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Moulds in spawn-run compost and their effect on mushroom production

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ABSTRACT: Spawn-run compost was routinely analysed for the presence of moulds. In a bulk spawn-running system, abundance of moulds was associated with low average yields and high levels of 25nm virus. Conversely when less moulds were present yields were higher and virus levels lower. We suggest that the presence of moulds in bulk spawn-run compost, other than *Agaricus* and *S. thermophilum,* indicate that hygiene practices are not 100% effective. Routine monitoring of mould levels may therefore highlight unnoticed breeches in hygiene. Inoculation of compost at spawning with *Acremonium murorum, Penicillium* sp., *Pythium oligandrum, Trichoderma atroviride* and *T. pseudokoningii* reduced first flush yields by 8 to 94%. Yields recovered to varying degrees in all cases except for *Penicillium* sp. This work highlights the potential for significant crop losses associated with covert moulds.

1 INTRODUCTION

Major developments associated with the commercial production of mushroom compost have occurred in recent years. As a result of environmental pressures concerning odour pollution, composters are increasingly adopting composting techniques that involve forced aeration of the Phase I process, to produce what is variously known as "indoor", or "controlled environment" compost (Perrin & Gaze 1989, Gerrits et al. 1993, Perrin & Macauley 1995). This results in a different spectrum of environmental conditions that can alter the degradation of the straw, compared to compost produced by traditional methods, due to changes in microbial populations and activity (Evered et al. 1995, liyama et al. 1995). It is envisaged that much of the compost in Europe will be produced by this method in the foreseeable future.

In addition to aerated Phase I, an increasing amount of compost is being spawn-run in bulk to produce what is known as 'bulk Phase III' compost. This process involves spawning a large volume of compost that is then put into a controlled-environment tunnel for spawn-running, rather than the more traditional methods of spawn-running in trays, bags, blocks or shelves. The technology associated with it is complex and costly but it is designed to produce a high-quality, uniform product (MacCanna 1997). Each bulk Phase III tunnel is, in effect, a giant 'solid substrate fermentor' concerned with the production of a 'pure' culture *(Agaricus)* after a period of incubation (spawn-run). As with all such systems, bulk Phase III requires stringent hygiene practices to maintain the purity of any given batch, as the system is intrinsically vulnerable to contamination. Should this occur, there is potential for serious outbreaks of virus or competitor moulds, and most bulk Phase III producers world-wide have experienced one or both types of problem at some time.

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 (Gro-

gan et al. 1996). If such a mould were to contaminate a bulk Phase III tunnel, the financial consequences would be enormous.

Trichoderma harzianum Th2 no longer seems to be a major problem in Britain, however, routine screening of bulk Phase III and other spawn-run composts, has revealed that a number of moulds regularly occur at this stage in the production cycle (Olivier & Guillaumes 1976, Fergus 1978). These moulds have sometimes, though not always, been associated with reductions in yield. Perhaps many of them are harmless but in view of the fact that some occur more often than others, and sometimes at high levels, it would be very useful to know whether or not they have any effect on the yield potential of the compost. This knowledge is also of importance when considering the vulnerability of the bulk Phase III system to contamination with virus-carrying propagules, as well as the great pressure on modern mushroom production units to be economically viable. Small reductions in yield cannot be sustained in a climate where profit margins are low and costs are always increasing.

The frequency and variety of moulds encountered during routine analysis of bulk spawn-run compost is presented and compared for compost from four different sources. Following on from this, a small-scale inoculation experiment was set up to examine the effects of six individual mould species on mushroom production.

2 MATERIALS AND METHODS

2.1 *Sites and sampling procedures*

Bulk spawn-run compost samples were obtained at regular intervals from three commercial producers, designated Sites 1, 2, and 3, respectively. For comparison, samples of compost from a tray spawn-run were also taken from the mushroom unit at Horticulture Research International (HRI), Site 4. Samples from Site 1 were obtained for the period 1995 to 1998 while samples for Sites 2, 3 and 4 were obtained for 1998 only. Samples from Sites 1, 2 and 3 were taken on the day when spawn-run tunnels were emptied and consisted of approximately 500 g of spawn-run compost placed in a clean polythene bag and dispatched by express post. A similar sample was taken at the end of a tray spawn-run at Site 4. All samples were processed within two days of receipt.

