86:1098-1105.

[111] Nguyen HTD et al. 2012. Characterization of bacteriophage ΦPto-bp6g, a novel phage that lyses *Pseudomonas tolaasii* causing brown blotch disease in mushrooms. J. Micro. Methods. 91:514-519.

[112] Sahin N. 2005. Antimicrobial activity of Streptomyces species against mushroom bacterial blotch pathogen. J. Basic Microbiol. 45:64-71.

[113] Todorovic B et al. 2016. Toxicity of twenty-two plant essential oils against pathogenic bacteria of vegetables and mushrooms. J. Environ. Sci. Health. 51:832-839.

- Application of antagonistic bacteria, including *Pseudomonas putida* and *P. fluorescens*. These bacteria reduced disease incidence in inoculated beds of mushrooms from approximately 90% to 12 – 25%[114].
- Soler-Rivas et al[115] reported that an extract from *Pseudomonas reactans* reduced the symptoms of brown blotch infection by 50% in inoculated mushrooms, mainly due to inhibition of browning.
- Bacteriophages can destroy host bacteria and multiply rapidly under suitable conditions. Nguyen et al[111] isolated 21 phages that infect *P. tolaasii*. Small scale laboratory tests indicated that a selected phage was highly effective against brown blotch, however larger scale testing is needed.
- A similar approach was taken by Saxon et al[116], who found that *P. tolaasii* is susceptible to attack by the δ-proteobacterium *Bdellovibrio bacteriovorus*. *B. bacteriovorus* invades the larger bacterium and replicates inside it. While this study was small, the results are promising. *B. bacteriovorus* is a natural soil dweller, so could potentially be added to casing material.

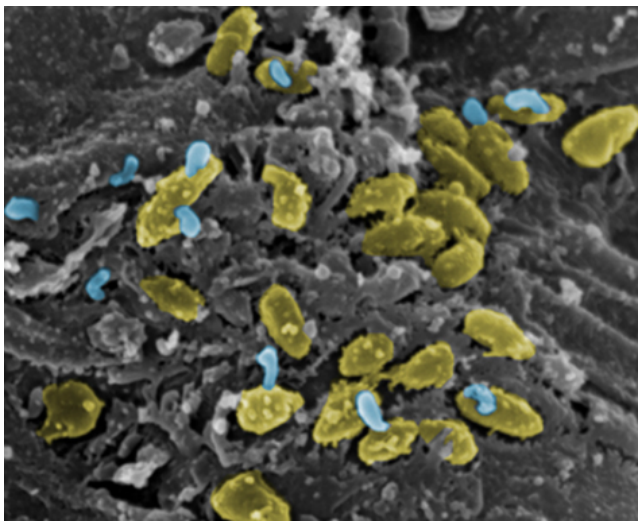

Figure 18. Electron microscope images showing attachment of *Bdellovibrio bacteriovorus* (blue) to *P. tolaasii* (yellow) on the surface of a mushroom cap. From Saxon et al, 2014.

- Postharvest incidence of bacterial blotch is increased if mushrooms are washed to remove casing soil. In some countries, mushrooms are commonly washed before slicing[117]. A number of research papers have investigated using anti-microbial washes such as citric acid or chlorine compounds to reduce populations of bacteria on mushrooms, thereby preventing or delaying appearance of brown blotch. These are discussed further in section 5.4 on postharvest washing treatments.

[114] Tajalipour S. et al. 2014. Biological control of mushroom brown blotch disease using antagonistic bacteria. Biocontrol Sci. Tech. 24:473-484.

[115] Soler-Rivas C. et al. 1999. WLIP, a lipopeptide of Pseudomonas 'reactans', as inhibitor of the symptoms of brown blotch disease of *Agaricus bisporus*. J. Appl. Microbiol. 86:635-641.

[116] Saxon EB et al. 2014. Bdellovibrio bacteriovorus HD100 guards against Pseudomonas tolaasii brown-blotch lesions on the surface of post-harvest *Agaricus bisporus* supermarket mushrooms. BMC Microbiol. 14:163

[117] Simon A, Gonzalez-Fandos E. 2009. Effect of wahsing with citric acid and antioxidants on the colour and microbiological quality of whole mushrooms (*Agaricus bisporus*). Food Sci. biotech. 44:2500-2504.

- After harvest, the best way to control bacterial blotch is to keep mushrooms cold and dry. Vacuum cooling effectively dries the surface of mushrooms compared to room cooling, and has been demonstrated to reduce subsequent growth of *P. tolaasii*[118].

4.4 Viruses

Key points

Far fewer published papers are concerned with virus diseases than other pathogens of mushrooms. In part this is because viruses are difficult to study and their symptoms are highly variable, but also because their pest status is relatively recent. As viruses are carried within mushroom mycelia they cannot be destroyed with disinfectants. The only control method is hygiene, particularly cook-out and thorough removal of organic materials.

- While a number of viruses can infect mushrooms, only a very few cause symptoms of disease. The most important of these is mushroom virus X (MVX). First reported in 1996, this has presented increasing problems over the last 10 years. The main symptom is cap browning, which can affect anything from a few mushrooms to up to 80% of a flush[119]. Symptoms also vary between flushes, with first flush mushrooms the most seriously affected[120].
- Other symptoms include barren patches, premature opening and distorted shape. Symptoms may worsen after harvest, particularly browning of the mushroom caps[121].

