Project title	Mushrooms: Influence of ammonia during compost pasteurisation and disinfectants on eradication of *Trichoderma aggressivum* (Th2)
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GROWER SUMMARY

Headline

- Disolite was the most effective disinfectant in killing Trichoderma spores, and Omnicide M was the most effective non-phenolic material
- Disolite, Environ and Prophyl were the most effective disinfectants in suppressing Trichoderma mycelial growth and Sporekill was the most effective non-phenolic material
- None of the disinfectants tested completely eradicated Trichoderma inoculum in infected compost but Disolite and Environ produced the greatest reduction.

Background and expected deliverables

Trichoderma aggressivum f. *europeum* is capable of causing severe or even complete mushroom crop loss when present in compost at levels that are at the detection limit of dilution plating methodology. *Trichoderma aggressivum* is known to have considerable tolerance to compost time-temperature treatments. In project M 50, a compost temperature of 60°C needed to be maintained for 12 hours to reduce spore and infected compost inocula to below a detectable limit. Although spores and infected compost inocula of *T. aggressivum* could survive an ammonia concentration of 300 ppm, there was evidence that survival declined with increasing ammonia concentration. However, excessively high ammonia concentrations, resulting from too high compost nitrogen content, can lead to delayed or incomplete clearance of ammonia in Phase II.

The withdrawal of formaldeyde as a gaseous disinfectant and fungicide tray dips has been a particular problem for farms without the facility to cook-out. There are several other disinfectants that are marketed in the mushroom industry for use as liquids and/or fogs but their effects at different concentrations on *Trichoderma aggressivum* are not established.

A Trichoderma selective medium is available that favours the growth of *Trichoderma* species over background moulds, and can be used for dilution plating of compost suspension. Results in M 50 showed that this method was capable of detecting about 10 propagules of *T. aggressivum* per g compost. A rapid real time molecular detection method (RT-PCR) for *T. aggressivum* has been developed by FERA. In project M 50, the method was found to be capable of detecting Trichoderma propagules in Phase III compost containing 0.01% infected compost inoculum. The Trichoderma detection limit of this molecular method has

not been compared with that of the semi-selective dilution plating method on Phase II and III samples containing spore or infected compost inoculum.

Project objectives

(a) Determine the influence of ammonia during compost 'pasteurisation' on the eradication of *Trichoderma aggressivum* (Th2).
(b) Confirm eradication of *Trichoderma aggressivum* (Th2) from Phase II and spawn-run compost using different detection methods.
(c) Obtain ammonia concentration data from commercial Phase II tunnels and compare the levels with those needed to achieve eradication.
(d) Determine the effect of different liquid and fogging disinfectants at different concentrations on the eradication of *Trichoderma aggressivum* (Th2).
(e) Determine the residues of disinfectants applied to cropping tray wood, with and without subsequent cook-out.
(f) Make recommendations on the optimum ammonia concentrations needed for eradication of *Trichoderma* in Phase II, and how they can be achieved practically.

Summary of the project and main conclusions

Compost pastuerisation treatments

- Spore and grain inoculum of *Trichoderma aggressivum* required compost pasteurisation at 60°C for 12 hours to achieve eradication although this treatment was not sufficient to completely eradicate compost inoculum containing a high level of Trichoderma.
- Addition of urea to compost increased the ammonia concentration during pasteurisation but did not affect *Trichoderma aggressivum*, the spores of which were able to withstand 5000 to 6000 ppm ammonia for 17 hours.
- The maximum level of urea which could be added to Phase I compost without adversely affecting mushroom yield was 0.5 g/kg (equivalent to 0.5 kg/tonne). The resulting level of ammonia during Phase II (300 ppm) did not affect the eradication of *Trichoderma aggressivum* inoculum.
- Ammonia concentration measured during pasteurisation of commercial Phase II tunnels ranged from 110 to 425 ppm.

Trichoderma detection methods

- There was general agreement between the results of RT-PCR, dilution plating and compost on agar methods for detecting low levels of *Trichoderma aggressivum*

inoculum in compost samples. Some discrepancies between the detection methods may have been due to the presence of other background *Trichoderma* species in the compost not detected by RT-PCR or the heterogeneity of the Trichoderma inoculum in the compost samples.

