Project Title	Mushrooms: Factors and practices influencing the susceptibility of composts to infection by different compost moulds and to subsequent crop loss
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Project leader:	Ralph Noble, Warwick HRI
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Key staff:	Andreja Dobrovin-Pennington William Turnbull
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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

[Name]
[Position]
[Organisation]

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[Name]
[Position]
[Organisation]

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Report authorised by:

[Name]
[Position]
[Organisation]

Signature ..... Date .....

[Name]
[Position]
[Organisation]

Signature ..... Date .....

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Grower Summary

Headline

A number of cultural practices in the composting process have been identified to reduce compost moulds.

Background and expected deliverables

Severe problems have been caused in recent times by *Trichoderma aggressivum* in the UK, the Netherlands and Poland (European form or Th2) and in North America (American form or Th4). However the reasons for these outbreaks have not been established, (e.g. faults in hygiene and/or compost factors etc).

Resistance of moulds to previously effective fungicides (carbendazim) has been reported in the USA.

Compost mould problems in the UK industry have been identified as green mould (Th2), 'smoky mould' (*Penicillium* species) and 'black compost' (*Pythium oligandrum*) although other moulds may also be responsible for crop loss. However, the level of mould growth in composts does not necessarily relate to the level of mushroom crop loss.

There have been significant changes in compost formulations in recent years, particularly with regard to the use of organic, fungicide-free straw, reductions in poultry manure and increased use of other nitrogen sources. Goody water nutrient and oxygen levels have also been shown to vary widely (Project M 3e). Composting technologies (windrow and bunker), durations, temperatures, aeration, and formulations vary widely not only in the UK, but also Dutch and Irish mushroom composting industries. Straw also varies in type and age. These compost factors may significantly affect the growth of moulds and subsequent crop loss.

Supplementation of Phase II is recognised as encouraging the growth of certain compost moulds, and supplementation of Phase III, which is more widely practiced, has also been shown to encourage the growth of *Trichoderma* Th4 [10, 11]. The effects of spawn rate on compost mould growth are less established.

The overall aim of this project was to identify the properties of Phase II compost, associated compost ingredients and composting conditions and practices that affect the susceptibility of composts to infection and mushroom crop loss resulting from different moulds.

The objectives and expected deliverables from this project are:

1. Obtain isolates of compost moulds from the WHRI culture collection, commercial farms and composters. Obtain molecular taxonomy of isolates.

2. Produce composts from a range of raw materials, water sources and amounts, under a range of environmental conditions and different levels of degradation.

3. Obtain commercially produced Phase II compost samples from a range of commercial composters in the UK on repeated occasions.

4. Test the growth of compost moulds (1 above) on experimentally prepared composts (02 above) and commercial Phase II composts (3 above), and assess effect on mushroom yield and quality. Relate compost mould growth and crop loss to compost factors and analysis.

5. Determine the effect of spawn-rate and supplementation on colonization of composts by different moulds.

6. Identify remedial action that can be taken to reduce the susceptibility of composts to colonisation by moulds and to subsequent crop loss.

Summary of the project and main conclusions

Spore suspensions of compost moulds were sprayed into spawned Phase II or Phase III compost contained within plastic bags. The effect of mould inocula on mushroom cropping was then compared with ‘clean’ compost of the same type in pot

experiments. The growth of the moulds in the composts was determined by plating compost extracts at casing and after the second flush.

Initial experiments demonstrated that *Trichoderma aggressivum* f. *europeum* (isolate 23443B) and *Penicillium implicatum* (isolate 1043D) resulted in more severe mushroom crop losses than *Acremonium murorum*, *Pythium oligandrum* and several other isolates of *Trichoderma.*

Further experiments were then conducted to examine the influence of *Penicillium* 1043D and *Trichoderma* 23443B on mushroom cropping in experimental and commercial Phase II and Phase III composts. Experimental composts were prepared from a range of straw, nitrogen and water sources. Commercial composts were obtained from four different commercial sites. The composts were analysed for moisture, nitrogen, ammonium-N, and ash contents and pH. The effect of different rates of spawn, using Phase III compost in place of spawn, and of spawn-running temperature on *Trichoderma* green mould infection was examined. The use of the supplement Natural Gold on the susceptibility of Phase III compost to *Trichoderma* infection was also examined.

Conclusions

Trichoderma aggressivum

- *Trichoderma aggressivum* f. *europeum* isolate 23443B applied as a spore suspension at spawning produced consistent green mould symptoms in the compost and on the casing surface, and suppressed mushroom yield across a wide range of experimental and commercial Phase II composts. Most of the mushrooms harvested from pots inoculated with this isolate had spotted cap symptoms.

- Green mould symptoms and subsequent mushroom crop loss occurred when *Trichoderma* was applied at 1.5×10^4 spores/kg compost. Green mould and crop loss became more severe when this concentration was increased.

- Several other *Trichoderma* isolates tested under the same conditions did not consistently produce green mould symptoms or suppress mushroom yields compared with an uninoculated control compost.

- *Trichoderma aggressivum* isolate 23443B applied to Phase III (spawn-run) compost at casing resulted in no visible green mould symptoms and did not significantly affect mushroom yield (Figure 1). The same inoculum of 23443B (8.8×10^5 spores/kg compost) applied to Phase II composts at spawning resulted in almost complete crop loss.

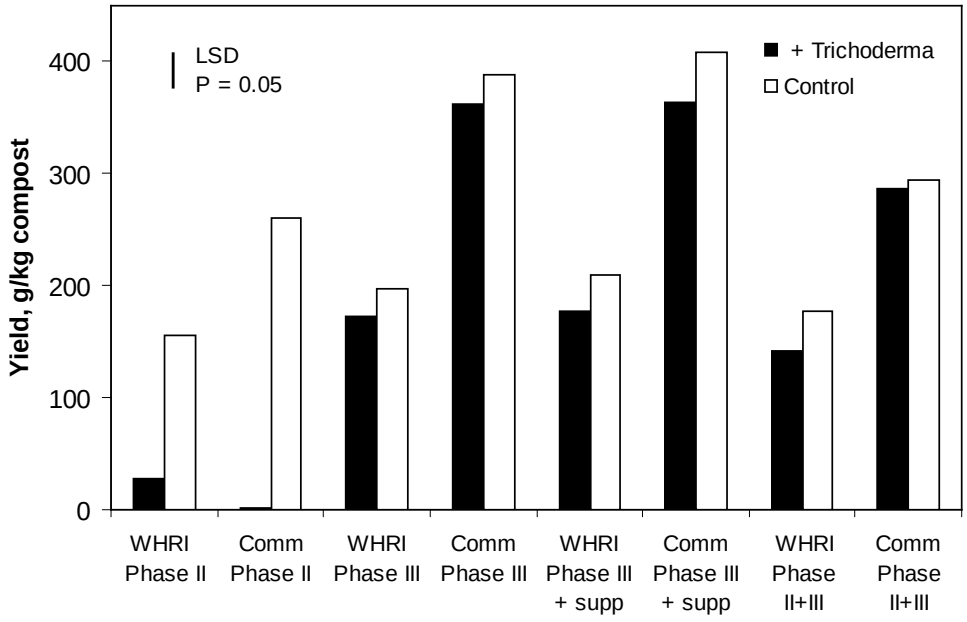

Figure 1. Effect on mushroom yield of inoculating different Phase II composts at spawning and Phase III composts (with and without supplementation) at casing with a spore suspension of *Trichoderma*.

- Mixing 33% Phase III compost with Phase II compost, in place of grain spawn, prevented the appearance of visible green mould symptoms following *Trichoderma* inoculation, and reduced mushroom yield loss to less than 15%, compared with a yield loss of over 95% when grain spawn was used. Mixing 25% Phase III compost with Phase II compost did not completely prevent the appearance of green mould symptoms following *Trichoderma* inoculation, and reduced mushroom yield loss to about 50%.

- The use of caccing (CI) in place of grain spawn reduced mushroom yield loss resulting from *Trichoderma* infection in one out of two composts. However, the population of *Trichoderma* propagules in both composts was increased compared with using grain spawn.

- Increasing the rate of grain spawn in compost from 0.25 to 0.85% reduced mushroom yield loss due to *Trichoderma* infection.

- Spawn-run temperature (20 or 25°C) or the use of Natural Gold supplement in Phase III compost had no significant effect on mushroom yield loss resulting from *Trichoderma* infection.