Figure 19. Brown cap disease of mushrooms, associated with mushroom virus X. From Fleming-Archibald et al, 2015.

- Viruses can exist within Agaricus at two levels, analagous to a transition in the virus 'life-cycle'. Symptoms may be very mild and persistent or acute. The adoption of bulk Phase III composting is thought to have triggered the transition of the virus into its acute strategy, instead of low-level persistence, possibly due to the mixing process and

[118] Varszegi T. Bacterial growth on the cap surface of *Agaricus bisporus*. ActaHort. 599:705-710.

[119] Fleming-Archibald C, Burton K, Grogan H. 2015. Brown cap mushroom virus (associated with mushroom virus X) prevention. MushTV Factsheet 02/15. Ag. Hort. Dev. Board. www.mushtv.eu.

[120] Fleming-Archibald C, Ruggiero A, Grogan HM. 2015. Brown mushroom symptom expression following infection of an *Agaricus bisporus* crop with MVX associated dsRNAs. Fungal biol. 119:1237-1245.

[121] Rao JR, Nelson DWA, McClean S. 2007. The enigma of double-stranded RNA (dsRNA) associated with mushroom virus X (MVX). Curr. Issues Mol. Biol. 9:103-121.

breakage of mycelia that occurs when transferring Phase III compost to growing houses[122].

- Detection of virus infection is difficult. Objective colour meters can detect subtle differences in cap colour that are not readily apparent to the human eye[122]. The virus can also be confirmed by the presence of four low molecular weight dsRNAs, which are detected using PCR techniques[120].
- The main source of virus infection is infected mycelium. If spent casing and compost are effectively cooked out then the mushroom mycelium – which carry the virus – will be killed, stopping infection spreading. The only way to control virus diseases is therefore through strict hygiene – cleaning and disinfecting all areas including removal of compost, casing and mushroom wastes[119].

[122] Eastwood D. et al. 2015. Viral agents causing brown cap disease of *Agaricus bisporus*. Appl. Env. Microbiol. 81:7125-7134.

4.5 Hygiene

Key points

It has been shown that microbes can persist even on farms with good hygiene practices. No disinfectants can kill pathogens in mushroom compost. Cook-out is the best way to kill microbes infecting growing rooms. As many disinfectants are neutralised on contact with organic materials it is essential to clean thoroughly before disinfecting surfaces. Disinfectants vary in the microbes they control and the contact time required. Disease surveys can indicate which hygiene procedures are working and where they need to be improved.

- Correct hygiene is the cornerstone of management of pests and diseases during mushroom production. It is therefore essential to use disinfectants that are effective against pathogens.
- The microbes that cause dry bubble, wet bubble and cobweb diseases can survive on surfaces or in debris for weeks or months. The aim of disinfection is to reduce the population to a level that no longer poses a threat to crop production. The best way to kill all pathogenic microbes is by steam cook-out at 65-70°C for 8 hours[123].
- Areas such as growing rooms, floors, equipment and picking aids should be cleaned of debris before disinfection. Disinfectants react with organic materials, so are ineffective if mushrooms, peat or compost is present[123].
- Dutch and Belgian growers have started to experiment with industrial foam based detergents for cleaning. These are ideally applied with a high-pressure supply of water and air. This equipment is expensive, however, and cheaper solutions have had mixed success[124].
- Disinfectants clearly need to be effective. However, other issues to consider include corrosiveness, application method, user safety, cost and the risk of accumulating residues that may contaminate mushroom crops. Based on this criteria, Baars[124] tested electrochemically activated water (ECAS), hypochlorite and a peractic acid + hydrogen peroxide mix under semi-commercial conditions. The ECAS was not effective, whereas the other two products worked well. Hypochlorite residues generally disappeared in less than an hour. Residues of peracetic acid on rubber surfaces were the slowest to degrade, but even these were undetectable within 4 hours[123].
- Gaseous ozone was tested for use in disinfecting rooms. Not only was it ineffective, it actually appeared to stimulate spore germination[124]!
- The MushTV project factsheet summarises the key features of a number of classes of disinfectants and is an excellent guide to on-farm hygiene (Table 2).

[123] O'Neill T, Lole M, Drakes D. 2015. Use of chemical disinfectants in mushroom production. MushTV Factsheet 01/15. Ag Hort Dev. Board. www.mushtv.eu.

[124] Baars J, Rutjens J. 2016. Finding a suitable biocide for use in the mushroom industry. Proc ISMS 19:114-117.

Table 2. Key features of different types of disinfectants. Derived from O'Neill et al, 2015.

Compound	Advantages	Disadvantages
Quaternary ammonium – cationic surfactants	Non corrosive and non irritating. Good penetration when combined with wetting agent.	Tend to foam and leave residue. More effective on bacteria. May have little to no effect on fungi. Inactivated by organic matter, oils and waxes. Expensive.
Chlorine based – halogens and halogen releasing	Inexpensive. Broad spectrum activity against viruses, bacteria, yeasts, fungi. Effective at cool temperatures.	Less effective against resting spores. Short residual effect. May be very corrosive. Inactivated by organic matter.
Peroxides – oxidising agents	Broad spectrum activity against viruses, bacteria and fungi including spores. Effective at cool temperatures and in the presence of organic materials. Environmentally benign.	May be corrosive. Most have short residual effect (some products contain silver, claimed to provide residual activity).
Phenols – high boiling point tar acids (HBTA)	Broad spectrum activity against viruses, bacteria, fungi and mycoplasmas. Effective at cool temperatures and in the presence of organic materials.	May be irritating (acidic). Corrosive to plastic and rubber. Strong odours. Risk of taint and residue if used on crop growing areas.
Phenols – synthetics.	Neutral pH, less odour and staining than HBTAs. Relatively broad spectrum.	Not as broad in activity as HBTAs. Questionable environmental safety. Risk of taint if used on crop growing areas.
Aldehydes – reducing agents	Broad spectrum activity.	Need a long contact time. Activity is temperature dependent. Potential hazards to operators from exposure.