- There were relationships between the levels of Trichoderma detected in compost samples using RT-PCR, dilution plating or compost on agar methods and the subsequent mushroom yield from the compost samples (Figures 1 and 2).

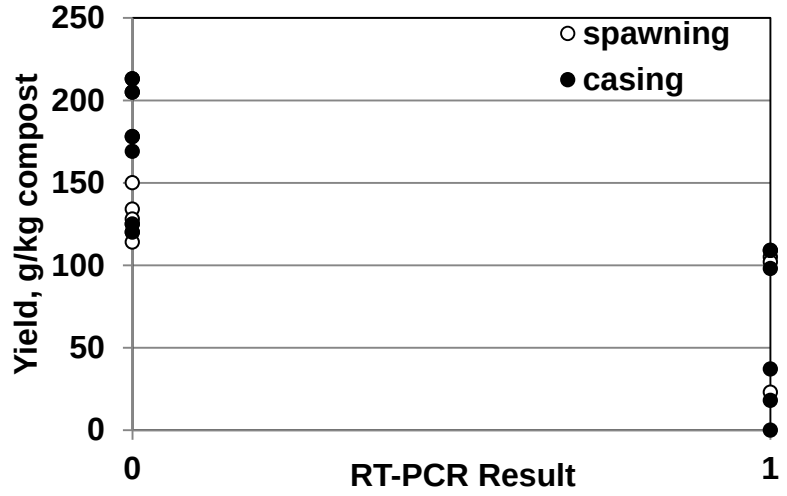

Fig. 1. Relationship between RT-PCR results at spawning and casing and mushroom yields from compost samples with different levels of Trichoderma inoculum, urea addition, and pasteurisation treatment.

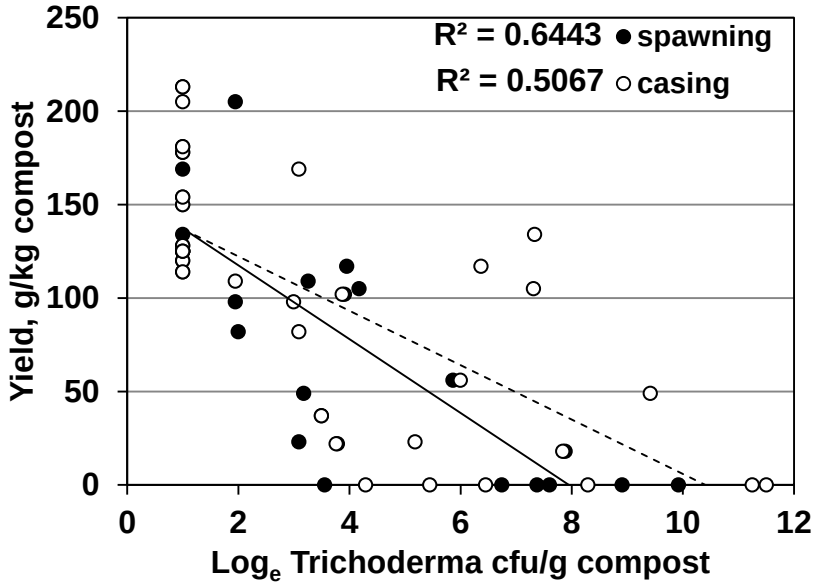

Fig. 2. Relationship between dilution plate counting of Trichoderma propagules at spawning and casing and mushroom yield from compost samples with different levels of Trichoderma inoculum, urea addition, and pasteurisation treatment.