- Counts of *Trichoderma* propagules in compost at the time of casing did not correspond with mushroom yield loss, although composts with more than 1000 cfu/g compost produced very few mushrooms.

Penicillium implicatum

- *Penicillium implicatum* isolate 1043D applied as a spore suspension at spawning (4.7 × 106 spores/kg compost) consistently produced 'smoky mould' symptoms and suppressed mushroom yield across a wide range of experimental and commercial composts.

- Mushroom yield losses resulting from *Penicillium* infection of compost were more severe at higher compost moisture content.

- Infection of compost with *Penicillium* resulted in smaller mushrooms which opened prematurely.

- Counts of *Penicillium* propagules in compost at the time of casing did not correspond with mushroom yield loss, although composts with more than 6 × 105 cfu/g compost produced very few mushrooms.

- Spore suspensions of *Acremonium murorum* and *Pythium oligandrum* did not produce symptoms or reduce mushroom yield compared with an uninoculated control compost.

Financial benefits

- The identification of techniques to reduce compost mould growth will help to increase yields of marketable mushrooms.

- The exact financial benefits will vary depending on the level of reduction of moulds using the techniques.

Action points for composters and growers

- In the event of a *Trichoderma* green mould outbreak, composters and growers should consider increasing the rate of spawn used in Phase II compost.

- The effect of mixing proportions of Phase III compost with Phase II should be considered. A ratio of 1 Phase III: 2 Phase II gave a good suppressive effect on green mould. Further work will be needed to produce a non-grain spawn mushroom inoculum for the Phase III compost.

- The supplement Natural Gold can be used without affecting the susceptibility of Phase III compost to *Trichoderma* infection.

- In the event of an outbreak of a *Penicillium* 'smoky mould', composters should consider reducing compost moisture content.

Science Section

Introduction

There have been recent severe problems caused by *Trichoderma aggressivum* in the Netherlands and Poland (European form or Th2) and in North America (American form or Th4), although the reasons for these outbreaks have not been established, i.e. faults in hygiene and/or compost factors [1, 2]. Resistance of moulds to previously effective fungicides (carbendazim) has been reported in the USA [2]. Compost mould problems in the UK industry have been identified as green mould (Th2), 'smoky mould' (*Penicillium* species) and 'black compost' (*Pythium oligandrum*) although other moulds may also be responsible for crop loss [3, 4, 5, 6]. However, the level of mould growth in composts does not necessarily relate to the level of mushroom crop loss [7, 8].

There have been significant changes in compost formulations in recent years, particularly with regard to the use of organic, fungicide-free straw, reductions in poultry manure and increased use of other nitrogen sources. Goody water nutrient and oxygen levels have also been shown to vary widely (M3e) [9]. Composting technologies (windrow and bunker), durations, temperatures, aeration, and formulations vary widely in the UK, Dutch and Irish mushroom composting industries. Straw also varies in type and age. These compost factors may significantly affect the growth of moulds and subsequent crop loss.

Supplementation of Phase II is recognised as encouraging the growth of certain compost moulds and supplementation of Phase III, which is more widely practiced, has also been shown to encourage the growth of *Trichoderma* Th4 [10, 11]. The effects of spawn rate on compost mould growth are less established.

The overall aim of this project was to identify the properties of Phase II compost and associated compost ingredients and composting conditions and practices that affect the susceptibility of composts to infection and mushroom crop loss resulting from different moulds.

Commercial objectives

01 Identify the properties of Phase II compost (physical, chemical and/or microbial) and associated compost ingredients (straw, nitrogen sources, recycled water) and composting conditions (temperature, oxygen, moisture) and practices that affect the susceptibility of composts to infection from different moulds.

02 Determine the effect of compost factors on mushroom yield and/or quality losses resulting from infection from compost moulds.

03 Determine the effect of spawning and supplementation practices on mould competition.

04 Identify remedial action to reduce the susceptibility of composts to colonization by moulds.

Materials and methods

Compost moulds and molecular taxonomy of isolates

Isolates of compost moulds were obtained from the Warwick HRI culture collection and from commercial farms (through FERA York, from project M46).

Table 1. Compost moulds used in the experiments

Species	Isolate	Source
Acremonium murorum	BPW24B	Warwick HRI
Penicillium implicatum	1043D	Warwick HRI
Pythium oligandrum	291	Warwick HRI
Trichoderma aggressivum f. *europeaeum* (Th2)	T7	Warwick HRI
Trichoderma aggressivum f. *europeaeum* (Th2)	23443B	FERA York
Trichoderma harzianum (Th1)	278	Warwick HRI
Trichoderma harzianum (Th1)	24651	FERA York

Depending on the experiment, spore suspensions of each mould containing between 1×10^3 and 1×10^8 spores/mL of each mould were prepared by flooding cultures produced on agar plates. The suspensions (35 mL) were then sprayed into 10 kg spawned Phase II or Phase III compost contained within plastic bags, which were closed and shaken periodically during spraying. The same volume of sterile water was used as a control.

Samples of compost were analysed for mould populations after inoculation, at casing, and at the end of the second flush. The procedure for analysis is described in Grogan & Harvey [4]. For each compost sample, a 20 g sub-sample was put in a sterile homegeniser bag with 360 mL sterile water. After soaking for 1 hour, the sample was homogenised in a 'Stomacher 400' laboratory blender for 2 × 1 min with a 5 min interval. The resulting compost extract was then serially diluted with sterile water to give a concentration of 1×10^0 to 1×10^5. A 1-mL aliquot of each dilution was pipetted into a series of sterile Petri dishes. Molten Ohio Agricultural

Experimental Station medium, held at 50˚C, was then poured into the dishes, which were then incubated at 25˚C. The numbers of colonies were recorded after 3, 5 and 7 days, which were then used to calculate the number of colony forming units (cfu) per g fresh weight of compost. Pieces of compost from the samples were also plated out on malt agar + Streptomycin + Chloramphenol to confirm whether mushroom mycelium and/or mould species would grow.

Production of composts and cropping procedure

Experimental composts were prepared in windrows (17 day pre-wet and Phase I) and bulk Phase II tunnels as described in Noble *et al*.[13]. The standard compost was prepared from new season wheat straw with broiler poultry manure and gypsum added at 600 kg and 70 kg per tonne of straw respectively. Fresh water was used throughout to produce compost with a moisture content at spawning of 71±1%. Analyses were conducted on samples of the Phase II and spawn-run composts. Dry matter (DM), Nitrogen (N), ammonium (NH_4^+) and ash contents and pH were determined as described in Noble & Gaze [14].

Unless otherwise stated, pasteurised compost (10 kg) was inoculated with rye grain mushroom spawn (spawned) at 0.5% of the fresh weight of compost with *Agaricus bisporus* spawn (Sylvan A15) and filled into plastic bags and spawn-run at 25°C unless stated. Depending on the treatment, a spore suspension or sterile water was sprayed into the bags at spawning as previously described. After 16 days, the compost from each bag was filled into three replicate plastic pots, 230 mm diameter x 220 mm depth. The pots were cased with a moist mixture of peat and sugar beet lime (850 g) containing casing inoculum of the strain A15 at 1% w/w. When mushroom mycelium was visible on the surface of the casing, the containers were transferred to a controlled environment chamber with an air temperature of 18°C, relative humidity of 90% and a CO_2 concentration of 0.1% to induce fruiting. Three flushes of mushrooms were harvested daily over a 17 day period (cap diameter 25-30 mm).

Commercially produced Phase II and spawn-run composts

Pasteurised (Phase II) compost samples were obtained from four UK composting sites on three occasions. Bulk spawn-run (Phase III) or tray spawn-run composts

were also obtained from two of the sites on two occasions.

Effect of Trichoderma isolates on mushroom cropping

Phase II composts from WHRI and a commercial site were inoculated at spawning with 35 mL of suspensions containing 2.6 – 8.3 $\times$ 10^7 spores/mL of the four *Trichoderma* isolates in Table 1. The same volume of sterile water was used as a control. Commercial Phase III compost was also inoculated at casing with a suspension containing 3.3 $\times$ 10^7 spores/mL of *Trichoderma aggressivum* f. *europeaeum* 23443B.

Effect of Acremonium, Penicillium and Pythium isolates on mushroom cropping

Phase II compost from a commercial site was inoculated at spawning with suspensions containing 2.9 $\times$ 10^7 spores/mL of *Acremonium murorum*, 3.1 $\times$ 10^7 spores/mL of *Penicillium,* or 1.7 $\times$ 10^5 spores/mL of *Pythium oligandrum* (Table 1). The *Pythium* isolate produced spores much less profusely than the other moulds so a much larger number of plates was needed to produce the inoculum.