- The MushTV project also tested a wide range of disinfectants for activity against green mould. Whereas 15 minutes contact time was sufficient for all disinfectants (except ozone) to kill conidiospores, only some disinfectants were effective against mycelial fragments and none were effective against mycelia in compost. The best result was obtained by 60 minutes exposure time to Menno Clean, which reduced the population of mycelia in compost by over 50%[123].
- Approximately 60% of the South African mushroom industry use Des-O-Germ disinfectant. In vitro testing was used to demonstrate that this product provides 100% mortality of cobweb conidia, even when large amounts of the pathogen are present (9,000,000 conidia.ml-1), if used according to label directions. However, these tests were in-vitro, rather than using the types of conditions that would be encountered during normal cleaning routines[125].

[125] Chakwiyaa A, Van der Linde E, Korsten L. 2013. In vitro efficacy testing of a disinfectant against Hypomyces species conidia survival. The Spawn Run December 2013 pp 12-17.

5 Harvest and Postharvest

5.1 Harvest

Key points

Manual harvesting of mushrooms is a major cost to growers. While machine harvesting has had a long history of development, the complexity of machines and software required, difficulty in picking mushrooms that are angled or clumped and damage to the product has limited adoption. New, single level production facilities can apparently make manual picking easier and faster. However no research papers were found that examine this option.

- Despite major advances in the efficiency of materials handling and composting, harvesting is still conducted by hand. Harvesting is therefore a major cost of production for mushroom growers.
- Various research groups have attempted to develop mechanical harvesting systems for mushrooms. As long ago as 1972 Penn State University developed a prototype harvester for 1.2m wide trays. Mushrooms were cut close to the bed surface, picked up using air and accumulated via a belt conveyor. Although the machine could harvest 3m.min^{-1}, 25% of mushrooms were damaged and others were left on the bed. It is noted that new varieties and changed control of environmental conditions are necessary to facilitate mechanical harvesting[126].
- Machine vision now offers improved opportunities for machine harvesting, with equipment able to determine size and quality and adjust accordingly. A prototype machine described by Reed et al uses machine vision to find mushrooms and suction cups to extract then from compost, reducing damage to 15% of the total harvested[127].
- Continuing this work, preliminary trials of mechanical harvesting were carried out at commercial facilities. Mushrooms could only be machine picked if they were growing vertically and not overly 'clumped'. Pre-picking the beds to remove overlapping mushrooms increased the success rate of the machine from 67 or 76% to 88%. However, machine picking speed was only 9 mushrooms.min^{-1}, compared to 18 mushrooms.min^{-1} for manual picking[128].
- Mechanical harvesters have been developed commercially. They are mostly used for processing mushrooms and have limited capacity to serve the fresh market due to bruising damage caused by suction cups[129]. Cost and complexity are also assumed to be major barriers to adoption.
- Some new farms developed by the Christiaens Group are known to be changing to single layer production. Although this clearly uses far more space, it increases the efficiency of

[126] Persson SPE. 1972. Mechanical harvesting of mushrooms.

[127] Reed JN, He W, Crook S. 1995. Harvesting mushrooms by robot. Proc. ISMS 14:385-391.

[128] Reed JN et al. 2001. Automatic mushroom harvester development. J. Agric. Eng. Res. 78:15-23.

[129] Weijn A et al. 2012. A new method to apply and quantify bruising sensitivity of button mushrooms. LWT Food Sci. Tech. 47:308-3014.

harvest. However, no scientific literature quantifying this effect or supporting this development was found in the course of this review.

Figure 20. Single layer production at a Christiaens Group constructed farm.

5.2 Bruising

Key points

Mushrooms are easily damaged during harvest and packing. This results in enzymic browning reactions. Bruising sensitivity varies by flush, size and maturity and increases after harvest. Although considerable research has evaluated use of NIR to measure enzyme activity and browning it is unclear how this technology could be used in the Australian supply chain. Another focus of recent work has involved determining the genes responsible for browning reactions. Identification of trait loci on the genome and next generation sequencing is facilitating development of new, non-browning strains.