2.2 *Extraction of mould propagules from compost*

Individual compost samples were transferred into a large clean polythene bag and shaken gently to break up the compost. A 20 g subsample was placed in a sterile polythene homogeniser-bag (182 x 310 mm x 72 µm), to which 360 ml sterile water was added. After soaking for 1 hour, the sample was homogenised for 1 minute in a 'Stomacher 400' laboratory blender, left to rest for 5 minutes then re-homogenised for 1 minute. The resulting compost extract was then serially diluted to give a concentration range of 1 x 10° to 1 x 10°. A 1 ml aliquot of each dilution was pipetted into a series of sterile Petri-dishes, and molten (50°C) OAES medium (Ohio Agricultural Experimental Station, Kaufmann et al. 1963), was then poured into each dish. When cooled, the Petri-dishes were incubated at 25°C, and examined after three and seven days. The number and identity of colonies was recorded for each dilution. The number of colony forming units/gram fresh weight of compost (c.f.u.'s/g.f.w.) was calculated for each mould based on the dilution(s) within a series that gave between 5 and 100 colonies.

2.3 *Compost inoculation*

Six compost moulds isolated from spawn-run composts were selected for a small scale cropping trial; *Acremonium murorum* (isolate 1003BJ; IMI 381 119), *Fusarium* sp. (isolate 919A), *Penicittium* sp. (isolate 1043D), *Pythium oligandrum* (isolate 1004C), *Trichoderma atroviride* (isolate T43; IMI 381 122); *Trichodermapseudokoningii* (isolate 924; IMI 381 121). The *T. atroviride* isolate was originally identified as *T. harzianum* molecular type Th3, but this group is now generally considered to be synonymous with *T. atroviride* (Dodd et al. 2000).

Three types of inoculum (spore suspension, grain, compost) were prepared for each mould in order to maximise the possibility that one or other would effectively cause the compost to be

colonised by the introduced mould. A 100 nil volume of a concentrated spore suspension (approx. 10^6 spores/ml), 250 g of spore-coated rye grain or approx. 60 g of inoculated compost was applied to 30 kg of Phase II compost. Control treatments were treated with sterilised water, grain or compost, respectively.

Thirty kg of Phase II was loosely filled into wooden trays measuring 91 cm x 61 cm x 17 cm (1 x b x h). Each tray was spawned individually with *Agaricus* (Sylvan A 15) at 0.5%. At the same time, compost was inoculated with one of the compost moulds, using one of the inoculum types. Care was taken to prevent cross contamination through the implementation of strict hygiene measures (changes of gloves, disinfecting vulnerable areas, spraying the air with a fine mist of water to precipitate any spores which may have become airborne). The spawned and inoculated compost was then spawn-run at 25°C for 20 days. After spawn-running, the compost from each tray was gently mixed up and filled into six 260-mm diameter pots at a rate of 3-kg compost per pot, compressed, then cased with a commercial casing (Tunnel Tech English) containing casing inoculum. Pots were positioned in a cropping chamber with three shelves, each taking 4 x 1 2 rows of pots, according to a split-plot design. Pots were case-run, aired and cropped according to standard procedures on the HRI mushroom unit. Three flushes were harvested. Yield data was analysed using the method of Restricted Maximum Likelihood (REML) to allow adjustment for any positional effects within the chamber. Treatment effects were determined using a Wald test and, where significant effects were determined, mould-treatment means were compared to the control mean using the least significance difference (LSD) value at $P = 0.05$, calculated from the standard error of differences between the means.

3 RESULTS AND DISCUSSION

3.1 *Moulds from spawn-run composts*

Despite the fact that spawn-run compost should be a relatively pure culture *of Agaricus*, a significant number of moulds were regularly isolated from spawn-run compost from four different sites over a period of one to four years. Some of these were thermotolerant moulds, probably derived from thermophilic activity during pasteurisation and conditioning of the compost in Phase II, and included *Scytalidium thermophilum, Aspergillus fumigatus, Absidia* sp., *Chrysosporium pruinosum, Coryneascus sepedonium* and *Paecilomyces variotii* but only *S. thermophilum* was isolated with any regularity. Since this is a beneficial organism associated with the conditioning of the compost during peak-heating, its frequent presence at relatively high levels is neither surprising nor particularly worrying as it is invariably recorded from phase II compost and is frequently recorded from spawn-run composts (Straatsma et al. 1989, 1994).

