Project Title	Survey of compost moulds in traditional and bulk Phase III (spawn-run) compost

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The results and conclusions in this report are based on an investigation conducted over a period of one year. The conditions under which the experiment was carried out, and the results obtained, have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

Table of Contents

PRACTICAL SECTION FOR GROWERS 1
Commercial benefits of the project 1
Background and objectives 1
Summary of results 1
Conclusions 2
Action points for growers 2
Anticipated practical and financial benefits 2

SCIENCE SECTION 5
Survey of compost moulds in traditional and bulk Phase III (spawn-run) compost 5

1. Introduction 5

2. Materials and Methods 6
2.1. Site selection 6
2.2 Compost sampling procedures 6
2.3 Extraction of mould propagules from compost 6
2.4 Data analysis 7

3. Results and Discussion 8
3.1 Organisms present in spawn-run compost 8
3.2 Tray spawn-run systems 13
3.3 Shelf spawn-run systems 13
3.4 Block Spawn-run systems 14
3.5 Bulk spawn-run systems 14
3.6 General Discussion 15

4. Conclusions 37

5. Technology transfer 38

6. References 39

7. Appendices. ..... **Error! Bookmark not defined.**

PRACTICAL SECTION FOR GROWERS

Commercial benefits of the project

It is important to know what moulds are present in spawn-run compost and what risk they pose to production. Some moulds in spawn-run compost (other than *Trichoderma harzianum* Th2) are known to cause significant yield depressions (see HDC report M 34). At only 5% yield depression, the cost to the industry would be in the region of £8 million. This project identifies the diversity of moulds present in spawn-run compost, from twelve sites, over a period of six months. This information will be used to identify the most commonly occurring mould organisms. Future research can establish if they pose a risk to compost productivity.

Background and objectives

Compost-mould-analysis carried out on traditional spawn-run and bulk Phase III compost over a period of five years, has revealed a high diversity of mould organisms in spawn-run compost at low to high levels. On several occasions the presence of moulds coincided with a cropping problem due, either to the moulds themselves, or some associated factor. Analysis for the presence of moulds on a continuous basis has monitored changes in the compost mycoflora over time and provided valuable insights into their population density and diversity. Spawn-run compost should be a virtually pure culture of *Agaricus* and any moulds that are present are potentially competing with *Agaricus*. The corollary of this is that *Agaricus* yields may suffer. The commercial objective of this work is therefore to identify the population of moulds that occur in spawn-run compost in order to assess their potential to reduce *Agaricus* yields.

Summary of results

This project identifies the diversity of moulds present in spawn-run compost, from twelve sites, over a period of six months. Mould populations from each of the sites are described. Differences in mould populations between spawn-run systems are commented upon.

A total of 27 different mould taxa (species or genera) were identified during the survey. Samples of tray spawn-run compost had a greater variety of moulds, occurring much more frequently, compared with compost from other spawn-run systems although there were distinct differences between sites with the same system (Table 1). For example Site 9 with tray spawn-run had fewer mould taxa than Sites 2 and 4, and Site 11 with shelf spawn-run had far fewer moulds than Sites 1 and 8. Similarly within the bulk systems Site 7 bulk spawn-run had only 3 mould taxa, compared with the 10 and 9 mould taxa recorded from Sites 3 and 6, respectively, however, Site 7 bulk also had the highest level of *Aspergillus* among the bulk spawn-run sites.

The survey provides good information on background levels of moulds in spawn-run compost according to site and also spawn-run system. It is useful for a grower to know what moulds regularly occur in his/her compost so that he/she can detect when that background population changes, for example at Site 1 (Figure 1). Such changes in mould populations can be associated with a drop in mushroom production. Monitoring compost moulds is another tool when trying to optimise and maximise mushroom production levels.

Conclusions

- The beneficial fungus, *Scytalidium thermophilum*, is frequently present in spawn run compost, along with (background) populations of bacteria and yeasts.
- Twenty-seven other mould groups were also isolated from apparently healthy spawn-run compost, the most common of which were *Aspergillus, Penicillium, Mucor, Cunninghamella, Fusarium, Geotrichum, Trichoderma* and *Scopulariopsis*
- Changes in background levels of moulds can indicate that mushroom production may be affected. For example, in M 34, a Trichoderma atroviride mould count of 2,643 propagules/gram fresh weight of spawn run compost, resulted in a 23% reduction of the 1st flush yield.
- Tray and shelf spawn-run systems tended to have more moulds, at higher levels, compared with bulk and block spawn-run systems although individual sites within each system differed.
- There is little information concerning the role of most moulds in spawn-run compost. Future work should aim to determine the impact of the most commonly encountered moulds on mushroom production.