Effect of disinfectants on Trichoderma inoculum

- Disolite was the most effective disinfectant in killing Trichoderma spores, and Omnicide M was the most effective non-phenolic material.
- Bleach also showed efficacy in killing spores, but it needed to be used at a dilution of 1:5 to eradicate a high concentration of spores.
- Jet 5 at 1:100 was not very effective in killing Trichoderma spores and Sporekill at 1:100 or activated Purogene at 1:20 were ineffective.
- Disolite, Environ or Prophyl added in dilutions of 1:750 (or more concentrated) to PDA medium completely suppressed the mycelial growth of *Trichoderma aggressivum* and *T. harzianum.*
- Sporekill suppressed mycelial growth of both *Trichoderma* species at a dilution of 1:250, whereas Omnicide M required a higher concentration (1:150) to achieve the same effect. Jet 5 was more inhibitory to the growth of *T. harzianum* than to that of *T. aggressivum* and required a concentration of 1:100 to completely suppress the growth of *T. aggressivum*.
- Bleach suppressed Trichoderma mycelial growth when added to PDA at a 1:5 dilution. Mycelial growth rate was reduced by activated Purogene at a concentration of 1:33.
- After 17 hours exposure, the vapour from Disolite at 1:250 dilution killed all Trichoderma spores, as did Prophyl at a 1:100 dilution.
- Trichoderma spores survived 70-240 ppm ozone but were killed after exposure to 300-400 ppm ozone. Activated Purogene at 1:33 resulted in an initial gaseous chlorine dioxide concentration of 12 ppm which killed all Trichoderma spores (all 17 hour exposures).
- None of the disinfectants tested completely eradicated Trichoderma inoculum in infected compost but Disolite and Environ produced the greatest reduction.
- The residues of phenolic disinfectants were detected on blocks of wood which had been dipped in 1:250 dilutions and then subjected to a simulated cook-out treatment.

Benefits to industry

The work has identified the pasteurisation conditions needed to eradicate moderate inoculum levels of *Trichoderma aggressivum* from compost and should reduce the occurrence of green mould from this source. The work identified the most effective phenolic and non-phenolic liquid and gaseous disinfectants in killing Trichoderma spores and mycelium. This should enable farms to improve the hygiene and reduce incidence of green mould.

Crop loss due to Trichoderma infection can be as high as 100% in individual composts (Catlin et al 2004). The costs of green mold to a medium-sized UK farm due to mushroom crop loss, cap spotting and additional monitoring has been estimated at around £70K annually (personal communication). This means that the cost of green mold to the entire UK mushroom industry could be £0.5M -1.0M annually. By improved compost pasteurisation treatment, detection methodology and selection of appropriate disinfectants and concentrations resulting from this project, this figure should be substantially reduced. Additional costs include longer pasteurisation treatment (increasing from 6 to 12 hours in the event of an outbreak), more frequent sampling for Trichoderma in compost, and more rigorous use of appropriate disinfectants. Costs to individual farms would depend on whether compost is made on-site or imported.

Action Points for Growers

1. In the event of a Trichoderma outbreak, compost should be pasteurised at 60°C for 12 hours; shorter periods at this temperature should only be used if Trichoderma is not a problem. Boosting ammonia levels in Phase II compost pasteurisation, for example by the addition of urea, will not improve Trichoderma kill or reduce the temperature/time requirement of the pasteurisation treatment.
2. To detect low levels of Trichoderma in heterogenous compost, a combination of compost on agar using a selective medium on a large number of samples (positive baiting), and molecular techniques (RT-PCR) to confirm *T. aggressivum* can be used. Combinations of test methods (selective plating and RT-PCR) are not essential. However, baiting tests on a larger number of samples, followed by RT-PCR on Trichoderma positives would reduce the cost of conducting RT-PCR analysis on all the samples, and improve the Trichoderma detection limit of only conducting RT-PCR on a single or small numbers of compost samples.
3. Disolite is an effective disinfectant for killing Trichoderma spores and phenolic disinfectants are the most suppressive to the growth of Trichoderma mycelium. However, they should not be used where they can come into contact with the crop since they are detectable at very low levels. They can be used on floors, in foot dips and for washing parts of machinery and vehicles that do not come in contact with the crop or substrates.
4. Of the non-phenolic disinfectants, Omnicide M was the most effective in killing Trichoderma spores and Sporekill was most suppressive to mycelial growth. They can be used for disinfecting trays and shelves, which should then be washed down.
5. The effect of disinfectants on other mushroom pathogens and mites, and the possibility of resistance must also be considered. In the event of a Trichoderma outbreak, the use

of concentrated bleach and fogging rooms with activated Purogene (chlorine dioxide) should also be considered, although they are corrosive materials.