Of greater concern is the regular, but less frequent isolation of non-thermotolerant moulds such as *Penicillium* spp., *Trichoderma* spp., *Geotrichum candidum, Fusarium* spp. and *Mucor* spp. These were isolated from 3 to 25% of spawn-run compost samples from Site 1 over a four year period, and from 10 to 81% of samples from three other sites during 1998. Analysis of the data from Site 1 shows that up to 18 different genera of fungi were isolated from bulk spawn run compost over a four year period from 1995 to 1998 (Table 1). As well as the beneficial *S. thermophilum,* which was isolated from approximately half of the samples, *Penicillium* spp. occurred in 22 to 32% of samples while *Aspergillus* spp. occurred in 6 to 9% of samples. Other moulds such as *Trichoderma* spp., *Geotrichum candidum, Fusarium* spp. and *Mucor* spp. occurred more irregularly in 4 to 17% of samples, depending on the year. For example, in 1995, 57% of samples contained moulds other than *S. thermophilum* while in 1997, only 33% of samples contained moulds (Fig. 1). These are also the years where average 25-nm virus levels are highest, and lowest, respectively. Conversely, these years have the lowest, and highest average yields, respectively, suggesting that there is some correlation between these variables (Fig. 1). In addition, most of the dominant moulds were generally present at higher levels in 1995 and 1996 (c.f.u.'s = $!O^{1,2,3,4}$) compared with 1997 and 1998 (c.f.u.'s = 10^{U}*0 (Table 2).

The results for the three remaining sites for 1998 are based on fewer samples. Nonetheless, all the dominant mould species present at Site 1 also occurred at Sites 2, 3 and 4 (Table 1). Again, *S. thermophilum* was the most commonly isolated mould however, in contrast to Site 1, a greater proportion of the samples from Sites 2 & 3, which also produce bulk spawn-run compost, had *Penicillium* spp. and *Trichoderma* spp. present. The tray spawn-run compost also had

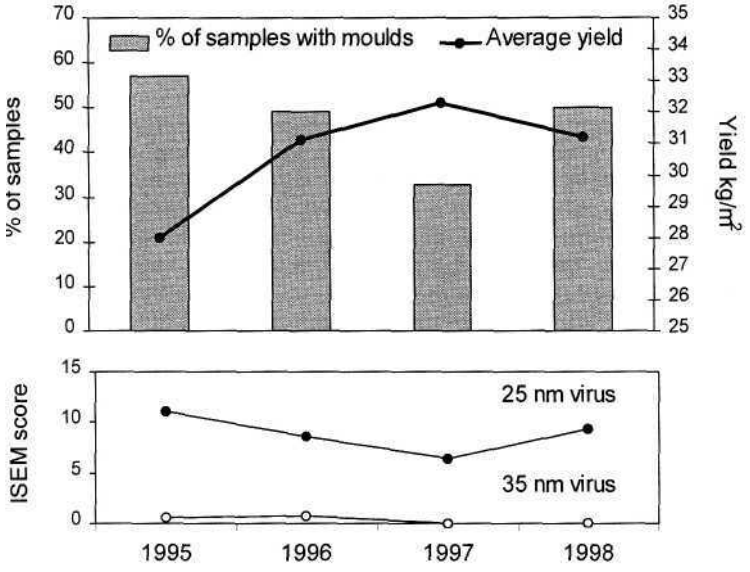

Figure 1. Yield, mould and virus data for bulk Phase III compost from Site 1 over four years. Percentage of samples with moulds excludes samples with *S. thermophilum* only. Average ISEM (immunosorbant electron microscopy) values based on analysis of first flush mushrooms from selected crops during each year by Central Science Laboratory, York, UK.

Table 1. List of moulds and frequency of isolation from spawn-run compost. Sites 1, 2 and 3 are commercial producers of bulk Phase III. Site 4 produces a tray spawn-run.