- Mushrooms are easily physically damaged during harvest and packing. Even light squeezes or vibration can trigger browning reactions, which greatly reduce their market value[130].
- Browning in mushrooms is caused by polyphenol oxidases (PPOs) and peroxidases. Enzymic reactions lead to the formation of the brown pigment melanin. Selection of strains which have reduced amounts of PPO, or use of treatments that deactivate these enzymes, have the potential to greatly reduce bruising sensitivity and improve visual quality[131].
- Bruising sensitivity is higher in small mushrooms than in large ones, especially those with open caps, and tends to be higher in first flush mushrooms than those from the third flush, with second flush intermediate. In all cases, bruising sensitivity increases during the first 24 hours after harvest[132].
- Considerable recent research has tested using near infrared (NIR) imaging to either determine enzyme activity in mushroom caps or measure browning reactions. While these are of scientific interest, it is difficult to see how this information could be used in the current Australian supply chain. For example;
    - Gaston et al[133] tested NIR for predicting PPO activity. Reasonable correlations were obtained for the model (R^2=0.78). The authors suggest that a sensor for PPO could help inform marketing decisions.
    - O'Gorman et al[134] examined using NIR to evaluate damage and estimate age of mushrooms. The model was able to detect damage on freshly harvested mushrooms but less able to discriminate old from new. It is proposed that the method could be used to classify mushroom grades and detect 'recycled' product (not likely to be an issue in Australia).

[130] Burton KS. 2004. Cultural factors affecting mushroom quality – cause and control of bruising. Mushroom Sci. XVI 397-402.

[131] Jolivet S. et al. 1998. *Agaricus bisporus* browning: A review. Mycol. Res. 102:1459-1483.

[132] Weijn A. et al. 2012. A new method to apply and quantify bruising sensitivity of button mushrooms. LWT Food Sci. Tech. 47:308-314.

[133] Gaston E. et al. 2010. Prediction of polyphenol oxidase activity using visible near-infrared hyperspectral imaging on mushroom (*Agaricus bisporus*) caps. J. Agric. Food Chem. 58:6226-6233.

[134] O'Gorman A. et al. 2010. Use of fourier transform infrared spectroscopy and chemometric analysis to evaluate damage and age in mushrooms (*Agaricus bisporus*) grown in Ireland. J. Agric. Food Chem. 58:7770-7776.

- Esquerre et al[135] also used NIR to detect bruising damage. Again, it is proposed that this could be used in an automated grading system. However it is unclear how damaged mushrooms could be removed.

- Other recent research has attempted to define differences between 'bruising sensitive' and 'bruising tolerant' strains. This information could then be used in breeding programs to ensure new strains have good agronomic properties. For example;
    - Weijn et al.[136] identified two specific compounds (GHB, GDHB) associated with bruising sensitivity. These compounds were 15x to 20x higher in bruising sensitive strains compared to tolerant strains.
    - Indian researchers have developed two high yielding strains with low enzymic activity. These strains showed did not react to mechanical injury, with no browning observed on cut surfaces two hours after slicing[137].
    - Gao et al.[138] used PCR techniques to identify and locate quantitative trait loci (QTLs) in the mushroom genome, which were associated with browning sensitivity. Major QTLs were identified on chromosomes 1 and 2. These explained 44–54% of variability in bruising between tolerant and susceptible strains.
    - Sylvan has adopted next generation sequencing (NGS) techniques, particularly the use of single nucleotide polymorphism markers (SNP chips), to identify the locations of important traits (QTLs) on the mushroom genome. The cost of such techniques has decreased logarithmically, while speed and ease of use has increased. The authors state that this advancement will greatly increase the speed and efficiency of development of new varieties[139].

[135] Esquerre C. et al. 2012. Wavelength selection for development of a near infrared imaging system for early detection of bruise damage in mushrooms (*Agaricus bisporus*). J. Near Infrared Spectrosc. 20:537-546.

[136] Weijn A. et al. 2013. Main phenolic compounds of the melanin biosynthesis pathway in bruising tolerant and bruising sensitive button mushroom (*Agaricus bisporus*) strains. J. Agric. Food Chem. 61:8224-8231.

[137] Singh M, Kamal S, Gupta M. 2016. Development and yield evaluation of non-browning hybrids in button mushroom (Agaricus bisporus). Proc. ISMS 19:309-312.

[138] Gao W. et al. 2015. Quantitative trait locus mapping for bruising sensitivity and cap colour of *Agaricus bisporus* (button mushrooms). Fungal Genetics biol. 77:69-81.

[139] Loftus M. et al. 2016. Next generation sequencing and *Agaricus bisporus* breeding. Proc. ISMS 19:305-308.

5.3 Modified atmosphere packaging

Key points

Unlike other fresh products, the respiration rate of mushrooms is relatively independent of O_2 and CO_2 concentrations in the surrounding atmosphere. Modified atmosphere packages are therefore very unlikely to extend storage life. Moreover, high CO_2 and low O_2 can be detrimental to quality. Despite reports of benefits of MAP in the peer-reviewed literature, the author does not consider this to be a worthwhile option for Australian growers.