Effect of Penicillium and Trichoderma on mushroom cropping in experimental and commercial composts

Composts were prepared according to the standard method previously described using new season wheat straw, and from 1-year old wheat straw and barley straw (Table 2). Compost was also prepared from horse manure in place of wheat straw and poultry manure. Composts at spawning had a moisture content of 66 - 75 %w/w. The following composts were also prepared using the ingredients of the standard wheat straw compost:

- ‘dry’ compost with moisture content at spawning of 65%
- ‘wet’ compost with a moisture content at spawning of 78%
- strawy compost, which was filled into Phase II after 7 days
- mature compost, which was composted for a 24-day Phase I.

Table 2. Percentage by weight of ingredients used in experimental composts, excluding added water. Gypsum (or lime for the inorganic treatment) was added at 4% of the total weight of the other ingredients.

Treatment name	Straw		Poultry manure	Other ingredient	
	Type	w/w		Type	w/w
Standard wheat	Wheat	63	38	none	0
Old wheat	Wheat	63	38	none	0
Barley	Barley	63	38	none	0
Horse manure	none	0	0	Horse manure	100
Inorganic	Wheat	75	23	Ammonium sulphate Urea	1.3 0.7

Two replicate batches of the standard wheat straw compost and a single batch of the other seven experimental composts were prepared. Phase II compost was also obtained from a commercial composting site for comparison with the experimental composts. Suspensions containing 2.8×10^6 or 2.8×10^7 *Penicillium* spores/mL, or 4×10^6 or 2.8×10^7 *Trichoderma* (23443B) spores/mL were applied to the composts at spawning.

A further three experimental composts were prepared, with the following modifications to the standard wheat straw compost:

- inorganic nitrogen (ammonium sulphate and urea) replacing 40% of the poultry manure (Table 2)
- 'goody water' compost in which fresh water was replaced by stored goody water
- anaerobic compost, which was not turned during the 17-day Phase I.

Each of these composts, together with a further three replicate batches of the standard compost, and five batches of Phase II compost from four different commercial sites were inoculated with a suspension of 4×10^6 *Trichoderma* 23443B spores/mL at spawning.

Effect of Trichoderma on Phase II, Phase III, and supplemented composts and blends of Phase II and Phase III composts

Phase II and Phase III composts were prepared according to the standard method previously described, and were also obtained from a commercial composter. For each source of composts, the following treatments were examined:

- Phase II compost, spawned at 0.5% w/w

- Phase III compost
- 2:1 Blend of Phase II and 1 Phase III composts
- Phase III compost + 0.8 % w/w Natural Gold supplement.

A suspension containing 2.5×10^5 *Trichoderma* 23443B spores/mL (35 mL) or similar volume of sterile water was applied to 10 kg bags containing the above treatments.

Effects of spawning rate and spawn-running temperature on Trichoderma infection

Phase II compost was prepared according to the standard method previously described, and was also obtained from a commercial composter. For both sources of composts, the following treatments were applied:

- Spawned with grain spawn at standard rate (0.5 % w/w)
- Spawned with caccing at 10% w/w
- 3:1 Blend of Phase II and Phase II composts.

A suspension containing 5.77×10^5 *Trichoderma* 23443B spores/mL (35 mL) or a similar volume of sterile water was applied to 10 kg bags containing the above treatments.

A further batch of the standard Phase II compost was prepared and the following treatments were applied:

- Spawned with grain spawn at standard rate (0.5 % w/w), spawn-run at 25°C
- Spawned with grain spawn at 0.25 % w/w, spawn-run at 25°C
- Spawned with grain spawn at 0.85 % w/w, spawn-run at 25°C
- Spawned with grain spawn at standard rate (0.5 % w/w), spawn-run at 20°C

Suspensions containing either 4.2×10^3 or 5.94×10^5 *Trichoderma* 23443B spores/mL (35 mL) or a similar volume of sterile water was applied to 10 kg bags containing the above treatments.

Results

Effect of Trichoderma isolates on mushroom cropping

Trichoderma counts following application of spore suspensions to the different Phase II and Phase III composts are shown in Table 1. Generally, there was an increase in *Trichoderma* counts in Phase II composts from the time of application at spawning until the end of the second flush. At the time of casing, the highest propagule counts

were in isolate *T. aggressivum* 23443B. For this isolate as well as Th1 278 and Th1 24561A, the propagule counts at casing were higher in the commercial compost than in the WHRI compost. No *Trichoderma* was detected in the control treatment.

Mushroom yield from the commercial compost was significantly higher than from the WHRI compost, with or without the *Trichoderma* inocula (Figure 2). Th1 isolates 278 and 24651A and *T. aggressivum* isolate 23443B applied at spawning resulted in severe green mould infection and almost complete suppression of mushroom yield in the WHRI Phase II compost (Figure 2). However, only isolate 23443B completely suppressed mushroom yield in the commercial compost, whereas isolates 278 and 24651A resulted in only a small reduction in yield. *T. aggressivum* isolate T7 had no significant effect on mushroom yield in either Phase II compost. During cropping, green spores of isolate 23443B became visible on the surface of the casing, and mushroom fruitbodies were severely spotted. *T. aggressivum* isolate 23443B applied to Phase III compost at casing resulted in a normal mushroom yield and no visible green mould in the compost or casing or cap spotting.

Table 1. *Trichoderma* spore counts in the initial inoculum and colony forming unit counts in composts at spawning, casing and end of second flush.

Isolate	Compost	Phase	Inoculum spores/mL	At spawning cfu/g	At casing cfu/g	End 2nd flush cfu/g
Control	WHRI	II	0	n.d.	0	0
Control	commercial	II	0	0	0	0
Th1 278	WHRI	II	4.24×10^7	n.d.	9.23×10^4	3.34×10^7
Th1 278	commercial	II	4.24×10^7	1.27×10^5	1.88×10^5	n.d.
Th1 24561A	WHRI	II	3.72×10^7	n.d.	6.53×10^3	1.33×10^7
Th1 24561A	commercial	II	3.72×10^7	8.60×10^4	9.36×10^5	n.d.
T a T7	WHRI	II	5.64×10^7	n.d.	6.30×10^4	4.95×10^5
T a T7	commercial	II	5.64×10^7	1.82×10^5	4.46×10^4	n.d.
T a 23443B	WHRI	II	6.76×10^7	n.d.	2.61×10^5	5.85×10^7
T a 23443B	commercial	II	6.76×10^7	2.14×10^5	2.35×10^6	n.d.
T a 23443B	WHRI	III	3.36×10^7	n.d.	1.92×10^4	n.d.
T a 23443B	commercial	III	3.36×10^7	n.d.	1.84×10^4	n.d.

n.d. not determined

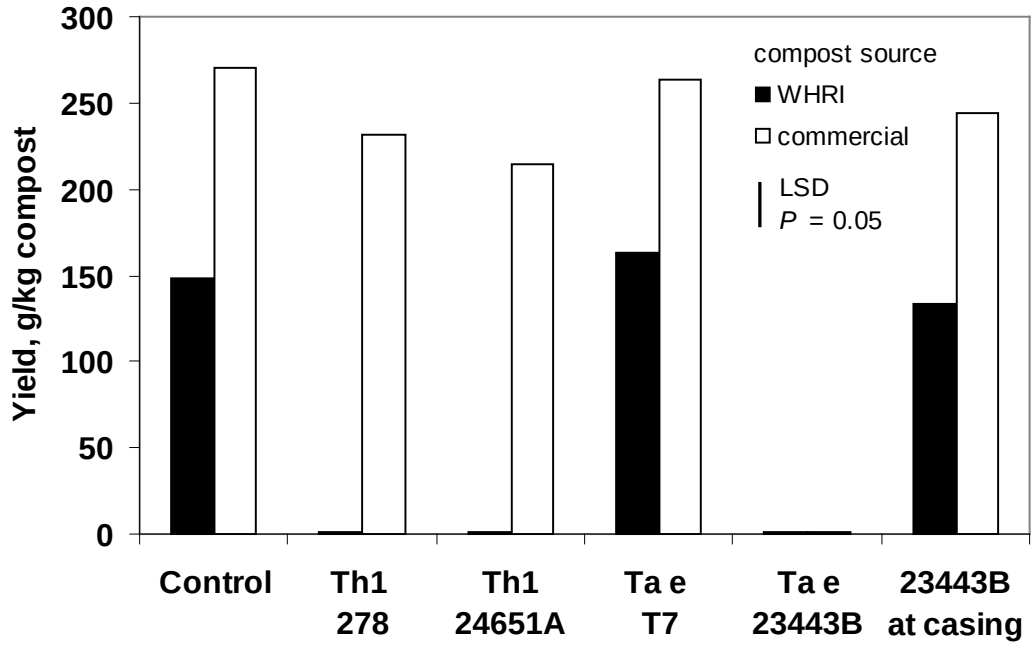

Figure 2. Effect on mushroom yield of *Trichoderma* isolates applied to Phase II composts at spawning, and *Trichoderma* isolate 23443B applied to Phase III composts at casing.