	Site 1					Site 2	Site 3	Site 4
Year:	1995	1996	1997	1998	Average	1998	1998	1998
Number of samples analysed:	47	51	45	46	189	16	20	10
	(% of samples)					(% of samples)		
Dominant moulds:								
Scytalidium thermophilum	53	39	51	59	51	44	85	50
Penicillium spp.	32	22	22	26	25	81	55	70
Trichoderma spp.*	9	14	2	13	9	75	25	30
Geotrichum candidum	17	8		4	7	31	10	10
Fusarium spp.	13	6	9		7	19	20	10
Aspergillus spp.**	9	6	7	7	7	25	20	10
Mucor spp.	4	8			3	50	20	10
Other moulds:								
Absidia sp.		4			1		20	
Acremonium murorum		4			1	13	10	
Alternaria sp.			2		<1			10
Botrytis cinerea				2	<1			
Chrysosporium pruinosum								20
Cladosporium spp.	2		7	9	4	19		10
Coryneascus sepedonium				2	<1			
Doratomyces spp.		2		4	2			10
Gliocladium roseum		2			<1			
Paecilomyces variotii			2		<1			
Rhizopus stolonifera	2				<1			
Scopulariopsis brevicaulis		2			<1			
Number of genera	9	12	8	9	18	9	9	11

* Predominantly *T. harzianum* Thl and *T. atroviride* (Th3) but also includes *T. longibrachiatum, T. koningii, T. viride* and unidentified *Trichoderma* spp.

** Includes *Aspergillus fumigatus.*

Table 2. Approximate number of propagules of various moulds isolated from spawn-run compost. Sites 1, 2 and 3 are commercial producers of bulk Phase III. Site 4 produces a tray spawn-run.

	Site 1				Site 2	Site 3	Site 4
Year	1995	1996	1997	1998	1998	1998	1998
Number of samples analysed:	47	51	45	46	16	20	10
	(Colony forming units /gram fresh weight of compost)						
Dominant moulds:							
Scytalidium thermophilum	10^{3-4}	10^{3+4}	10^{3-4}	10^{2+3}	10^{4-5}	10^{4+5}	10^{3+4}
Penicillium spp.	10^{2+3}	10^{2+3}	10^{1+2}	10^{1+2}	10^{2+3}	10^{1+2}	10^{2+3}
Trichoderma spp.*	10^{2+3}	10^{3+4}	10'	10^{1+2}	10^{3}	10^{2}	10^{2}
Geotrichum candidum	$10^{2,3}$	10^{1+2}		10^{1+2}	10^{1+2}	10^{2}	10^{3}
Fusarium spp.	10^{2}	10^{2}	10^{1+2}		10^{3}	10^{U}	10^{2}
Aspergillus spp.**	10^{U}	<10'	10^{2+3}	10^{1+2}	10^{2+3}	10^{1+2}	10^{1}
Mucor spp.	10^{2}	10^{2+3}			10^{2+3}	10^{2}	10^{3}
Other moulds:							
Absidia sp.		10^{4}				10^{1}	
Acremonium murorum		10^{2}			10^{4}	10^{4}	
Alternaria sp.			10^{1}				10'
Botrytis cinerea				10^{1}			
Chrysosporium pruinosum						10'	
Cladosporium spp.	10'		10^{1+2}	10^{1+2}	10^{1+2}		10^{2}
Coryneascus sepedonium				10^{4}			
Doratomyces spp.		10'		10^{3}			10^{2}
Gliocladium roseum		10^{2}					
Paecilomyces variotii			10^{2}				
Rhizopus stolonifera	10^{2}						
Scopulariopsis brevicaulis		10'					

* Predominantly *T. harzianum* Thl and *T. atroviride* (Th3) but also includes *T. longibrachiatum, T. koningii, T. viride* and unidentified *Trichoderma* spp.

** Includes *Aspergillus fumigatus*

Table 3. Yield of mushrooms for composts inoculated with different mould species. Yields for each mould are the average of all inoculation treatments and are expressed as a % of the control yield.