- Modified atmosphere packaging (MAP) uses the respiration rate of the product to reduce O_2 and increase CO_2 inside a sealed package. The atmosphere that develops depends on the respiration rate of the product as well as the gas permeability of the film. The objective is generally to inhibit respiration (usually reported as O_2 consumption), this being an indicator of metabolic activity. This in turn can increase storage life – whether due to conservation of carbohydrates used to fuel metabolism, or through the slowing down of pre-programmed ageing processes.
- Australian research on MAP[140] has shown that, unlike other fresh products, increasing CO_2 concentrations do not affect the rate of O_2 consumption. Respiration rate was also relatively unaffected by O_2 concentration, remaining constant until O_2 fell to approximately 0.2%. Low O_2 levels (~10%) combined with low CO_2 increased stipe elongation, while high CO_2 increased browning and cap yellowing. It was concluded that any benefits from MAP were likely to be slight and therefore not cost-effective.
- These results were supported by Varoquaux et al[141], who confirmed that mushroom respiration was unaffected by CO_2 and O_2 concentrations within the range attainable through MAP. It was concluded no extension of shelf life is attainable through MAP, with management of RH far more important to storage life and quality.
- Despite this, numerous researchers have continued to examine MAP for mushrooms, with many reporting apparent extension of storage life[142]. For example, Oz et al[143] found that mushrooms packaged in polyethylene with 12% O2 plus 5% CO2 were whiter than the controls after 16 days at 5°C. However, the effects were slight, and appear unlikely to be commercially significant.
- An alternative method was proposed by Lin et al[144] involving gas flushing packages with CO_2, then puncturing the film to return to normal air storage after 12 to 48 hours. While the authors report a significant reduction in browning from the 12h treatment, again the effects are relatively small.

[140] Bower J. 1996. Modified atmosphere packaging of mushrooms. HAL report and UWSH Honours thesis.

[141] Varoquaux P. et al. 1999. Respiratory parameters and sugar catabolism of mushroom (*Agaricus bisporus*). Postharvest Biol. Technol. 16:51-61.

[142] Ares G, Lareo C, Lema P. 2007. Modified atmosphere packaging for postharvest storage of mushrooms. A review. Fresh Produce 1:32-40.

[143] Oz A. et al. 2015. The postharvest quality, sensory and shelf life of *Agaricus bisporus* in active MAP. J. Food Proc. Pres. 39:100-106.

[144] Lin Q. et al. 2017. Effects of high CO_2 in-package treatment on flavor, quality and antioxidant activity of button mushroom (*Agaricus bisporus*) during postharvest storage. Postharvest Biol. Technol. 123:112-118.

- It should be noted that anaerobic atmospheres inside packages of mushrooms can permit growth of food safety pathogens, particularly *Clostridium botulinum*. *Staphylococcus aureus* has also been shown to grow and produce toxins inside PVC-overwrapped mushroom packages that were not refrigerated[145].

5.4 Washes (processing aids)

Key points

In many countries mushrooms are washed before sale, especially if they are going to be processed. Two-stage washing processes have been developed in the USA and are used commercially. These have been demonstrated to reduce browning and improve food safety of mushrooms. While a range of other compounds have also been tested, with promising results for citric acid (as an alternative for hydrogen peroxide), only those that involve two-stage processes appear to provide commercially significant outcomes. This is a key area of recent research, but one that has not been investigated for Australian mushrooms.

- Mushrooms are not washed in Australia. However in other countries they are, particularly if they are going to be sliced or otherwise processed. Washed mushrooms are more susceptible to bacterial blotch due both to mechanical injury and water absorption causing high internal humidity. They can also suffer increased enzymic browning[146]. However some washing solutions can whiten and sterilize, reducing food safety risks as well as blotch development.
- The most common wash previously used for mushrooms was a solution of sodium metabisulfite. This removed casing particles and reportedly enhanced whiteness but did not reduce bacterial growth, so the effects were transitory. The practice was banned in the USA in 1986 due to allergic reactions[147]. Sodium metabisulfite dipping prior to slicing continued to be widely practiced in Ireland into the 1990's, despite being shown to be ineffective in research trials [148].
- A two stage washing process was developed and patented by Beelman-Duncan (US Patent no. 5,919,507). This involved a first-stage high pH antibacterial wash (pH 9.0 to 10 or higher) followed by a neutralizing wash in a buffered solution of erythorbic acid and sodium erythorbate (browning inhibitors).
- Sapers et al[149] further developed the two-step process for washing mushrooms. The optimum was determined to involve a pre-wash to remove soil and casing containing

[145] Martin ST, Beelman RB. 1996. Growth and enterotoxin production of Staphylococcus aureus in fresh packaged mushrooms (*Agaricus bisporus*). J. Food Prot. 59:819-826.

[146] Sapers GM et al. 1994. Enzymic browning control in minimally processed mushrooms. J. Food Sci. 59:1042-1047.

[147] Chikthimmah N, Beelman RB. 2016. Microbial spoilage of mushrooms. In "Microbiology of fruits and Vegetables" Eds GM Sapers, JR Gorny, AE Yousef. CRC Press.

[148] Brennan M et al. 1999. The effect of sodium metabisulphite on the whiteness and keeping quality of sliced mushrooms. LWT 32:460-463.

[149] Sapers GM et al. 2001. Shelf-life extension of fresh mushrooms (*Agaricus bisporus*) by application of hydrogen peroxide and browning inhibitors. J. Food Sci. 66:362-366.

0.5% hydrogen peroxide (H_2O_2), followed by a 30 second dip in 5% H_2O_2 then a spray with 4% sodium erythorbate + 0.1% NaCl. The system was tested using a continuous, commercial scale washing facility. Mushrooms were vacuum cooled then stored at 4°C. The treatment was demonstrated to reduce development of lesions due to brown blotch as well as enzymic browning.