SCIENCE SECTION

Introduction

The *Trichoderma aggressivum* f. *europeum* (Tafe or previously Th2) isolate 23443B was capable of causing severe or even complete mushroom crop loss when present in compost at levels that were at the detection limit of the sampling and dilution plating methodology used (about 10 cfu per g compost). The results of HDC project M 50 showed *Trichoderma aggressivum* (Th2) isolate 23443B to have considerable tolerance to compost time-temperature treatments. A compost temperature of 60°C needed to be maintained for 12 hours to reduce spore and infected compost inocula of isolate 23443B to below a detectable limit. This confirmed earlier work showing *Trichoderma aggressivum* types *europeum* and *aggressivum* (Th2 and Th4) to be extremely temperature tolerant (Morris et al, 2000; Rinker & Alm, 2000, Catlin et al. 2004).

Gaseous ammonia (NH_3) is known to be toxic to several soil-borne plant pathogens and the addition of urea to soil can be used to suppress them (Chun & Lockwood, 1985). A concentration of only 17 ppm was sufficient to kill mycelium and prevent germination of zoospores of *Phytophthora cinnamomi* (Gilpatrick, 1969).

Project M 50 showed that *Trichoderma aggressivum* isolate 23443B spores and infected compost inocula of the isolate could survive an ammonia concentration of 300 ppm, although there was evidence that survival declined with increasing ammonia concentration. Other work has shown that *Trichoderma aggressivum* type *aggressivum* (Th4) spores can withstand up to 200 ppm gaseous ammonia in laboratory conditions (Rinker & Alm, 2000). Fletcher & Gaze (2007) recommend a minimum ammonia concentration of 450 ppm during compost pasteurisation.

Ammonia concentrations during Phase II can range from 200 to over 900 ppm (Gerrits, 1988; Noble & Gaze, 1996; Noble et al. 2002). Miller et al. (1990) recorded peak ammonia concentrations of 100 to 1500 ppm in bunker composts. Ross & Harris (1983) showed that ammonia concentrations during pasteurisation increased with compost temperature, with ammonia levels at 55°C being three times higher (450 vpm) than those at 40°C. Cool zones in tunnel composts, in combination with low ammonia concentrations, may increase the risk of *Trichoderma aggressivum* (Th2) surviving a Phase II process. However, excessively high ammonia concentrations, resulting from too high compost nitrogen content, can lead to

delayed or incomplete clearance of ammonia in Phase II and subsequent problems such as ink-caps (*Coprinus* spp) (Noble & Gaze, 1996).

The withdrawal of formaldeyde as a gaseous disinfectant and fungicide tray dips has been a particular problem for farms without the facility to cook-out. Abosriwil & Clancy (2002) found that Environ and Purogene were more effective than Sudol in limiting the recovery of *Trichoderma* spp. and *Cladobotryum dendroides*. Ammonia is used as a disinfectant in the poultry industry (Hermans & Morgan, 2007). Chlorine dioxide (Purogene) and ozone have also been used as a disinfectant in mushroom culture and used to eradicate other pathogens from seeds (Abosriwil & Clancy 2002; Sankaran et al. 2008; Trinetta et al. 2011; Allen 2012a). These materials can be used as liquid or gaseous applications. Romaine et al (2003) found the phenolic disinfectants Environ and Premisan as well as concentrated bleach and alcohols were effective in killing *Trichoderma aggressivum* spores. There are several other disinfectants that are marketed in the mushroom industry for use as liquids and/or fogs but their effects at different concentrations on *Trichoderma aggressivum* are not established.

Romaine et al (2003) found that the efficacy of isopropanol, bleach, Environ and Permisan against *Verticillium fungicola* spores was not affected by the presence of organic matter, although the efficacy of some other disinfectants was compromised by the presence of mushroom compost. The effect of organic matter on the efficacy of disinfectants against Trichoderma spores was not investigated.

Phenolic disinfectants have been used in the mushroom industry for several years including Disolite, Environ and Prophyl. The EU minimum residue level for 2-phenylphenol in fresh produce (including mushrooms) is 0.05 mg/kg and the level for benzy-chlorophenol and chloro-methylphenol is 0.01 mg/kg. Although phenolic disinfectants are known to be effective against Trichoderma spores and mycelium (JT Fletcher, WA Hayes unpublished data), the relative efficacy of non-phenolic disinfectants against Trichoderma inoculum is not established. Work in Australia (Allen 2012b) has shown that two quarternary ammonium chloride disinfectants (Tri-Clean and Tri-Fog) have efficacy against Trichoderma mycelium at a dilution of about 1:50.