Effect of Acremonium, Penicillium and Pythium isolates on mushroom cropping

The counts of *Penicillium* were higher than those of *Acremonium* throughout the mushroom culture period (Table 2). Plates for *Pythium* became contaminated with other moulds so could not be counted. None of the moulds were detected in the control treatment.

The mushroom spawn run of composts inoculated with suspension of *Acremonium* or *Pythium* spores at spawning looked healthy and subsequent mushroom yields were not significantly different from the control yields (Figure 3). Compost inoculated with *Penicillium* spores at spawning produced characteristic clouds of spores at casing (smoky mould) and mushroom mycelial growth was poor. Subsequent mushroom yields were severely reduced compared with the untreated control. Mushrooms harvested from the *Penicillium* inoculated pots were usually small and tended to open early. There was no visible mould growth in the control treatment.

Table 2. Spore counts in the initial inoculum and colony forming unit counts in

composts at spawning, casing and end of second flush.

Compost mould	Inoculum spores/mL	At spawning cfu/g	At casing cfu/g	End 2nd flush cfu/g
Control	0	0	0	0
Acremonium murorum	2.88×10^7	2.88×10^3	6.93×10^4	1.27×10^4
Penicillium implicatum	3.12×10^7	3.34×10^4	2.34×10^5	2.57×10^4
Pythium oligandrum	1.67×10^5	not detected	not detected	not detected

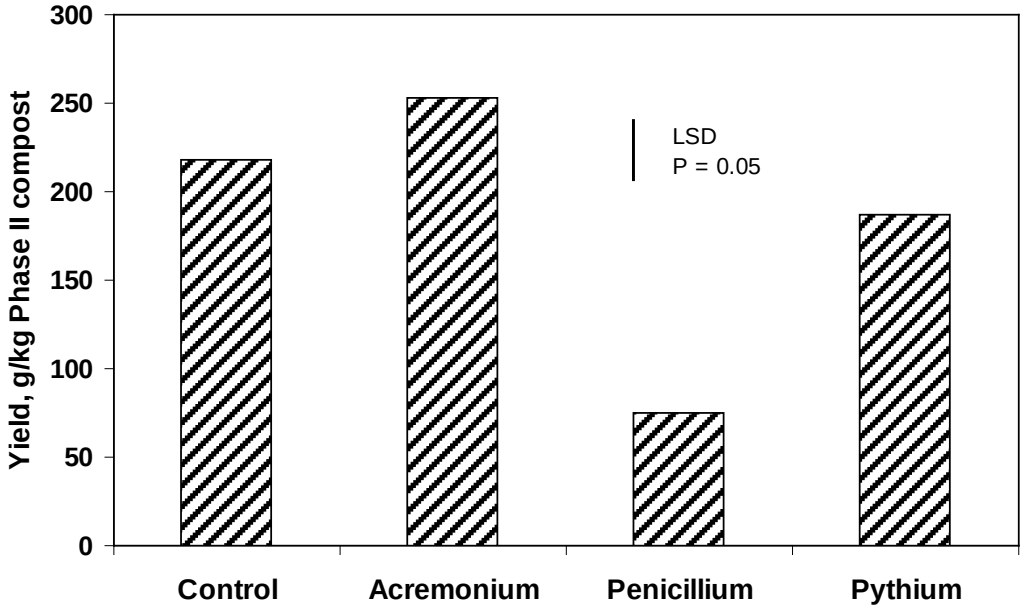

Figure 3. Effect on mushroom yield of different compost moulds applied to Phase II compost at spawning.

Effect of Penicillium and Trichoderma on mushroom cropping in experimental and commercial composts

At the time of casing, differences in compost counts for *Penicillium* and *Trichoderma* between the low and high inoculation rates were generally small (Table 3). *Penicillium* counts in the different composts were fairly similar. *Trichoderma* counts were highest in the two WHRI standard wheat straw composts and lowest in the wet, strawy and commercial composts (Table 3). *Trichoderma* could not be detected in the commercial composts following the low inoculation rate, or in any of the uninoculated control composts.

Table 3. *Penicillium* and *Trichoderma* counts in different composts (cfu/g) at casing, in the control and following inoculation at low and high spore rates at spawning.

Compost	*Penicillium*		*Trichoderma* 23443B		Control
	Low	High	Low	High	
Wheat standard 1	1.83×10^5	2.83×10^5	2.39×10^8	4.60×10^8	0
Wheat standard 2	-	1.25×10^6	-	5.99×10^8	0
Old wheat	1.91×10^5	3.22×10^4	3.63×10^6	1.63×10^7	0
Barley	1.67×10^5	5.31×10^5	2.07×10^5	4.36×10^5	0
Horse manure	1.77×10^5	2.52×10^5	7.79×10^5	5.72×10^6	0
Dry (wheat)	-	7.06×10^5	-	1.17×10^3	0
Wet (wheat)	-	9.95×10^5	-	9.00×10^1	0
Stawy (wheat)	-	7.79×10^5	-	9.90×10^2	0
Mature (wheat)	-	8.96×10^5	-	7.74×10^5	0
Commercial Phase II	1.63×10^5	2.88×10^5	0	9.36×10^2	0

The *Trichoderma* inoculum resulted in a poorer spawn-run than the control, and the high rate resulted in a poorer spawn-run than the low rate. At the time of casing, green mould symptoms were generally only visible in the compost inoculated with the high rate of spores. However, both the low and high *Trichoderma* inoculation rates resulted in almost complete suppression of mushroom yield in all the composts, except in the commercial compost where a yield loss of 20-48% occurred (Figures 4 and 5). Green spores were visible on the surface of the casing of most of the Trichoderma inoculated pots, and most of the harvested mushrooms were spotted.

The high and low rates of *Penicillium* inoculum also severely suppressed yields in most of the composts except the dry or strawy experimental composts and the commercial compost (Figures 4 and 5). Mushroom yields (with or without *Trichoderma* or *Penicillium* inoculum) were significantly higher from the commercial compost than from the experimental composts (Figure 4). Yield from the wheat straw composts (new or old straw, standard moisture, dry, mature) were significantly better

than from the barley or horse manure composts and the wet or strawy wheat straw composts (Figure 5).

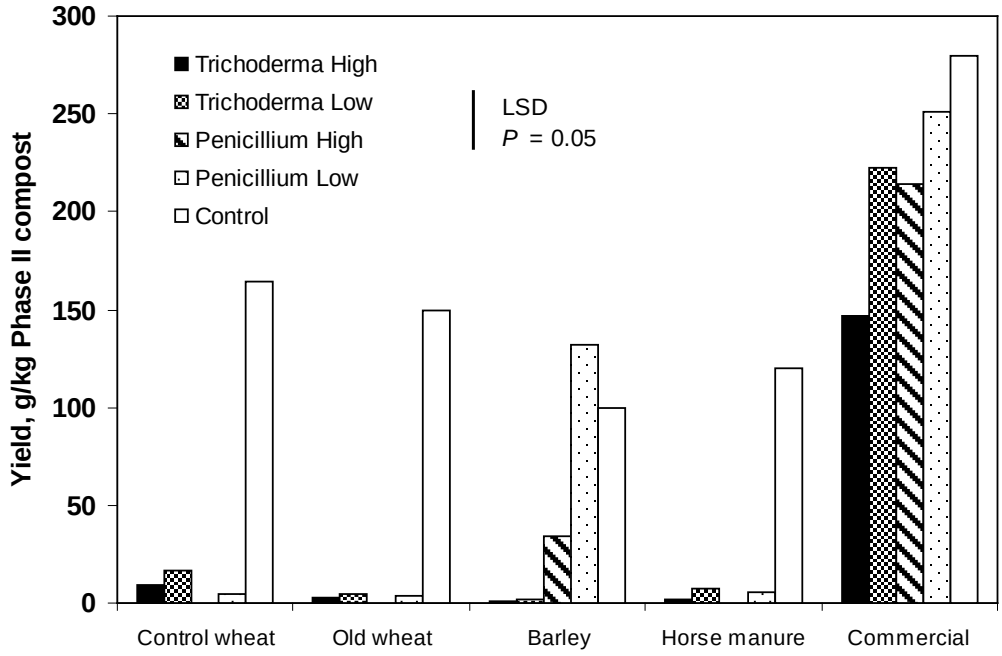

Figure 4. Effect on mushroom yield of inoculating different experimental and commercial composts with two different concentrations of spore suspensions of *Penicillium* or *Trichoderma* at spawning.