Action points for growers

There is little to be done until there is more information available on specific moulds in relation to potential losses in mushroom production. To prevent moulds proliferating in spawn-run compost growers should ensure that

- Phase 1 and Phase 2 compost is of good quality and homogeneous
- environmental controls are effective and accurate
- vulnerable processes of spawning and spawn-running are adequately protected from spore-laden dust

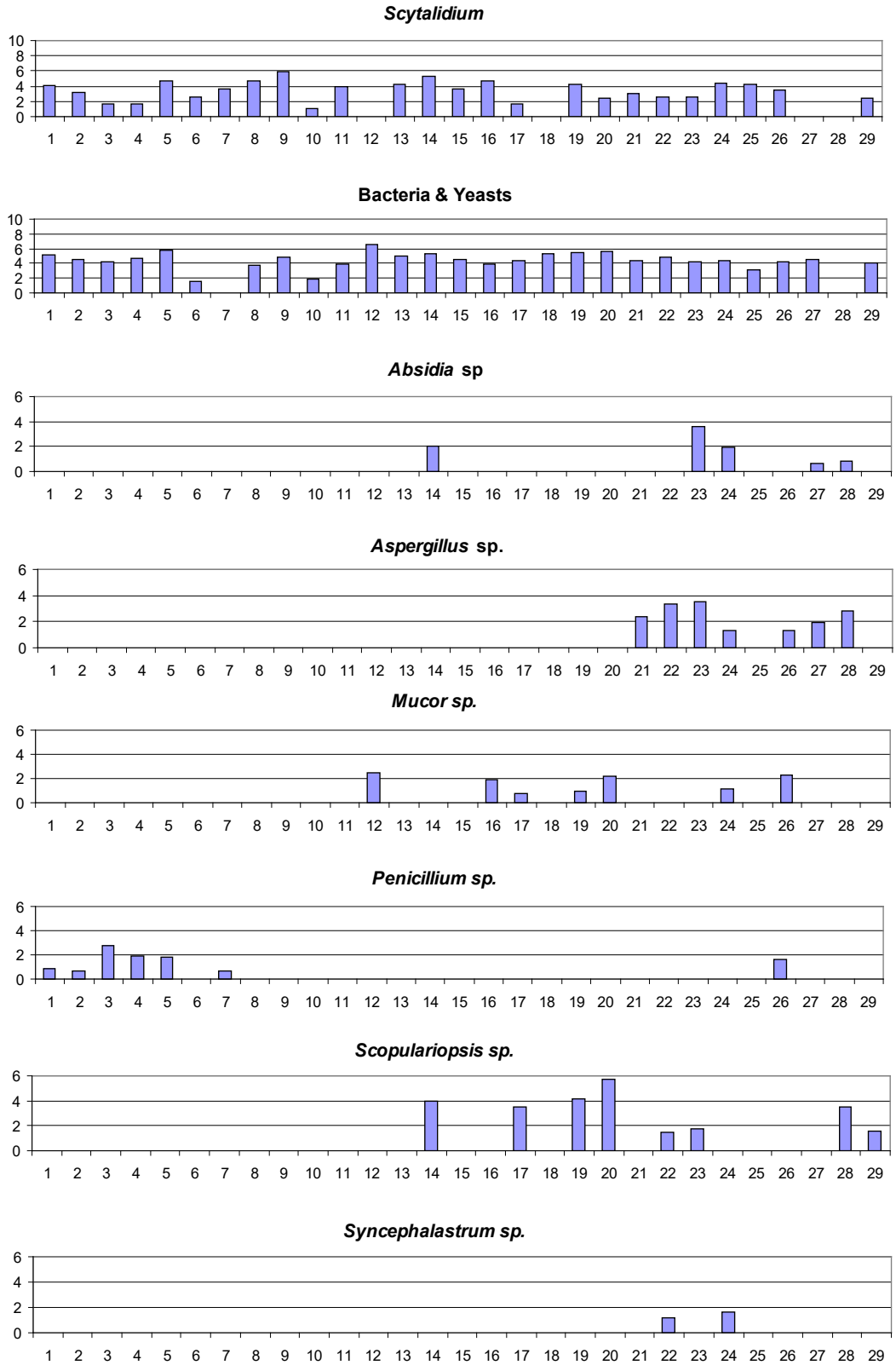
Anticipated practical and financial benefits

Tray and shelf spawn-run systems appear to harbour more moulds than block and bulk systems, though the latter are more vulnerable to contamination in view of the extended periods of exposure associated with the bulk handling of these products. If future work shows that specific moulds have a negative impact on mushroom production then yields and revenue should increase once effective control measures are identified and put in place.

Table 1. Percentage of spawn-run compost samples containing various mould organisms.