- Other authors have tried to improve on this process using simpler methods and/or approved additives. For example;
    - Soaking whole mushrooms in 40g.L^{-1} citric acid or 50ml.L^{-1} H_2O_2 increased the shelf life of the sliced product from 11 days to 15 and 14 days respectively at 4°C. However, treatment time was relatively long at 10 minutes[150].
    - Simon and Gonzalez-Fandos[151] also trialed citric acid, in this case combined with an anti-browning treatment of 1.5% sodium ascorbate. Citric acid immediately resulted in a 2.8 log reduction in *Pseudomonas* bacteria. The result was that mushrooms were still free of bacterial blotch after 13 days at 5°C, whereas controls had moderate to severe infection.
    - Two minute dips in citric acid combined with calcium chloride and sorbitol were shown by Khan et al[152] to retain antioxidant enzymes in stored mushrooms, but in this case the effects on colour were minor.
    - Salicylic acid (aspirin) is a safe, antioxidant material that can stimulate disease resistance. Treatment with 250µM salicylic acid increased activity of enzymes that competitively inhibit PPO, with the result that cap browning was reduced[153]. However, it should be noted this work was un-replicated.
    - Electrolyzed water is currently being heavily promoted to many Australian horticultural industries. Chlorine is produced by electrolysis of salt (NaCl) in the water. Mushrooms immersed in electrolyzed water with chlorine content ≥25mg.L^{-1} for 3 minutes retained whiteness and texture better than mushrooms washed in water alone[154]. However, in this case the trial did not include an unwashed control, so it is impossible to determine whether this was a true positive effect.

[150] Brennan M, Le Port G, Gormley R. 2000. Postharvest treatment with citric acid or hydrogen peroxide to extend the shelf life of fresh sliced mushrooms. LWT 33:285-289.

[151] Simon A, Gonzalez-Fandos E. 2009. Effect of washing with citric acid and antioxidants on the colour and microbiological quality of whole mushrooms (*Agaricus bisporus*). Int. J. Food Sci. Tech. 44:2500-2504.

[152] Khan ZU et al. 2015. Integrated treatment of $CaCl_2$, citric acid and sorbitol reduces loss of quality of button mushroom (*Agaricus bisporus*) during postharvest storage. J. Food Proc. Pres. 39:2008-2016.

[153] Dokhanieh AY, Aghdam MS. 2016. Postharvest browning alleviation of *Agaricus bisporus* using salicylic acid treatment. Scientia Hort. 207:146-151.

[154] Aday MS. 2016. Application of electrolyzed water for improving postharvest quality of mushroom. LWT Food Sci. Tech. 68:44-51.

5.5 Irradiation and pulsed light treatments

Key Points

Irradiation with gamma radiation, electron beams and pulsed UV-C light have all been demonstrated to reduce microbial loads on mushrooms. This can increase shelf life, especially in the presence of brown blotch causing bacteria. Cost and penetration into packed punnets may be issues with these technologies.

- Irradiation can be conducted using a huge range of different wavelengths of energy. It encompasses everything from simply exposing mushrooms to sunlight to dosing them with gamma irradiation from a cobalt-60 source.
- Irradiation with 1kGy can delay or prevent cap opening, stipe elongation browning and softening without having any negative impacts on flavor or texture[155]. Cost and public resistance to irradiated foods are generally declining. Despite this, this treatment seems unlikely to be acceptable in the near future.
- Electron beam 'pasteurisation' does not require a nuclear source, with wavelengths generated from electricity. Exposure of sliced mushrooms to >0.5kGy has been shown to extend shelf life by destroying pathogenic bacteria, yeasts and mould[156]. This technology is not yet available in Australia, but there is interest in constructing electron beam facilities in major cities (Steritech).
- Exposure to UV-C light is well established as generating Vitamin D in mushrooms, with significant health benefits for consumers. Some growers have incorporated intense pulsed light sources into their production systems, creating value added, 'nutraceutical' products[157]. Although UV-C light can induce slight cap browning initially, it reduces populations of both human pathogenic, and *Agaricus*-pathogenic bacteria. As a result, bacterial blotch development is slowed, remaining less than half that on untreated mushrooms even after 21 days at 4°C[158]. Similar results with UV-C light have been reported for whole mushrooms by Lu et al[159] and Wu et al[160] as well as for sliced mushrooms by Oms-Oliu et al[161]. This last study found 0.6 to 2.2 log reductions in the natural microflora of mushrooms, resulting in 2-3 days extension of shelf life.

[155] Kramer ME, Doores S, Beelman RB. 1987. The effect of radiation processing on mushroom (*Agaricus bisporus*) shelf life at two storage temperatures. Proc. 4th Eastern Food Sci Tech Symposium, Lancaster PA.

[156] Koorapati A et al. 2004. Electron beam irradiation preserves the quality of white button mushroom (*Agaricus bisporus*) slices. J. Food Sci. 69:25-29.

[157] Simon RR et al. 2013. Safety assessment of the post-harvest treatment of button mushrooms (*Agaricus bisporus*) using ultraviolet light. Food Chem. Toxicol. 56:278-289.

[158] Guan W, Fan X, Yan R. 2012. Effects of UV-C treatment on inactivation of Eschericia coli O157:H7, microbial loads and quality of button mushrooms. Postharvest Biol. Technol. 64:119-125.

[159] Lu Y et al. 2016. Effects of UV-C irradiation on the physiological and antioxidant responses of button mushroom (*Agaricus bisporus*) during storage. Food Sci. Tech. 51:1502-1508.