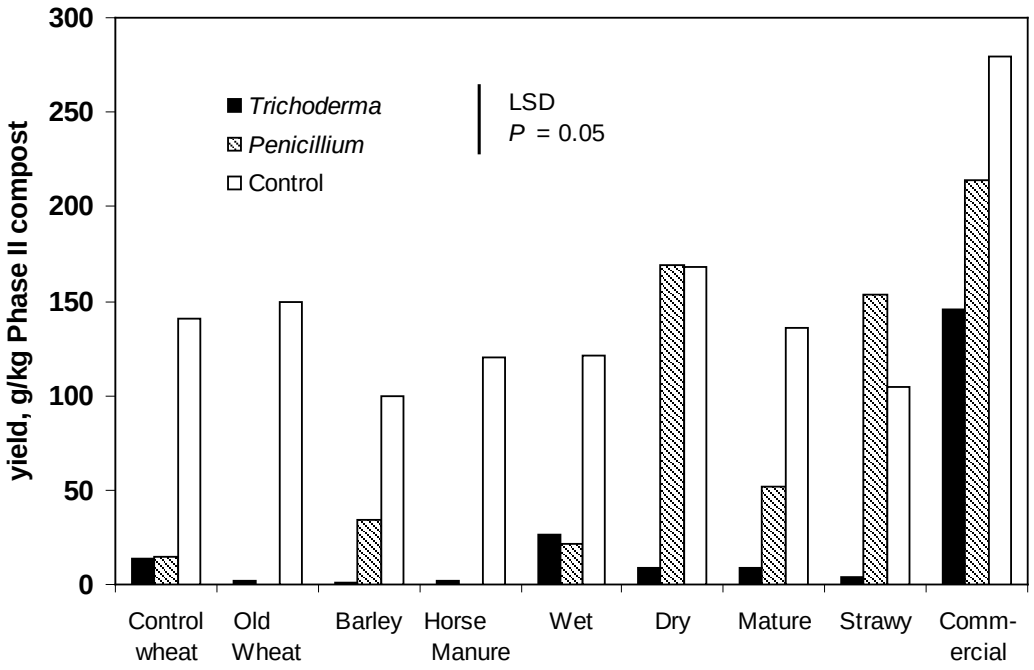

Figure 5. Effect on mushroom yield of inoculating different experimental and commercial composts with spore suspensions of *Penicillium* or *Trichoderma* at spawning.

Trichoderma counts at casing in further experimental composts (standard wheat straw/ poultry manure compost, anaerobic compost, and composts prepared with inorganic nitrogen sources or goody water) are shown in Table 4. Colony forming unit counts in the standard, inorganic nitrogen and goody water composts were similar, but the count in the anaerobic compost was significantly higher. No *Trichoderma* was detected in any of the uninoculated composts.

Mushroom yield was higher in the standard wheat straw/ poultry manure compost than the anaerobic compost and composts prepared with inorganic nitrogen or goody water instead of fresh water (Figure 6). *Trichoderma* inoculum severely reduced mushroom yield in the standard compost and almost completely suppressed mushroom production in the other experimental composts. The effect was most severe in the anaerobic compost, which failed to produce any fruitbodies.

Table 4. *Trichoderma* counts at casing (cfu/g compost) in different standard and experimental composts inoculated with *Trichoderma* spores at spawning.

Compost	Standard	Anaerobic	Inorganic nitrogen	Goody water
cfu/g compost	3.36×10^3	1.05×10^7	3.65×10^3	1.81×10^4

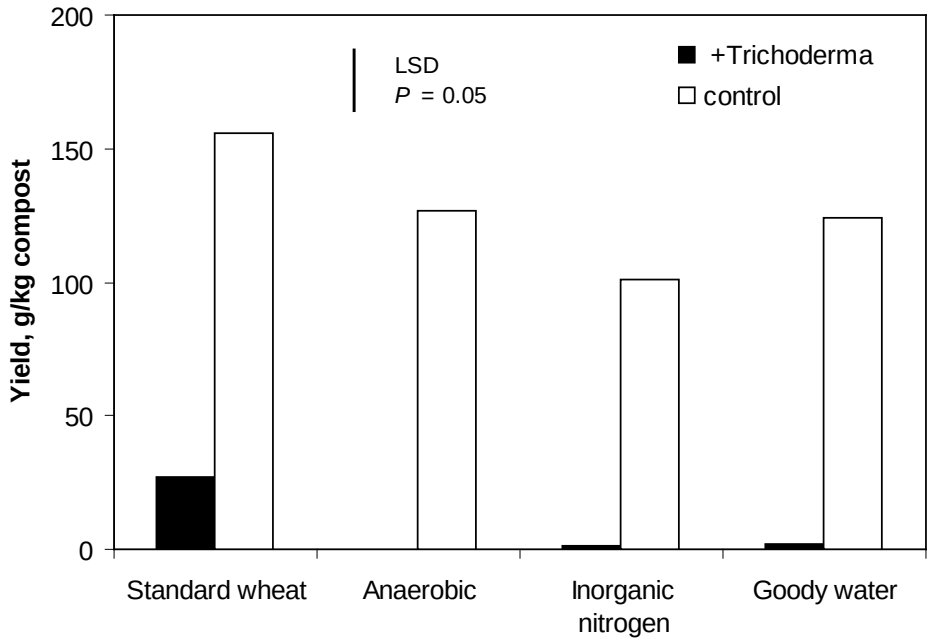

Figure 6. Effect on mushroom yield of inoculating different standard and experimental composts with a spore suspension of *Trichoderma* at spawning.

Trichoderma counts at casing in standard and commercial composts are shown in Table 5. The levels were variable, even between batches of the same source of compost. The counts did not correspond with the mushroom yield loss resulting from green mould since the first batch of compost A had the highest *Trichoderma* counts at casing but was not the most severely affected compost in terms of mushroom yield loss (Figure 7). No *Trichoderma* was detected in any of the uninoculated composts at casing. Spawn-run of the *Trichoderma* inoculated composts was poorer than that of the uninoculated composts at casing.

Table 5. *Trichoderma* counts at casing (spores/g compost) in different standard and commercial composts inoculated with *Trichoderma* spores at spawning.

Compost	Standard	A	B	C	D
Batch 1	2.68×10^5	2.81×10^8	5.22×10^4	4.50×10^7	4.77×10^7
Batch 2	3.36×10^3	1.22×10^6	_	_	_

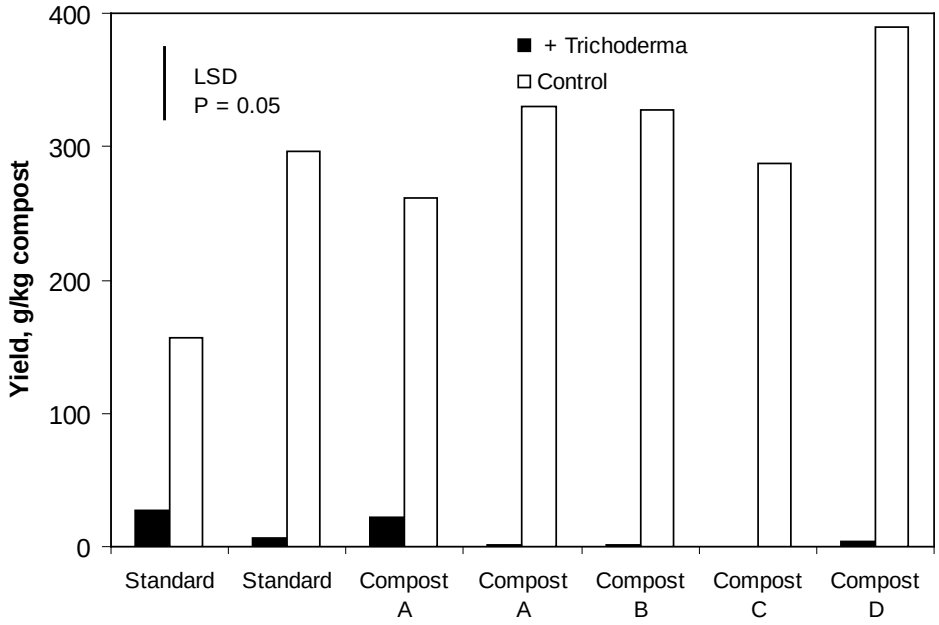

Figure 7. Effect on mushroom yield of inoculating different standard and commercial composts with a spore suspension of *Trichoderma* at spawning.