Spawn-Run System:	Trays			Shelves			Blocks			Bulk			No. of sites with mould present
Site No.	2	4	9	1	8	11	5	10	12	3	6	7	
No. of Samples	26	26	26	29	26	27	17	26	9	26	26	26	
Organisms present	**(% of samples)**												
Scytalidium	42	46	96	86	58	100	94	88	89	100	92	100	12
Bacteria & Yeasts	92	96	65	93	88	89	88	81	100	100	85	77	12
Most common moulds													
Aspergillus spp.	23	65	69	24	42	7	18	8	11	19	4	50	12
Penicillium spp.	77	46	65	24	81	11	35	8	33	31	23	8	12
Trichoderma spp.	15	4	27	3	4	7	24		22	4	12		10
Mucor spp.	62	15	8	24	38		12			31			7
Scopulariopsis spp.	8			28	4	4	12		22	4			7
Geotrichum sp.	8	12			46				11	23	4		6
Fusarium spp.	50	12			19		18	4					5
Cladosporium spp.						4	29	8			12	4	5
Cunninghamella sp.	42	92		3	4								4
Paecilomyces sp.		4	35						11				3
Other Moulds (alphabetically)													
Absidia spp.				17						15			2
Acremonium spp.			4				18			8			3
Alternaria spp.											8		1
Ascomycete sp.			4										1
Basidiomycete sp.								4					1
Chrysosporium spp.	4	4	4		8								4
Doratomyces spp.		19			4								2
Gliocladium roseum				3									1
Graphium sp.		8											1
Humicola type	8		19								4		3
Mortierella spp.	15						6						2
Nectria inventa				3									1
Oedocephalum sp.	12						6			12			3
Papulospora sp.								4					1
Phoma type				3	4		12			4	4		5
Syncephalastrum sp.				7									1
Unidentified mould							6				4		2
Total number of moulds/site (excluding *Scytalidium,* Bacteria & yeasts)	12	11	9	11	11	5	12	6	6	10	9	3	
Average/system	10.7			9			8			7.3			

Figure 1. Presence of selected moulds in spawn-run compost at Site 1 (Shelf spawn-run) over 29 weeks (Week 1 = 10 Oct 2000; Week 29 = 27 April 2001).



SCIENCE SECTION

Survey of compost moulds in traditional and bulk Phase III (spawn-run) compost

1. Introduction

The colonisation of pasteurised Phase II compost by *Agaricus bisporus* is a key step in the production of mushrooms. It is important that *Agaricus* colonises the compost well in order to maximise the production of mushrooms. If there is any competition for the compost from other moulds, then *Agaricus* yields can be lower than maximum. Competitive moulds, which severely compete with *Agaricus*, have been recorded from spawn-run compost before with devastating effects on production levels. Two well-known examples are (1) *Trichoderma* green mould (*Trichoderma harzianum* Th2) and (2) *Penicillium* "smoky mould" species.

In the late 1980's and early 1990's, *Trichoderma harzianum*, strain Th2, caused widespread economic losses to mushroom growers in Britain and Ireland. This strain had not been encountered within the mushroom industry before, nor did it show similarity with *T. harzianum* isolates from culture collections (Muthumeenakshi *et al*. 1994, Ospina-Giraldo *et al.* 1999). It is an aggressive competitor of *Agaricus* in compost, which results in very significant crop losses (Grogan *et al.* 1996).

Penicillium-related problems seem to occur sporadically, often associated with bulk spawn-run systems, but they are also less well documented than green mould outbreaks. Nonetheless, they have resulted in very severe cropping problems (Fletcher, White & Gaze 1989), with at least one species capable of wiping out a complete crop (Grogan *et al.* 2000, Grogan & Harvey, 1999 - HDC report M 34)

Routine compost-mould-analysis carried out on spawn-run compost over a period of five years, has revealed a high diversity of mould organisms in spawn-run compost at low to high levels (Grogan *et al.* 2000). In one well-studied case, the average yield of mushrooms was highest when the level of moulds recorded was lowest. Cropping experiments (HDC report M 34) showed that a number of mould species were capable of causing significant reductions in yield, while others had no effect, and it is important that this information is acquired for all mould species which are encountered in spawn-run compost. Reduced yields, however small the reduction, cannot be sustained in a climate where profit margins are small, and costs are increasing.

Analysis for the presence of moulds on a continuous basis will monitor changes in the compost mycoflora over time and provide valuable insights into their population density and diversity. Spawn-run compost should be a virtually pure culture of *Agaricus* and any moulds that are present are potentially competing with *Agaricus.*

The commercial objective of this work is to identify the frequency and diversity of the moulds that occur in spawn-run compost in Britain. This will be based on a six-month survey of spawn-run compost from 12 sites, which include traditional tray and shelf spawn-run systems as well as block and bulk spawn-run systems.

2. Materials and Methods

2.1. Site selection

Following discussions with a biomatrician, twelve commercial sites, which represented the most common spawn-running systems found throughout the British mushroom industry, were chosen for the survey. Three sites were chosen to represent each of the following four spawn-running systems:

Spawn-run system	Phase 2 system	Sites
Wooden trays	Wooden trays	Site 2, Site 4, Site 9
Aluminium shelves	In situ on shelves Bulk	Site 1, Site 8, Site 11
Shrink-wrapped blocks	Bulk	Site 5, Site 10, Site 12
Bulk tunnels	Bulk	Site 3, Site 6, Site 7

At each site, one person was instructed on how to take the compost samples so as to standardise the sampling across all the sites and to minimise any chances of undesirable contamination.