A Trichoderma selective medium is available that favours the growth of *Trichoderma* species over background moulds, and can be used for dilution plating of compost suspension. Results in M 50 showed that this method was capable of detecting about 10 propagules of *T. aggressivum* per g compost. A rapid real time PCR detection method for *T. aggressivum* has

been developed by FERA. In project M 50, the method was found to be capable of detecting Trichoderma propagules in Phase III compost containing 0.01% infected compost inoculum. The Trichoderma detection limit of this molecular method has not been compared with that of the semi-selective dilution plating method on Phase II and III samples containing spore or infected compost inoculum.

Project objectives

(a) Determine the influence of ammonia during compost 'pasteurisation' on the eradication of *Trichoderma aggressivum* (Th2).
(b) Confirm eradication of *Trichoderma aggressivum* (Th2) from Phase II and spawn-run compost using different detection methods.
(c) Obtain ammonia concentration data from commercial Phase II tunnels and compare the levels with those needed to achieve eradication.
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(e) Determine the residues of disinfectants applied to cropping tray wood, with and without subsequent cook-out.
(f) Make recommendations on the optimum ammonia concentrations needed for eradication of Trichoderma in Phase II, and how they can be achieved practically.

Materials and methods

Flask-scale composting

Commercially produced Phase I compost was used for the tests. Except where stated, the *Trichoderma aggressivum* (Th2) isolate 23443B obtained from FERA York, was used. Trichoderma spore suspensions containing 1×10^7 spores/ml were prepared by flooding cultures produced on agar plates and exposed to light to encourage sporulation. The suspensions (28 ml) were then sprayed into 8 kg Phase I compost contained within plastic bags, which were closed and shaken periodically during spraying to give a concentration of inoculum of 3.5×10^4 spores per gram fresh weight. The Trichoderma propagule count in the inoculated compost was 7.9×10^6 cfu/g compost. The same volume of sterile water was used as a control. The control had a background Trichoderma count of 6.3 to 9.0×10^1 cfu/g compost.

Where infected compost inoculum was used, clean Phase II compost was spawned with *Agaricus bisporus* spawn (Sylvan A15) at 0.5% w/w and inoculated with a Trichoderma spore suspension in plastic bags as described above. The compost was then incubated at

25°C for 16 days. The bags were then exposed to light for several days until green spores became visible. The compost inoculum therefore contained both Trichoderma mycelium and spores. The infected compost inoculum was added at 10% w/w to Phase I compost to produce a Trichoderma propagule count of 7.9×10^6 cfu/g compost.

Where Trichoderma infected spawn grains were used as inoculum, samples of A15 spawn were rolled in sporulating agar plates of *T. aggressivum* 23443B. The spawn grains were then added at 1% w/w into the Phase I compost to produce a Trichoderma propagule count of 1.1×10^6 cfu/g compost.

Phase II composting was conducted in 'Quickfit' multiadapter flasks immersed in thermostatically controlled water baths, each holding four 2 L flasks (Noble et al, 1997). The prepared Phase I composts (about 1.1 kg samples) were placed on a perforated stainless steel platform within each flask and the flasks immersed in the water baths such that the water level was above the level of the enclosed substrate. Each flask was connected to ancillary equipment providing independent aeration of the compost. The oxygen concentration in the substrate was controlled regularly by adjusting the airflow through the compost in each flask within the range 8-16 L kg^{-1} substrate h^{-1} by means of flow meters. The temperature of the substrate in the flasks was monitored with Squirrel multipoint temperature loggers (Grant Instruments Ltd, Cambridge, UK).

For the first 48 hours of the Phase II composting process, the compost temperature was controlled at 49°C to allow a natural succession and gradual build-up of microorganisms. The substrate temperature was then increased to the required pasteurisation temperature (50, 55 or 60˚C) for the required time (6 or 12 hours). The temperature was then reduced to 49°C for the remainder of the composting period, which was between five and six days, until the air in the flask was clear of ammonia. A minimum oxygen concentration of 11 (± 1.5)% v/v was maintained in the substrate. The material in each flask was then sampled (2 × 50 g samples per flask) and analysed for viable Trichoderma inoculum and spawned with mushroom spawn as described later.