Effect of Trichoderma on Phase II, Phase III, and supplemented composts and blends of Phase II and Phase III composts

At the time of casing, both inoculated Phase II composts showed symptoms of green mould infection and the spawn run was poor. In the absence of *Trichoderma*, mushroom yield was significantly better in the commercial Phase II compost than the WHRI Phase II compost (Figure 1 repeated below). However, the *Trichoderma* inoculum applied at spawning depressed yields in both composts to a very low level. Conversely, the *Trichoderma* inoculum applied to the other treatments at casing had only a small or non-significant effect on mushroom yield. Mushroom yields from the Phase II+III treatment were intermediate between the yields obtained from the individual Phase II and III composts. The commercial Phase III compost produced a higher yield than the WHRI Phase III (spawn-run) compost. The Natural Gold

supplement did not have a significant effect on mushroom yield in either Phase III compost and did not affect the susceptibility of the compost to green mould infection.

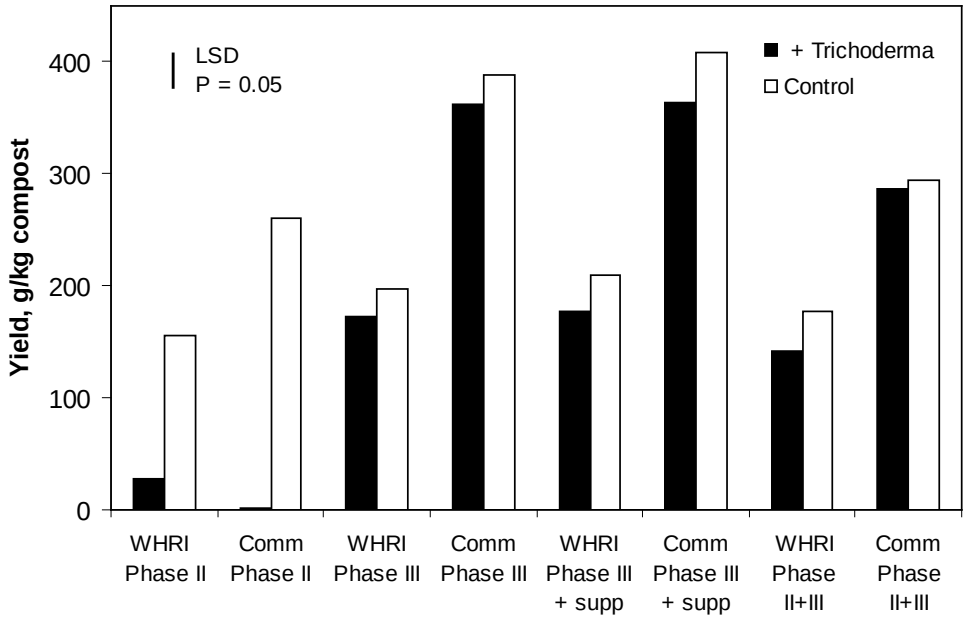

Figure 1. Effect on mushroom yield of inoculating different Phase II composts at spawning and Phase III composts (with and without supplementation) at casing with a spore suspension of *Trichoderma*.

Effect of Mushroom inoculum on Trichoderma infection

Inoculating WHRI and commercial Phase II composts with caccing (CI) at 10% w/w resulted in a higher *Trichoderma* count at casing than inoculation with grain spawn at 0.5% w/w (Table 6). Both of these inoculation methods resulted in visible green mould symptoms at the time of casing.

In the absence of *Trichoderma* inoculum, mushroom yields from the commercial compost were slightly higher than those from the WHRI compost. No *Trichoderma* was detected in the uninoculated composts. Mushroom yield from both composts spawned with grain spawn at 0.5% and the commercial compost spawned with 10% caccing was almost completely suppressed by inoculation with *Trichoderma*. Yield from the WHRI compost with 10% caccing was reduced by 50% compared with uninoculated control (Figure 8). Mushroom yields from the composts inoculated with spawn-run compost were lower than those inoculated with grain spawn. However, the effect of *Trichoderma* on these composts was less severe, with

the *Trichoderma* inoculated compost producing more than 50% of the yield of uninoculated control composts.

Table 6. *Trichoderma* counts at casing (spores/g) in composts inoculated with *Trichoderma* spores at spawning with different types of mushroom inoculum.

Mushroom inoculum, % w/w	WHRI compost	Commercial compost
Grain spawn, 0.5	2.67×10^5	5.22×10^5
Caccing, 10	1.98×10^8	5.49×10^7
Spawn-run compost, 25	9.90×10^2	0

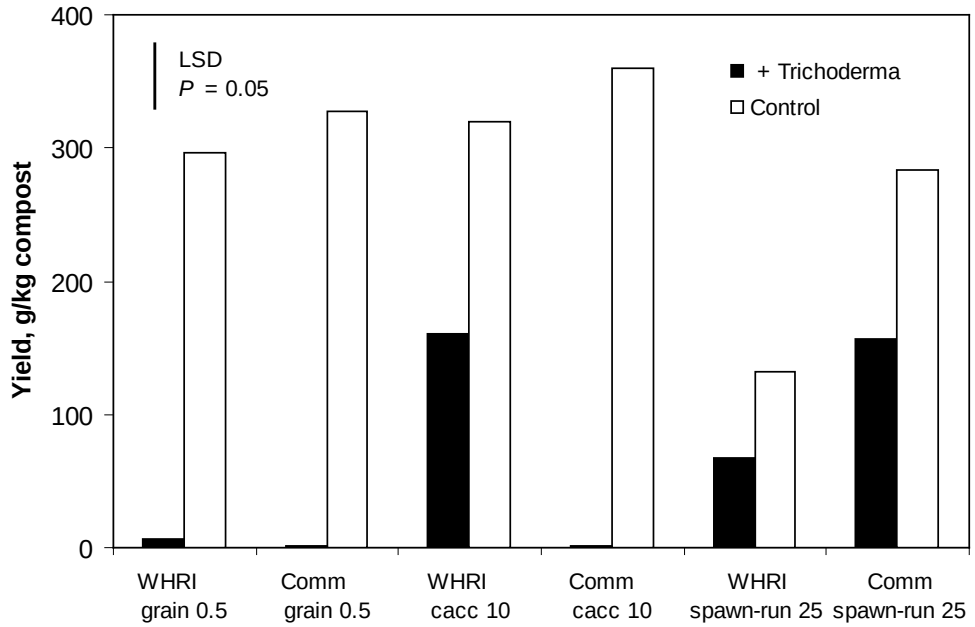

Figure 8. Effect of *Trichoderma* on mushroom yields from composts inoculated with different types of mushroom inoculum (0.5% grain spawn, 10 caccing, CI or 25% spawn-run compost)

Effects of spawning rate and spawn-running temperature on Trichoderma infection

The *Trichoderma* inoculum with a higher concentration of spores resulted in a higher propagule count in the compost at casing than the less concentrated inoculum (Table 7). Green mould symptoms in the compost and subsequent mushroom crop loss occurred at both rates of *Trichoderma* spore inoculum, but were more severe at the higher rate (Figure 10). At the higher rate of *Trichoderma* inoculation, the rate of spawning with grain spawn and the spawn-running temperature had no significant effect on the *Trichoderma* propagule count in the compost at casing (Table 7). At the lower rate of *Trichoderma* inoculation, *Trichoderma* propagule counts in the compost

at casing were higher after spawn-run at 25C and at the intermediate rate of spawning (0.5%). No *Trichoderma* was detected in the uninoculated composts at casing.

Mushroom yields in the absence of *Trichoderma* from the different spawning rate and spawn-run temperatures were not significantly different (Figure 9). Spawn-running temperature also had no effect on yields in the presence of *Trichoderma* inoculum. The effect of *Trichoderma* on mushroom yield declined with increasing spawn rate from 0.25 to 0.85%.