2.2 Compost sampling procedures

Spawn-run compost samples were received on a weekly basis, for a period of twenty-six weeks, from the twelve commercial sites. Every week, each site provided two samples of spawn-run compost, taken shortly before casing. Each sample was taken wearing clean disposable gloves. A small area of the surface compost was first removed to expose fresh undisturbed spawn-run compost below from which two large handfuls were taken, and put into a clean, sealable, polythene bag. All bags were pre-labelled for each site and had four small holes for air exchange to prevent samples going anaerobic in transit. For many sites the two samples were taken from two locations within a spawn-running house usually one from the top shelf/tray and one from the middle or bottom shelf/tray. The two samples were then sent by 1st class post to arrive in the laboratory for processing within a few working days. The majority of samples were processed on the day of receipt, or after 1 to 3 days' storage in the fridge. Samples received over the Christmas period were stored for up to seven days.

2.3 Extraction of mould propagules from compost

- The two samples from each site were labelled “A” and “B” or “top” and “bottom” where appropriate. Bags of compost were briefly opened to allow air in, then resealed and shaken to loosen up the compost.

- For each sample received, a 20g sub-sample of spawn-run compost was weighed out into a clean polythene (stomacher) bag. A fresh disposable glove was used per sample. Sterile water (400ml) was added to each sub-sample and the compost was left to infuse for 1 hour. Each bag of infused compost was put into a "Stomacher 400 Circulator" laboratory blender for 1 minute at 230 revolutions/minute and then left to sit for 5 minutes before being blended for a further minute.
- A 5 ml volume of compost extract was removed from each infused and blended

sample and placed in a sterile universal glass bottle. From this, a serial dilution of compost extract was made ranging from 10^0 (the original extract) through to 10^{-5}, by successively adding 1 ml of extract (or preceding dilution) to 9 ml of sterile water Plate 1, Appendix 1.

- For each dilution series, 1 ml of each dilution was placed in a separate sterile Petri-dish and molten (50°C) OAES medium (Appendix 2) poured into each dish. The dishes were then gently swirled to mix the compost extract with the molten medium. This gave six dishes for each sample ranging from 10^0 to 10^{-5}. Once the medium had set, the Petri-dishes were labelled and incubated for 1 week at 25°C.

- In addition to the serial dilutions, pieces of compost were taken from each sample bag and placed onto pre-poured Malt Agar (Appendix 3) plates containing antibiotics. These "compost" plates were placed in a small sealable bag and incubated along with the dilution plates at 25°C.

- All Petri dishes were examined initially after 2 days to count any very fast growing colonies, and again after 7 days when the plates were removed from the incubator and recorded (Plate 2, Appendix 1). The number and identity of colonies were recorded for each dilution on a record sheet. The number of propagules per gram fresh weight of spawn-run compost (props/gfw) was calculated for each mould based on the dilution(s) within a series that ideally gave between 5 and 100 colonies. Propagule numbers were converted to their corresponding $\log_{10}$ value to take into account the logarithmic nature of dilution plate counts.

- For each site, a graph for each mould organisms was drawn up, and added to each week, to show the range of organisms encountered in spawn-run compost at a given site, and to monitor how the range and frequency changed over the sampling period.

2.4 Data analysis

The count data for mould propagules was converted into the corresponding $\log_{10}$ number to take into consideration the logarithmic nature of dilution count data. The log data were used to calculate the average count for the two samples received on a weekly basis and this figure was plotted on the weekly graph for each mould. The overall frequency of occurrence of any given mould at each site was expressed as a percentage of the samples from which it was isolated. Average percentage data for each spawn-run system were also calculated.

3. Results and Discussion

3.1 Organisms present in spawn-run compost

Scytalidium thermophilum

The beneficial thermophilic fungus, *Scytalidium thermophilum,* was isolated from spawn-run compost at all sites irrespective of system. This is to be expected as pasteurised compost at spawning is thoroughly colonised by this fungus, leaving mycelium and spores in the compost. Although it is a thermophile, it can still grow slowly at spawn-running temperatures. Its ubiquitous presence in spawn-run compost is likely to be a result of a high population of spores following pasteurisation and conditioning, along with a significant presence of slow growing mycelium. *Scytalidium* occurred consistently in most samples from the block and bulk spawn-run samples but was recorded for fewer samples for some tray and shelf spawn-run sites, usually as a result of the presence of fast growing moulds, which would have obscured its presence.

Bacteria and yeasts

Bacteria and yeasts were also isolated from spawn-run compost at all sites, irrespective of system, and despite the fact that anti-bacterial ingredients were included in the growth medium to minimise their number. Bacteria and yeasts are also present in high numbers in compost after pasteurisation and conditioning, so they will be present in compost during spawn-running also. Very high levels of bacteria and yeasts may suggest a wetter compost but their significance is not dealt with within this report.