Effect of adding urea to Phase I compost on ammonia and mushroom cropping

An experiment was conducted to examine the effect of adding urea or ammonia solution to Phase I compost on the ammonia levels in the compost. Urea was added at 0.5, 1.0, 1.5 or 2.0 g/kg compost The urea was first dissolved in 100 ml water before addition to 8 kg Phase I compost. Ammonia solution (2M in propanol) was added at 4.5 ml in 100 ml to a separate 8 kg compost sample. The control treatment consisted of 100 ml water without urea added to 8

kg Phase I compost. The composts were pasteurised at 50 or 60°C for 6 hours. Two replicate batches of compost were prepared with two replicate flasks and cropping pots of each treatment in each batch.

Effect of ammonia and pasteurisation time and temperature on Trichoderma

The effect of four pasteurisation treatments (50°C for 6 hours, 55°C for 12 hours and 60°C for 6 or 12 hours) was examined on Phase I compost containing Trichoderma spore-infected compost or grain inoculum-infected compost as described above. Phase I composts without Trichoderma inocula were used as control treatments. Sufficient flasks were prepared so that all the pasteurisation treatments were examined with and without urea added at 0.5 g/kg Phase I compost. For each pasteurisation treatment × inoculum type x urea treatment, two replicate flasks were prepared.

The ammonia during all of the above pasteurisation treatments was measured with Draeger gas detector tubes.

Cropping procedure

At the end of the flask composting period, compost from each flask (1 kg) was spawned at 1% w/w of the fresh weight of compost with *Agaricus bisporus* rye grain spawn (Sylvan A15) and filled into plastic pots, 150 mm diameter x 130 mm depth, placed in a vented polythene bag and spawn-run at 25°C. After 16 days, the pots were cased with a moist mixture of peat and sugar beet lime (700 g), watered, and again placed in vented polythene bags. When mushroom mycelium was visible on the surface of the casing, the air temperature was reduced to 18°C, and a relative humidity of 90% and a CO_2 concentration of 0.1% to induce fruiting were maintained. Two flushes of mushrooms were harvested over a 14 day period (cap diameter 25-30 mm). The number and weight of mushrooms from each pot was recorded.

Trichoderma analysis of composts

Samples of compost were analysed for Trichoderma populations at spawning and at casing. The procedure for analysis is described in Grogan & Harvey (1999) and HDC project report M 50. For each compost sample, a 20 g sub-sample was put in a sterile homogeniser 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. For low concentrations, a 3 ml aliquot of diluted compost extract was used. 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. Three replicate pieces of compost from the samples were also plated out on malt agar + Streptomycin + Chloramphenol and kept at 25˚C. The growth of *Trichoderma* species was assessed under a binocular microscope after 7 days as follows: 0 no Trichoderma present; 1 Trichoderma just detectable; 2 moderate Trichoderma growth and/or sporulation visible; 3 extensive growth and sporulation of Trichoderma.

Real time PCR

A rapid real time PCR protocol for the detection of *Trichoderma aggressivum* in spawn-run compost samples was developed and described in project report M48 (Lane, 2010). Nucleic acid was extracted from all the compost samples using the protocol developed in M48 and then tested for presence of *T. aggressivum* by real time PCR. Compost samples (24) were selected from the flask experiments which were close to the Trichoderma detection limit using the plating tests. Positive and negative control samples were also included.

Ammonia levels in commercial pasteurisation tunnels

Data on ammonia levels during peak heating of Phase II was obtained from four composting sites on two occasions by measuring with Draeger tubes in the exhaust ducts.

Testing efficacy of disinfectants against Trichoderma inoculum

The disinfectants used in the tests are listed in Table 1. These consisted of three phenolic-based materials, three chlorine based compounds and five other materials or compounds. Liquid disinfectants were tested against Trichoderma spores, mycelium and infected mushroom compost and gaseous disinfectants were tested against Trichoderma spores. Citric acid was used to activate Purogene at 10% w/v according to the manufacturers recommendations.