	Yield of mushrooms			
	1st Flush	2nd Flush	3rd Flush	Total
Control (grams or kg/plot)	584.6 g	504.1 g	157.2g	1.25kg
	100%	100%	100%	100%
Inoculated Mould species:				
Fusarium sp.	99	100	99	100
T. pseudokoningii	92*	102	115*	99
Acremonium murorum	82*	101	111	93*
Pythium oligandrum	82*	96	119*	93*
T. atroviride (T43)	74*	98	110	88*
Penicillium sp. (1043D)	16*	3*	0*	9*
LSD at ^{5} = *0.05*	39.6 g (6.8%)	23.6 g (4.7%)	17.1 g (11%)	43.7 g (3.5%)

Yield is significantly different from the control based on statistical analysis of raw data.

a higher proportion of samples with these two moulds, however the sample sizes for these sites were considerably smaller so percentage data are less precise. Propagule counts for Site 2 were generally higher than Site 3 but unfortunately there is no yield data available for these sites. Propagule counts for the tray spawn-run were also moderate to high.

The reductions in (1) the proportion of samples with moulds, (2) the number of mould-c.f.u.'s isolated and (3) 25-nm virus levels, corresponded to a major effort at Site 1 to prevent contamination of the Phase II compost at spawning, following a serious 35-nm virus episode in 1994. Thus, monitoring spawn-run compost for the presence of unintentional mould species would appear to be a good hygiene strategy for Phase III producers. Their presence indicates that non-thermophilic moulds are present during spawn-run and since they must have had a route of en-

try, so too will there be an entry for propagules containing 35-nm virus, the devastating consequences of which are well documented (Geels et al. 1988; Fletcher et al. 1989).

3.2 *Effects of moulds on mushroom yield*

The regular occurrence of moulds in spawn-run compost, described in 3.1 above, raised the question as to whether or not any of them could successfully compete with *Agaricus* at a level that would reduce yields significantly. Their regular isolation from spawn-run compost suggested that they could happily co-exist with *Agaricus,* without being particularly noticeable or aggressive. This is in contrast with *T. harzianum* Th2 or Th4, which have a devastating effect on yield and regularly produce green mould symptoms in spawn-run compost (Grogan et al 1996; Anderson et al. 1998; Rinker & Aim 1998).

Five out of six moulds tested in the inoculation experiment had a significant inhibitory effect on the yield of first flush mushrooms compared to the uninoculated control treatment (Table 3). The average reduction in first flush yield was 8% for *Trichoderma pseudokoningii,* 18% for *Acremonium murorum* and *Pythium oligandrum,* 26% for *T. atroviride* and 84% for *Penicillium* sp. The second flush yields for all treatments, except *Penicillium* sp., were similar to the uninoculated controls, while the third flush yields for a number of the mould treatments were higher than the controls. The yield improvements in the second and third flushes were not sufficient to prevent an overall yield reduction by *Acremonium murorum, Pythium oligandrum,* and *T. atroviride,* whereas the yield from *T. pseudokoningii-moculated* compost fully recovered by the third flush. *Fusarium* sp. did not have any yield-reducing effect while *Penicillium* sp. caused almost complete crop failure.

The *Penicillium* isolate 1043D used in this study originated from compost with "smoky mould" symptoms, which include severe yield reductions and "spore-smoke" emanating from the compost when disturbed (Fletcher et al. 1989). Exact identification of the species involved in smoky mould problems is uncertain. Initially it was thought to be *P. chermesinum,* then *P. implicatum.* Comparison of ITS sequence data for four smoky mould isolates (including isolate 1043D) and culture-collection isolates of P. *chermesinum* and *P. implicatum* indicates considerable confusion. Only one smoky mould isolate was similar to a *P. implicatum* isolate, but culture-collection isolates of the same species from different sources appeared to be different (McKay, pers. comm.). Further work is therefore required to firmly establish the identity of these problematical *Penicillium* moulds.

4 SUMMARY & CONCLUSIONS

The regular isolation of mould species from bulk spawn-run composts, despite the high level of hygiene implicitly associated with bulk systems suggests that many moulds can co-exist with *Agaricus* during the spawn-running process. Results from the inoculation studies indicate that a number of apparently non-aggressive moulds can significantly reduce first flush yields by up to 25% and that one particular *Penicillium* sp. can result in almost complete crop failure. Monitoring mould-levels in spawn-run compost, particularly from bulk systems, should identify when breeches in hygiene occur and so direct attention towards rectifying them in order to prevent large-scale problems developing.

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