Table 7. *Trichoderma* counts at casing (spores/g) in composts inoculated with *Trichoderma* spores at spawning with different rates of mushroom grain spawn and spawn-run at 20 or 25°C.

	Spawn-running compost temperature			
	20°C		25°C	
Spawn/*Trichoderma* rate	4.20×10^3	5.94×10^5	4.20×10^3	5.94×10^5
0.25%	-	-	2.21×10^3	7.11×10^8
0.50%	2.48×10^5	6.12×10^8	5.40×10^7	1.17×10^8
0.85%	-	-	3.11×10^5	1.97×10^8

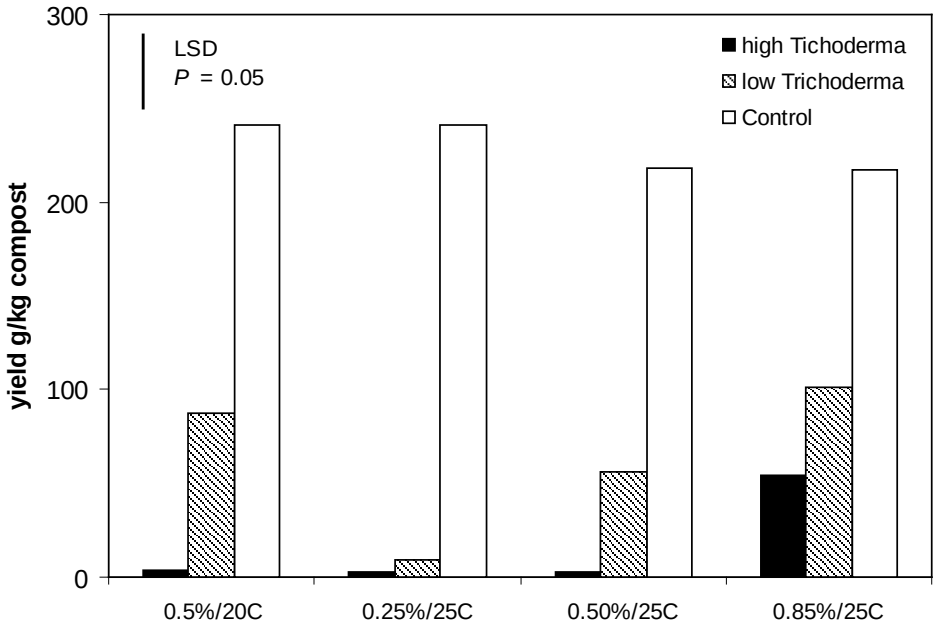

Figure 9. Effect of *Trichoderma* on mushroom yields from composts inoculated with different rates of grain spawn and spawn-run at 20 or 25°C.

Relationships between compost analysis and infection by Penicillium and Trichoderma

Data from the above experiments was combined in order to test if there were relationships between the analysis of the Phase II composts and the yield loss resulting from inoculation with *Penicillium* or *Trichoderma*. Only treatments in which grain spawn was used as mushroom inoculum were included.

There was a significant negative relationship between compost moisture content at spawning and yield loss resulting from *Penicillium* infection (Figure 10). There were no significant relationships between compost pH or nitrogen or ammonium contents and mushroom yield loss due to *Penicillium*. There was no significant relationship between the *Penicillium* propagule count in compost (cfu/g compost) at casing and subsequent mushroom yield loss. However, composts with more than 6×10^5 cfu/g compost at casing produced very few mushrooms (Figure 11).

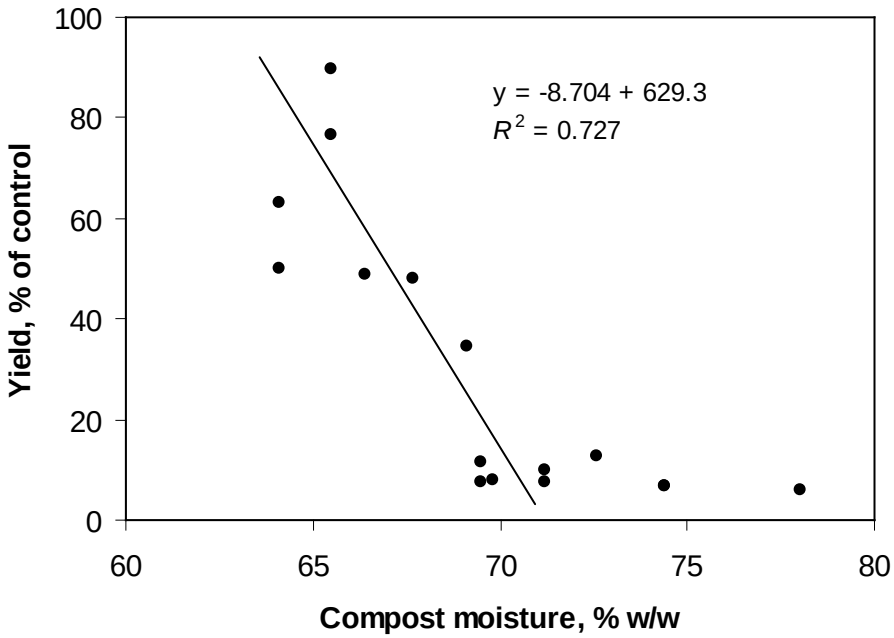

Figure 10. Relationship between compost moisture at spawning and mushroom yield loss from *Penicillium*, expressed as percentage yield from uninoculated control. The equation is for compost moisture between 64.1 and 72.6%.

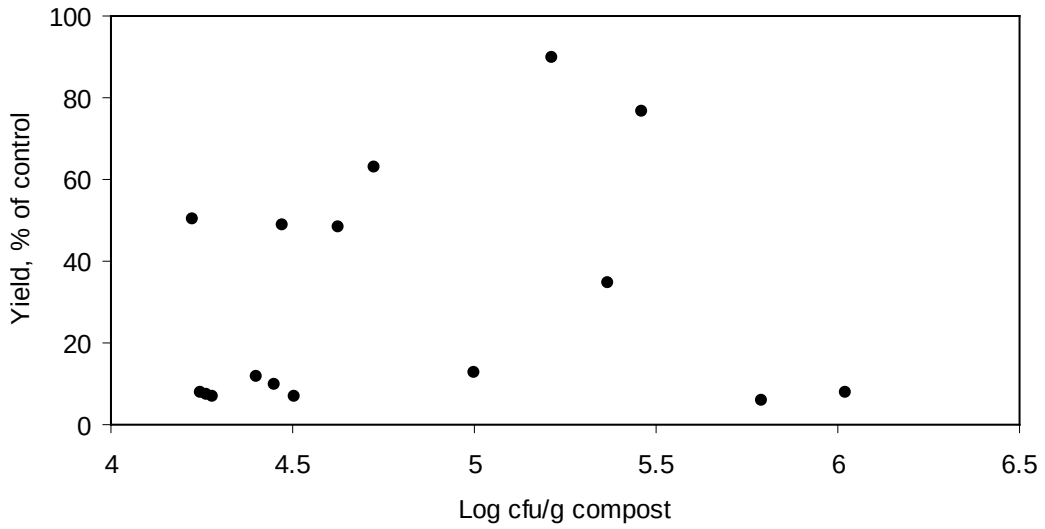

Figure 11. Relationship between *Penicillium* propagule count in compost at casing (log scale) and mushroom yield loss, expressed as percentage yield from uninoculated control.

Trichoderma propagule counts in compost at casing greater than 1000 cfu/g compost resulted in almost complete suppression of mushroom yield (Figure 12).