[160] Wu X et al. 2016. Effects of UV-C on antioxidant activity, total phenolics and main phenolic compounds of the melanin biosynthesis pathway in different tissues of button mushroom. Postharvest Biol. Technol. 118:51-58.

[161] Oms-Oliu G et al. 2010. Effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (*Agaricus bisporus*). Postharvest boil. Technol. 56:216-222.

5.6 Other treatments to maintain quality

Key Points

Pre-harvest irrigation with calcium chloride has been shown by several researchers to improve postharvest quality of mushrooms by increasing the stability of cell membranes.

Fumigants and other new compounds have not previously been a viable option for treating mushrooms postharvest. However new technologies can incorporate compounds directly into packaging materials, or encapsulate them for slow release. This could allow them to be integrated with normal packing practices.

Finally, natural plant cytokinins can retard cap opening, but do not appear to have been studied for postharvest use since this was first reported in 2001.

- Postharvest quality is affected by pre-harvest practices. Viral or bacterial infections and poor nutrition can result in faster than normal postharvest deterioration. Conversely, some pre-harvest practices can extend postharvest shelf life.
- Mushrooms irrigated with 0.3% calcium chloride ($CaCl_2$) are whiter at harvest and develop less browning during storage[162]. The effects are increased if the mushrooms are bruised. This is due to higher levels of calcium in the mushrooms. Calcium is linked to cell wall integrity, so higher levels of Ca result in stronger cell membranes[163] (Figure 21).

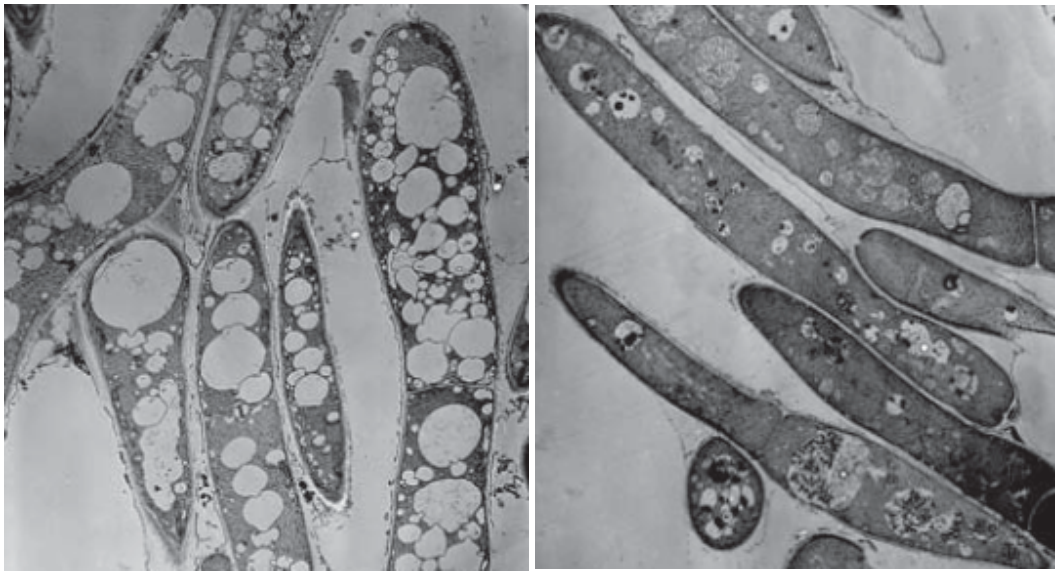

Figure 21. Electron micrographs of bruised tissue in mushrooms irrigated with water only (left) or irrigated with water containing 0.3% CaCl2 (right). The vacuoles in the CaCl2 irrigated tissue have remained intact, limiting the opportunity for enzymic reactions. From Kukura et al, 1998.

- Cytokinins are plant (and fungal) growth substances that promote cell division, and can increase shelf life of some products. Cytokinins, applied to cut stipes, have been

[162] Miklus MB, Beelman RB. 1996. $CaCl_2$ treated irrigation water applied to mushroom crops (*Agaricus bisporus*) increases Ca concentration and improves postharvest quality and shelf life. Mycologia 88:403-409.

[163] Kukura JL. 1998. Calcium chloride added to irrigation water of mushrooms (*Agaricus bisporus*) reduces postharvest browning. J. Food Sci. 63:454-459.

demonstrated to retard cap opening[164]. In this case the study was motivated by academic interest in the mechanisms of cap opening; use of cytokinins has not been tested as a postharvest treatment.

- Several researchers have tested fumigants to extend mushroom storage life. Candidates include methyl jasmonate[165], essential oils such as cinnamaldehyde[166], sulphur dioxide (released from sodium metabisulfite pads) and green tea extract[167]. Fumigation would not normally fit into normal supply chain operations. However, it is now possible to incorporate these products into packaging material using slow release pads or films, which could provide sustained release during storage. This appears to be a growing area of research and could provide useful and novel materials in the future.
- Perhaps continuing the positive results gained for cinnamaldehyde, Hu et al tested 4 methoxy-cinnamic acid dips. A naturally derived extract of cinnamic acid in plants, this compound has been shown to inhibit tyrosinase – involved in browning reactions in mushrooms. A 60 second dip significantly reduced browning, cap opening and weight loss of mushrooms, largely due to inhibition of PPO activity[168].
- After harvest, the best way to maintain mushroom quality is to keep mushrooms cold and dry. Vacuum cooling effectively dries the surface of mushrooms compared to room cooling, and has been demonstrated to reduce development of bacterial blotch[169].