Most common moulds

Any mould that occurred in more than 20% of the samples for at least one site, and was recorded for at least three sites, was considered to be a common mould. Ten moulds fell into this category and are listed in Tables 1 & 2.

***Penicillium* and *Aspergillus* spp.**

Penicillium and *Aspergillus* spp. were by far the most common moulds encountered across all spawn-run systems (Table 1), occurring in 37, and 30 % of all samples, respectively (Table 2). None the less there were differences between different sites with from 4 to 81% of samples from a given site contain these moulds (Table 1). Both these moulds produce antibiotics that can inhibit the growth of other organisms. A number of *Penicillium* spp., similar to "smoky mould", are capable of reducing *Agaricus* yields however many *Penicillium* species occur in compost which seem to have little or no effect. No "smoky mould" isolates were recorded during the survey, however, there is no clear consensus as to the exact identity of the *Penicillium* species responsible for "smoky mould". The small amount of work that has been done has shown that no two authorities agree on the same identification. In addition identified isolates of a given species in several culture collections have turned out not to be identical when subjected to genetic fingerprinting. More work is needed to accurately identify problematical *Penicillium* spp.

Aspergillus fumigatus was one of the most commonly isolated *Aspergillus* spp. It is a thermophilic mould, frequently present during pasteurisation and conditioning however under optimum conditions of composting, pasteurisation and conditioning, it should not be present at high levels. Some individual sites had a high background of *Aspergillus* (eg tray spawn run Sites 4 & 9, and bulk spawn-run site 7; Figures 4, 9 and 7, respectively).

As *Aspergillus fumigatus* can cause serious respiratory problems as well as allergic responses, it would be prudent for farms with high background levels to monitor staff health. They should also endeavour to reduce the mould level by optimising composting, pasteurisation and conditioning processes, and by minimising dust levels when Phase II or Phase III compost is being handled.

Many species of *Aspergillus* and *Penicillium* also occur quite widely in the air.

***Trichoderma* spp.**
Trichoderma moulds occurred at 10 of the 12 sites tested, across all spawn-run systems, being present in from 4 to 27% of samples (Table 1). The species recorded included *T. atroviride, T. harzianum, T. longibrachiatum, T. parceramosum* and *T. viride*. They were more common at some sites than others, especially those with tray and block spawn-run systems (Tables 1 & 2). *T. harzianum* and *T. viride* were the most common species encountered.

A number of *Trichoderma* spp. are commonly associated with mushroom compost and compost ingredients, most of which are non-aggressive. In addition to the aggressive *T. harzianum* Th2 however, *T. atroviride* and *T. pseudokoningii* are capable of causing reduced yields (HDC report M 34). In addition, several members of this group of moulds are capable of causing spotting symptoms on growing mushrooms. Consequently moderate levels of Trichoderma spp in spawn-run compost on a site should give cause for some concern.

Zygomycete fungi
(*Mucor, Cunninghamella*, also *Absidia, Mortierella, Syncephalastrum* spp.)
The Zygomycete fungi, *Mucor* and *Cunninghamella* spp. occurred at 7 and 5 sites, respectively, out of the 12 sites tested. They were present in 17, and 13% of all samples, respectively, but tended to be more common in tray and shelf systems than block or bulk systems (Tables 1 & 2). Most Zygomycete fungi cannot utilise cellulose but need simpler forms of carbohydrate such as sugars or starch. Many species can tolerate moderately warm temperatures, with some being thermophilic, and such moulds can be present during conditioning and pasteurisation. It is likely that in tray and shelf pasteurisation systems there may be greater variations in temperature profiles, resulting in less uniform conditioning and pasteurisation. There may therefore be a carryover of some Zygomycete propagules into the spawn-running stage. It is not known what effects Zygomycete fungi, present in spawn-run compost, will have on mushroom production.

***Scopulariopsis* spp**.
Scopulariopsis spp. occurred at 7 of the 12 sites tested and were recorded from 6% of all samples, across all systems (Tables 1 & 2). In general they were present in very few samples but at two sites, Site 1, a shelf spawn-run, and Site 12, a block spawn-run they occurred in more than 20% of samples. At Site1 several other moulds were also present in the samples over the same period of time (Figure 1). Several *Scopulariopsis* spp. were identified in the survey and included *S. brevicaulis*, the most common, along with *S. brumptii, S. candida* and *S. fimicola* (a white plaster mould). *S. candida* was the most common type at Site 1 (Figure 1) although *S. brevicaulis* was also present. The presence of *Scopulariopsis fimicola* has been associated with incomplete or over composting. All these *Scopulariopsis* spp. can grow well at 30^{o}C so that they can benefit from any unevenness of temperature during spawn-running.