(a) Liquid disinfectants against Trichoderma spores

A method based on that developed by Romaine et al (2003) was used for the tests. Spore suspensions of *T. aggressivum* 23443B were prepared by washing two agar plate cultures with 30 ml sterile distilled water (SDW) and counting spores with a haemocytometer. Suspensions containing either 2.3×10^7 spores/ml (low rate, equivalent to 5.2×10^8 cfu/ml) or 2.2×10^8 spores/ml (high rate, equivalent to 1.7×10^9 cfu/ml) were obtained by suitable dilution with SDW. Spore suspensions (1 ml) were centrifuged for 10 minutes at 13000 x *g*. The supernatant was discarded and the spores were resuspended in 0.2 ml of disinfectant

solution of the required strength. After a specified time interval (0.5, 2, 8 or 14 minutes), 5 µl of the disinfectant spore suspension was diluted in 1.5 ml of sterile distilled water and then centrifuged for 10 minutes at 13000 x *g*. The spore 'pellet' was then resuspended in 0.1 ml SDW and plated on to three replicate plates of potato dextrose yeast agar (PDYA). PDYA was used instead of the conventional PDA to follow the method of Romaine et al (2003). The plates were incubated at 25˚C and the number of colony forming units recorded after 3 and 7 days. If the number of colony forming units was less than 300, the number was recorded, otherwise the plates were only recorded as positive (more than 300 cfu per plate) or negative (no surviving spores).

Table 1. Disinfectants used in the tests. Details of manufacturers and UK suppliers are shown in the Appendix.

Disinfectant	**Active ingredient**	**Recommended dilution rate**	**Manufacturer/ UK Supplier**
Activated Purogene	chlorine dioxide	1:200 to 1:20	Tristel Technologies Ltd
Ammonia	ammonia	n/a	Sigma Aldrich Co. Ltd
Bleach	sodium hypochlorite available chlorine	n/a	Staples Disposables Ltd
Disolite	2-phenylphenol benzyl-chlorophenol propan-2-ol	1:250 to 1:50	Progress Products
Environ	o-phenylphenol o-benzyl-p-chlorophenol isopropanol	1:250 to 1:100	Steris Ltd
Jet 5	peroxyacetic acid	1:500 to 1:125	Certis
Omnicide M	glutaraldhehyde cocobenzyldimethyl ammonium chloride	1:150 to 1:50	Progress Products
Ozone	ozone	n/a	Onnic International Ltd
Prophyl	4-chloro-3-methyl phenol 2-benzyl-4-chlorophenol	1:250	J.F. McKenna Ltd
Sporekill	potassium salts of fatty acids	1:1000 to 1:100	IPP Ltd

The following disinfectants were tested in solutions with sterile water at 1:100 and 1:250 dilution strengths: Disolite, Environ, Jet 5, Omnicide M, Prophyl and Sporekill. Purogene was tested at 1:20 and bleach was tested at 1:5 and 1:9 strengths. Sterile water was used as a control. Exposure of both concentrations of spores (low or high) to each disinfectant and

concentration was tested for 0.5, 2, 8 and 14 minutes. Ammonia and ozone were only used a gaseous disinfectants (see later section).

(b) Liquid disinfectants against Trichoderma mycelial growth

Potato dextrose agar (PDA) containing the following disinfectants at 1:150 (i.e. most concentrated), 1:250, 1:500 and 1:750 (i.e. most dilute) was prepared: Disolite, Environ, Jet 5, Omnicide M, Prophyl, Purogene and Sporekill. Jet 5 was also incorporated at 1:100 and Purogene at 1:20, 1:50 and 1:100. Bleach was incorporated into PDA at 1:5, 1:10, 1:20 and 1:50. PDA plates prepared only with water, without disinfectant, were used as a control.

Three replicate plates of each disinfectant treatment were prepared and inoculated in the centre with a 5 mm plug of a sporulating culture of either *T. aggressivum* 23443B or *Trichoderma harzianum* (Th1) obtained from a commercial mushroom culture. The radial growth of the mycelium on the plates was recorded at daily intervals for four days. The growth of Trichoderma mycelium on any plates that were not fully colonised by this time continued to be recorded at 2-3 day intervals for up to 20 days. The growth rate was determined from the radial growth in mm divided by the number of days.