[164] Braaksma A, Schaap DJ, Donkers JW. 2001. Effect of cytokinin on cap opening in *Agaricus bisporus* during storage. Postharvest Biol. Technol. 23:171-173.

[165] Meng D et al. 2012. Postharvest application of methyl jasmonate for improving quality retention of *Agaricus bisporus* fruit bodies. Food Chem. 60:6056-6062.

[166] Gao M, Feng L, Jiang T. 2014. Browning inhibition and quality preservation of button mushroom (*Agaricus bisporus*) by essential oils treatment. Food Chem. 149:107-113.

[167] Wrona M, Bentayeb K, Nerin C. 2015. A novel active packaging for extending the shelf-life of fresh mushrooms (*Agaricus bisporus*). Food cont. 54:200-207.

[168] Hu Y-H et al. 2015. Postharvest application of 4-methoxy cinnamic acid for extending the shelf life of mushroom (*Agaricus bisporus*). Postharvest Biol. Technol. 104:33-41.

[169] Varszegi T. Bacterial growth on the cap surface of *Agaricus bisporus*. ActaHort. 599:705-710.

6 Current projects on mushrooms

The following table lists current projects on mushrooms being conducted in the Netherlands, Ireland and South Africa. Information has also been requested from USA and other locations in Europe. Chinese research is less clearly defined by research group and has not been identified.

It is important to note that the majority of projects listed here are student activities – PhDs, Masters etc. – rather than fully funded projects conducted by experienced research teams.

Title	Researcher	Institution	Objective	Complete
Netherlands				
How mushrooms feed on sugar	Prof Han Wösten and PhD student	Dept Microbiology Utrecht University.	Identification of the carbon nutrient sources utilised by *Agaricus bisporus* from compost	mid 2017
Push the white button	Prof Han Wösten and PhD student	Dept Microbiology Utrecht University.	Identification of the early molecular triggers for mushroom formation	mid 2017
Weerbare dekaarde	Dr. Jan van der Wolf and PhD student	Wageningen Plant Research, BioInteractions	Finding out which biotic and abiotic factors in casing soil determine the severity of bacterial blotch or wet bubble disease	2020
Ecophysiological behaviour and risk assessment for Listeria monocytogenes in mushroom cultivation	Dr Leo van Overbeek and PhD student	Wageningen Plant Research, BioInteractions	Finding out which risks are associated with the presence of Listeria monocytogenes in *Agaricus bisporus* cultivation and how they can be managed	2020
Input-output	Dr Anton Sonnenberg and PhD student	Wageningen Plant Research, Plant breeding	Preparation of mass balances for cultivation of a white commercial strain of *Agaricus bisporus*, trying to identify which factors limit production	2020
Biological efficiency (biological efficiency is expressed as dry matter produced as mushrooms per kg of dry matter lost from compost)	Dr Johan Baars	Wageningen Plant Research, Plant breeding	Using a collection of genetically dissimilar wild isolates of Agaricus bisporus, try to identify which genetic factors determine the biological efficiency of A. bisporus	2016
Recombination landscape of the button mushroom	Dr Anton Sonnenberg	Wageningen Plant Research, Plant breeding	Identification of the genetic factors regulating the recombination rate in the genome of Agaricus bisporus during meiosis	June 2017

Title	Researcher	Institution	Objective	Complete
Ireland				
Genomic, transcriptomic and proteomic analyses of *A. bisporus* strains showing some resistance/tolerance to mushroom virus X		Teagasc (Ashtown) in collaboration with National University of Ireland, Maynooth.	The aim of this project is to determine the antiviral genetic attributes of a number of *A. bisporus* strains	September 2019
A Genomic Approach to Understanding and Improving Compost Utilisation	Dr Kerry Burton (EMR)	Teagasc (Ashtown) in collaboration with Sligo Institute of Technology	Investigate the mechanisms underlying the processes of nutrient utilisation by *Agaricus bisporus* with the ultimate aim of improving mushroom crop yields, particularly in the third flush.	May 2018
South Africa				
Maintenance and expansion of the Global Mushroom culture collection		University of Pretoria, ARC and Wageningen University	Provide researchers with access to germplasm of the diseases of mushrooms	ongoing
The mushroom microbial biome - Investigating the total microbiome of mushrooms from casing to the ready to sell product	Dr Nazareth Siyoum	University of Pretoria	Assess microbial population density and fluctuations during different stages of growth	2018
Prevalence of *Trichoderma* species and detection of *T. aggrressivum* from commercial white button mushroom production farms	Ms Kololwetu Cetyiwe: [BSc Hons student]	University of Pretoria	As per title	2017
Incidence of *Lecanicillium* species in commercial white button mushroom production farms	Ms Minah Molomo: BSc Agric final	University of Pretoria	As per title	2017
Morphological and molecular diversity of *Cladobotryum mycohpilum* isolates associated with cobweb disease of *Agaricus bisporus* in the South African mushroom industry	Ms Alinesie Chakwiya PhD student	University of Pretoria	As per title	2017
Mushroom disease prevention services.	Dr Noncy Gomba	University of Pretoria	Monitoring mushroom farms for pathogen prevalence	2018