***Geotrichum (candidum)* sp.**
Geotrichum sp., (not lipstick mould which is *Sporendonema purpurescens*), occurred at 6 of the 12 sites tested and was present in 9% of all samples, across all systems, (Tables 1 & 2). It was recorded from 46% of samples from Site 8 which had a shelf spawn-run system (Figure 8), and 23% of samples from Site 3, a bulk spawn-run system (Figure 3). It is not known what effect *Geotrichum (candidum)* sp. has on mushroom production.

***Fusarium* spp.**
Fusarium spp. occurred at 5 of the 12 sites tested and were isolated from 9% of all samples (Tables 1 & 2). They were not recorded from bulk spawn-run systems. They were recorded in 50% of the samples from tray spawn-run Site 2, sometimes at quite high levels (Figure 2). A number of *Fusarium* spp. are associated with diseases of wheat, and the wheat straw used in mushroom compost may be the source of inoculum for the *Fusarium* spp. recorded during the survey. One *Fusarium* sp., inoculated into a crop in HDC project M34, had no effect on mushroom yield, but it wasn't clear from that work if the inoculum was too weak to cause an effect or if the mould itself was benign. It is not known what effect other *Fusarium* spp. have on spawn-run compost.

***Cladosporium* spp.**
Cladosporium occurred at 5 of the 12 sites tested and was present in 4% of all samples tested (Tables 1 & 2). It is a common airborne fungus and is found regularly at low levels wherever substrates are exposed to the air. It is not considered to pose any significant threat to mushroom production.

***Paecilomyces (variotii)* sp.**
Paecilomyces variotii was recorded from 3 of the 12 sites tested, two of which were tray spawn-run systems, and in 4% of all samples (Tables 1 & 2). It occurred in 35% of samples from Site 9, but usually at relatively low levels. It is a thermophilic fungus that is occasionally isolated from pasteurised compost but it is seldom present at high levels. It is not known however, what effects high levels would have in spawn-run compost.

Other Moulds
A number of other moulds occurred irregularly in samples of spawn run compost throughout the duration of the survey. Most of them usually occurred along with other moulds (eg *Absidia* at Site 1 - Figure 1, *Acremonium* at Site 5 - Figure 5, *Oedocephalum* at Site 3 - Figure 3). The presence of more than one mould species in a spawn-run compost sample can indicate that the compost substrate still has residual soluble carbohydrates and nitrogenous molecules to support some mould growth.

Table 1. Percentage of spawn-run compost samples containing various mould organisms.

Spawn-Run System:	**Trays**			**Shelves**			**Blocks**			**Bulk**			No. of sites with mould **present**
Site No.	2	4	9	1	8	11	5	10	12	3	6	7	
No. of Samples	26	26	26	29	26	27	17	26	9	26	26	26	
Organisms present						**(% of samples)**							
Scytalidium	42	46	96	86	58	100	94	88	89	100	92	100	12
Bacteria & Yeasts	92	96	65	93	88	89	88	81	100	100	85	77	12
Most common moulds													
Aspergillus spp.	23	65	69	24	42	7	18	8	11	19	4	50	12
Penicillium spp.	77	46	65	24	81	11	35	8	33	31	23	8	12
Trichoderma spp.	15	4	27	3	4	7	24		22	4	12		10
Mucor spp.	62	15	8	24	38		12			31			7
Scopulariopsis spp.	8			28	4	4	12		22	4			7
Geotrichum sp.	8	12			46				11	23	4		6
Fusarium spp.	50	12			19		18	4					5
Cladosporium spp.						4	29	8			12	4	5
Cunninghamella sp.	42	92		3	4								4
Paecilomyces sp.		4	35						11				3
Other Moulds (alphabetically)													
Absidia spp.				17						15			2
Acremonium spp.			4				18			8			3
Alternaria spp.											8		1
Ascomycete sp.			4										1
Basidiomycete sp.								4					1
Chrysosporium spp.	4	4	4		8								4
Doratomyces spp.		19			4								2
Gliocladium roseum				3									1
Graphium sp.		8											1
Humicola type	8		19								4		3
Mortierella spp.	15						6						2
Nectria inventa				3									1
Oedocephalum sp.	12						6			12			3
Papulospora sp.								4					1
Phoma type				3	4		12			4	4		5
Syncephalastrum sp.				7									1
Unidentified mould							6				4		2
Total number of moulds/site (excluding *Scytalidium,* Bacteria & yeasts)	12	11	9	11	11	5	12	6	6	10	9	3	
Average/system		10.7			9			8			7.3		

Table 2. Average percentage of compost samples from different spawn-run systems containing various mould species