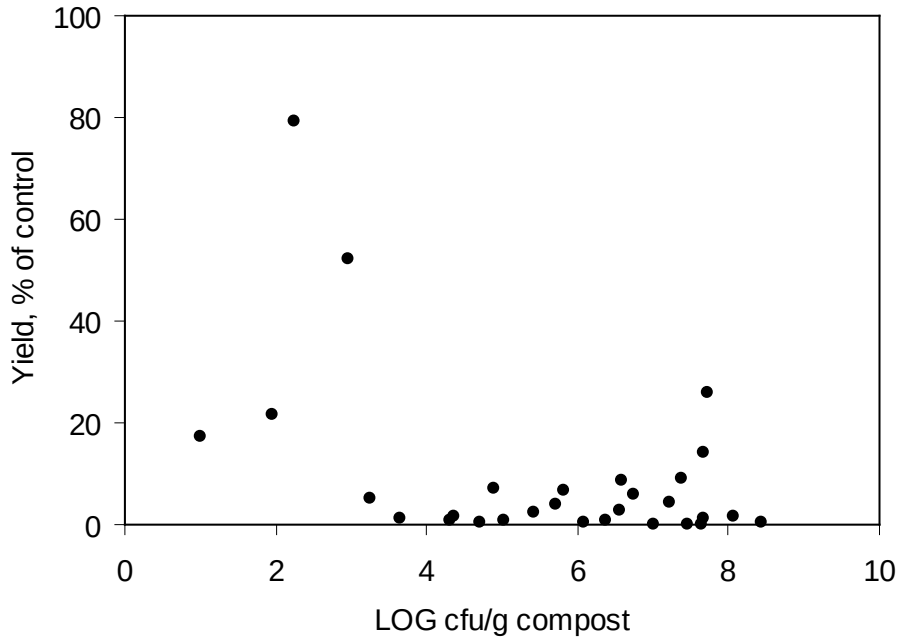

Figure 12. Relationship between *Trichoderma* propagule count in compost at casing (log scale) and mushroom yield loss, expressed as percentage yield from uninoculated control.

There were no significant relationships between any of the Phase II compost factors analysed (moisture, nitrogen, ammonium N and ash contents or pH) and the susceptibility of composts to green mould infection. Only one of the Phase II

composts tested (a commercial compost used in the first experiment) showed any tolerance to *Trichoderma* green mould infection. However, the analysis of this compost was similar to other '*Trichoderma* susceptible' Phase II composts. The average and range of Phase II and Phase III compost analytical factors in these experiments is shown in Tables 8 and 9.

Table 8. Average, maximum and minimum analytical values of Phase II composts used in the *Penicillium* and *Trichoderma* experiments.

	Moisture %	Nitrogen % of DM	NH_4+ % of DM	Ash % of DM	pH
average	69.9	2.68	0.043	27.2	7.44
maximum	78.0	3.30	0.191	36.5	7.92
minimum	61.2	1.92	0.003	19.8	6.59

Table 9. Average, maximum and minimum analytical values of Phase III composts used in the *Trichoderma* experiments.

	Moisture %	Nitrogen % of DM	NH_4+ % of DM	Ash % of DM	pH
average	69.6	2.90	0.34	29.0	6.13
maximum	73.5	3.22	0.51	32.3	6.33
minimum	65.8	2.64	0.20	27.1	5.92

Discussion

The results of this work confirm previous work[15] that has shown mushroom grain spawn to be an important factor in green mould infection of Phase II compost. Phase III compost was relatively tolerant of infection by *Trichoderma* spores, at spore concentrations that caused almost complete crop loss in Phase II compost at spawning. Avoiding the use of grain spawn by mixing a proportion of colonised Phase III compost with Phase II compost reduced or prevented the ingress of *Trichoderma* and subsequent green mould symptoms. Previous work[16] has shown that a non-grain spawn inoculum (Speedy Inoculum) may have a similar effect. Increasing the rate of grain spawn (from 0.25 to 0.85% w/w) also reduced mushroom

crop loss from *Trichoderma*, presumably by increasing the rate at which mushroom mycelium colonises the compost before *Trichoderma* can have a negative effect.

The isolate of *T. aggressivum* 23443B, produced green mould symptoms and significant mushroom crop loss when applied at 1.5×10^4 spores/kg compost. Previous work[15] with a different isolate of *T. aggressivum* showed no effect of 100 spores/kg compost but almost complete mushroom crop loss at 10^5 spores/ kg compost.

This work has shown that *Trichoderma aggressivum* isolate 23443B can cause green mould symptoms on a wide range of experimental and commercial Phase II composts. Only one (commercial) compost showed any tolerance to infection at the spore concentrations used, although the analysis of this compost was within the range of other '*Trichoderma* susceptible' composts. Previous work [2,7] has indicated that certain composts (such as very degraded or anaerobic composts) are more susceptible to *Trichoderma* infection. The present work indicates that such factors are unnecessary for *Trichoderma* to gain a foothold in the compost. The presence of grain spawn appears to be a much more important factor in stimulating *Trichoderma* growth.

This work has confirmed previous work[4] that *Penicillium* is a more aggressive competitor mould in mushroom compost than *Acremonium* or *Pythium*. However, the spore concentrations needed to produce a comparable negative effect on mushroom yield are in the order of 100 times higher than those for *Trichoderma aggressivum*. The mushroom symptoms resulting from *Penicillium* infection of compost (small size and premature opening) were similar to those previously reported [4].

Conclusions

Trichoderma aggressivum

1. *Trichoderma aggressivum* f. *europeum* isolate 23443B applied as a spore suspension at spawning produced consistent green mould symptoms in the compost and on the casing surface, and suppressed mushroom yield across a wide range of experimental and commercial Phase II composts. Most of the mushrooms harvested from pots inoculated with this isolate had spotted cap symptoms.

2. Green mould symptoms and subsequent mushroom crop loss occurred when *Trichoderma* was applied at 1.5×10^4 spores/kg compost. Green mould and crop loss became more severe when this concentration was increased.
3. Several other *Trichoderma* isolates tested under the same conditions did not consistently produce green mould symptoms or suppress yields compared with an uninoculated control compost.
4. *Trichoderma aggressivum* isolate 23443B applied to Phase III (spawn-run) compost at casing resulted in no visible green mould symptoms and did not significantly affect mushroom yield. The same inoculum of 23443B (8.8×10^5 spores/kg compost) applied to Phase II composts at spawning resulted in almost complete crop loss.
5. Mixing 33% Phase III compost with Phase II compost, in place of grain spawn, prevented the appearance of visible green mould symptoms following *Trichoderma* inoculation, and reduced mushroom yield loss to less than 15%, compared with a yield loss of over 95% when grain spawn was used. Mixing 25% Phase III compost with Phase II compost did not completely prevent the appearance of green mould symptoms following *Trichoderma* inoculation, and reduced mushroom yield loss to about 50%.
6. The use of caccing (CI) in place of grain spawn reduced mushroom yield loss resulting from *Trichoderma* infection in one out of two composts. However, the population of *Trichoderma* propagules in both composts was increased compared with using grain spawn.
7. Increasing the rate of grain spawn in compost from 0.25 to 0.85% reduced yield loss due to *Trichoderma* infection.
8. Spawn-run temperature (20 or 25°C) or the use of Natural Gold supplement in Phase III compost had no significant effect on mushroom yield loss resulting from *Trichoderma* infection.
9. Counts of *Trichoderma* propagules (colony forming units, cfu) in compost at the time of casing did not correspond with mushroom yield loss, although composts with more than 1000 cfu/g compost produced very few mushrooms.

Penicillium implicatum

1 *Penicillium implicatum* isolate 1043D applied as a spore suspension at spawning (4.7×10^6 spores/kg compost) consistently produced ‘smoky mould’

symptoms and suppressed mushroom yield across a wide range of experimental and commercial composts.

2. Mushroom yield losses resulting from *Penicillium* infection of compost were more severe at higher compost moisture content.
3. Infection of compost with *Penicillium* resulted in smaller mushrooms which opened prematurely.
4. There was no significant relationship between the *Penicillium* propagule count in compost (cfu/g compost) at casing and subsequent mushroom yield loss, although composts with more than 6×10^5 cfu/g compost produced very few mushrooms.
5. Spore suspensions of *Acremonium murorum* and *Pythium oligandrum* did not produce symptoms or reduce mushroom yield compared with an uninoculated control compost.

Technology transfer

The effect of mixing proportions of Phase II and Phase III compost on mushroom cropping is currently being examined as a separate HDC project. The results of this work will have direct implications for control of *Trichoderma* by avoiding the use of grain spawn. A future HDC project due to start October 2009 will examine non-grain spawn mushroom inoculum that can be used for producing Phase III compost for such a Phase II+III system.

Acknowledgements

The preparation and liquid nitrogen storage of cultures of *Acremonium, Penicillium, Pythium* and *Trichoderma* in the Warwick HRI culture collection by Helen Grogan (Kinsealy Research Centre, Dublin) is acknowledged. Thanks are due to Charles Lane, FERA, for providing two of the *Trichoderma* isolates used in these experiments. Thanks are also due to Agricultural Supply Co. Ltd, J. Rothwell & Son Ltd, Snowcap Mushrooms Ltd, and Tunnel Tech Ltd for providing compost samples for the experiments.

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