Spawn-Run System:	**Trays**	**Shelves**	**Blocks**	**Bulk**	**Average for all samples**
Organisms present	**% of samples**				
Bacteria & Yeasts	85	90	90	87	**87**
Scytalidium	61	81	91	97	**82**
Most common moulds					
Penicillium sp.	63	37	25	21	**37**
Aspergillus sp.	53	25	12	24	**30**
Mucor sp.	28	21	4	10	**17**
Cunninghamella sp.	50	2			**13**
Trichoderma sp.	15	5	15	5	**9**
Geotrichum sp.	6	15	4	9	**9**
Fusarium sp.	21	6	7		**9**
Scopulariopsis sp.	3	12	11	1	**6**
Cladosporium sp.		1	12	5	**4**
Paecilomyces sp.	13		4		**4**
Other Moulds (alphabetically)					
Absidia sp.		6		5	**3**
Acremonium sp.	1		6	3	**2**
Alternaria sp.				3	**<1**
Ascomycete sp.	1				**<1**
Basidiomycete sp.			1		**<1**
Chrysosporium sp.	4	3			**2**
Doratomyces sp.	6	1			**2**
Gliocladium roseum		1			**<1**
Graphium sp.	3				**<1**
Humicola type	9			1	**3**
Mortierella sp.	5		2		**2**
Nectria inventa		1			**<1**
Oedocephalum sp.	4		2	4	**2**
Papulospora sp.			1		**<1**
Phoma type		2	4	3	**2**
Syncephalastrum sp.		2			**<1**
Unidentified mould				1	**<1**
Number of moulds/system (excluding *Scytalidium,* Bacteria & yeasts)	17	16	15	14	**27**

3.2 Tray spawn-run systems

Tray spawn-run systems had the most moulds during the survey, with all three sites having several individual mould species occurring in more than 50% of samples. This is likely to reflect the pasteurisation and conditioning processes at these sites which was also in trays, or in situ on shelves. Maintaining uniform pasteurisation and conditioning temperatures throughout the compost is more difficult in trays and on shelves than in bulk, as the air can circulating around trays and shelves more easily than penetrate through the denser compost. This results in a temperature gradient within the compost resulting in a more heterogeneous pasteurisation and conditioning. Such heterogeneity is likely to result in localised areas of compost where soluble carbohydrates and nitrogenous compounds are available for a wide variety of mould species, especially those that can tolerate moderately high temperatures. Low levels of such moulds are unlikely to pose serious problems as once the compost has been exhausted of the more readily available nutrients, they will die off. However, high levels of these moulds are unlikely to be beneficial.

Site 2 (Figure 2).
A number of moulds occurred regularly in the tray spawn-run compost from Site 2, at moderate to high levels. They were dominated by *Penicillium, Mucor, Fusarium* and *Cunninghamella*

Site 4 (Figure 4).
Spawn-run compost from Site 4 had continuously high levels of *Cunninghamella,* a Zygomycete fungus closely related to *Mucor*. It can tolerate temperatures of 45^{O}C and high CO_2 levels. *Aspergillus* and *Penicillium* also occurred quite regularly during the survey period.

Site 9 (Figure 9).
A number of moulds occurred regularly at low to moderate levels in the tray spawn-run compost from Site 9. They were dominated by *Aspergillus* and *Penicillium,* but *Humicola* type, *Paecilomyces* and *Trichoderma* also occurred regularly at low levels. *Trichoderma parceramosum* was the most common *Trichoderma* spp. found at this site; a species not encountered at any other site.

3.3 Shelf spawn-run systems

The same comments relating to tray spawn-run systems equally apply to shelf spawn-run systems, where pasteurisation has been done *in situ.* In addition, shelf spawn-run systems can be more vulnerable to contamination from the air, as doors into the rooms can be open for long periods at a time.

Site 1 (Figure 1).
Mould levels at this site were quite low up to week 12 when high levels of *Mucor* and *Trichoderma* were recorded. *Mucor* continued to occur regularly to the end of the survey period. In addition, other moulds such *as Absidia, Aspergillus, Scopulariopsis* and *Syncephalastrum* occurred regularly at moderate levels in the latter half of the survey period.

Site 8 (Figure 8).
A high proportion of spawn-run compost samples from Site 8 contained moulds throughout the survey period. The most frequent were *Penicillium, Geotrichum, Aspergillus* and *Mucor* of which *Penicillium* and *Geotrichum* occurred at quite high levels.

Site 11 (Figure 11).
Spawn-run compost from Site 11 was relatively mould free during the period of the survey. Only five mould genera were recorded, occurring in 11% of samples or less. This probably reflects the fact that Site 11 fills bulk Phase II compost onto its shelves whereas Sites 1 and 8 pasteurise and condition their compost on shelves *in situ*.

3.4 Block Spawn-run systems

Blocks of spawned compost are prepared by custom composters using bulk pasteurised and conditioned compost. The high technological investment in bulk composting and pasteurisation usually results in a more homogenous product, which should perform well (all other things being taken into consideration). Shrink wrapping blocks in polythene also protects the compost from contamination during spawn-running so that there should be few moulds present in spawn-running compost. This was largely the case during this survey, with relatively few moulds occurring, usually at relatively low levels. However, there is a strong need for the composter to ensure the quality of his blocks, and to maintain their relatively mould-free status, by ensuring that all precautions are taken to prevent contamination of compost at spawning.

Site 5 (Block spawn-run) Figure 5.
Block spawn-run compost from Site 5 was relatively clean in the first half of the survey period. From week 24 onwards however, a number of moulds occurred regularly but usually at relatively low levels.

Site 10 (Figure 10)
Block spawn-run compost from Site 10 was very clean throughout the period of the survey with very few mould species occurring at relatively low levels.

Site 12 (Figure 12)
Site 12 only sent in samples up to week 10. In that time a small number of moulds occurred, usually at low levels.

3.5 Bulk spawn-run systems

Bulk Phase III compost, which is spawn-run in large tunnels, is considered to be a major technological advance in the British mushroom industry, and one which has been steadily growing in the past ten years. It is a high-cost, technologically-advanced system that produces a uniform product, which generally performs well. It enables mushroom growers to bypass the spawn-running stage and to grow more crops per year. Despite it's advantages however, it is also inherently very vulnerable to contamination as bulk loads (80-100 tonnes) can be lost or severely compromised if a contaminant succeeded in entering and colonising a bulk tunnel. Bulk spawn-run compost should therefore be **exceptionally** clean at all times, and systems should be in place to continually monitor

mould populations as the risks due to, not only mould contamination but also virus contamination, are extremely high.

Site 3 (Bulk spawn-run) Figure 3.
A number of moulds occurred at regular intervals, and at low to moderate levels in the bulk spawn-run compost from Site 3. The dominant species *were Penicillium, Mucor, Geotrichum* and *Aspergillus,* with *Absidia* and *Oedocephalum* also occurring. This level of mould contamination of bulk spawn-run compost should give rise to serious concern.

Site 6 (Bulk spawn-run) Figure 6.
Bulk spawn-run compost from Site 6 was relatively clean throughout the survey period. Occasionally, common airborne contaminants were picked up such as *Alternaria, Cladosporium,* and *Penicillium.* On one occasion, week 16, *Trichoderma (atroviride)* was present at a moderate level. This mould has the potential to reduce yields significantly (HDC Report M 34) and should not be tolerated within a bulk spawn-run facility.

Site 7 (Bulk spawn-run) Figure 7.
Bulk spawn-run compost from Site 7 had the fewest number of mould genera of any site in the survey, however, 50% of all samples had low to moderate levels of *Aspergillus* throughout the survey period. *Aspergillus* spp can cause respiratory illnesses and allergic reactions and operators should be duly protected from this health hazard. This level of mould contamination of bulk spawn-run compost should give rise to concern.

3.6 General Discussion

A total of 27 different mould taxa (species or genera) were identified during the survey. Samples of tray spawn-run compost had a greater variety of moulds, occurring much more frequently, compared with compost from other spawn-run systems although there were distinct differences between sites with the same system. For example Site 9 tray spawn-run had fewer mould taxa than Sites 2 and 4, and Site 11 shelf spawn-run had far fewer moulds than Sites 1 and 8. Similarly within the bulk systems Site 7 bulk spawn-run had only 3 mould taxa, compared the 10 and 9 mould taxa recorded from Sites 3 and 6, respectively, however, Site 7 bulk also had the highest level of *Aspergillus* among the bulk spawn-run sites.

The data presented in Tables 1, 2 and Figures 1-12 provide good information on background levels of moulds in spawn-run compost according to site and also spawn-run system. It is useful for a grower to know what moulds regularly occur in his compost so that he can detect when that background populations changes, for example Sites 1 and 5 (Figures 1 and 5), and if such mould population changes are associated with a drop in mushroom production. Monitoring compost moulds is another tool when trying to optimise and maximise mushroom production levels, in addition to the standard and routine chemical analyses of compost.

Insert figures 1-12 from excel file "M34a Report Figures" pages 16-36

4. Conclusions

- The beneficial fungus, *Scytalidium thermophilum*, is frequently present in spawn run compost, along with (background) populations of bacteria and yeasts.
- Twenty-seven other mould groups were also isolated from apparently healthy spawn-run compost, the most common of which were *Aspergillus, Penicillium, Mucor, Cunninghamella, Fusarium, Geotrichum, Trichoderma* and *Scopulariopsis*
- Changes in background levels of moulds can indicate that mushroom production may be affected.
- Tray and shelf spawn-run systems tended to have more moulds, at higher levels, compared with bulk and block spawn-run systems although individual sites within each system differed.
- There is little information concerning the role of most moulds in spawn-run compost. Future work should aim to determine the impact of the most commonly encountered moulds on mushroom production.

5. Technology transfer

- A poster presentation of interim results was on display during the HRI/HDC Mushroom Day, at Horticulture Research International on June 21st 2001.

- Technical staff at participating sites were frequently in contact with the project leader throughout the duration of the project

- A HDC News article provisionally entitled "Moulds in spawn-run compost" is scheduled for publication in 2001.

- A presentation will be given at the HRI/HDC Mushroom Day in 2002.

6. References

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