(c) Liquid disinfectants against Trichoderma in infected mushroom compost

Spawn-run mushroom compost infected with *T. aggressivum* 23443B (4 g) was placed into 6 x 6 mm plastic mesh bags. Two replicate bags were immersed into 300 ml of each of the following disinfectants for 14 minutes: Disolite, Environ, Jet 5, Prophyl and Sporekill at 1:100 and Purogene at 1:20. SDW was used as a control. The bags were then rinsed for 5 minutes in 350 ml SDW and drained on tissue paper for 3 minutes. Compost (1 g) from each bag was made into a serial dilution for plating on PDA + chlorotetracyline. The remaining compost was put on to plates containing malt agar + streptomycin + chloramphenicol.

(d) Gaseous disinfectants against Trichoderma spores

The tests were conducted in 2 litre Quickfit flasks. The flasks contained 5 ml of the following disinfectants which evaporated from inside the walls and base: Disolite, and Prophyl at 1:100 and 1:250; Purogene at 1:33 and 1:100. Ammonia solution (2 ml or 50 µl) was sprayed into two flasks. Ozone from an ozone generator (Onnic International Ltd, Southampton) was filled into flasks at 240 and 400 ppm. The concentration of ammonia or ozone in the flasks was monitored at the start and end of the test with Draeger gas detector tubes (types 5/a and 0.5%/a for ammonia and type CH 21001 for ozone). The concentration of chlorine dioxide was measured with Gastec tubes type GAS23M. The gaseous concentration of Disolite and Prophyl was determined from the calculation that all of the disinfectant in the 5 ml applied to

flask walls evaporated during the test (no liquid remained on the sides of the flasks). Two flasks with 5 ml of water were used as controls.

Two 10 µl volumes of *T. aggressivum* 23443B spore suspension (7.5 x 10^7 spores/ml) were pipetted into the wells of microslides and allowed to dry for 30 minutes. The slides were then placed on upturned sterilised glass jars inside the flasks. The flasks were then sealed for 17 hours, after which the slides were retrieved and the spores resuspended in 100 µl of SDW and plated on to PDA agar.

Residues of phenolic disinfectants applied to cropping tray wood

Blocks of dry tray wood, 25 x 50 x 110 mm, 370 g, were dipped into the following phenolic disinfectants for 5 seconds: Disolite, Environ and Prophyl at 1:250 dilution in water. Water was used as a control. The blocks were then drained and reweighed, so that they had absorbed about 30 ml of the disinfectant solution or water. The blocks were then placed in glass flasks and heated at 65°C for 12 hours to simulate a cook-out treatment. The blocks were then soaked in 1 litre of water for 2 hours. The washing waters, together with samples of the disinfectants at 1:250 dilution and a water sample were then analysed by Mountainheath Analytical for residues of the disinfectants.

Results

Effect of adding urea to Phase I compost on ammonia and mushroom cropping

Fig. 3 shows that addition of 1.5 g or 2 g urea per kg Phase I compost immediately before Phase II resulted in a large increase in the ammonia concentration during pasteurisation and the ammonia levels were not fully cleared after conditioning. Addition of 1 g urea per kg Phase I resulted in a smaller increase in ammonia and the effect of adding 0.5 g urea was negligible compared with the control compost. Ammonia levels were higher when the compost was pasteurised at 60°C than at 50°C.

The effect of adding urea to compost before Phase II on subsequent mushroom yield is shown in Fig. 4. Addition of 1.5 or 2 kg urea per kg Phase I compost significantly reduced mushroom yields, probably due to low levels of residual ammonia in the compost. The mushroom yield obtained from compost with addition of 1 g urea per kg Phase I was also lower than from the control compost although the difference was not significant at $P = 0.05$. There was no significant effect of adding 0.5 g urea per kg Phase I compost on subsequent mushroom yield.

The result showed that the maximum amount of urea which can safely be added to Phase I compost before pasteurisation is 0.5 g/kg (equivalent to 1 kg/tonne compost) although the effect of this addition on ammonia levels is small.

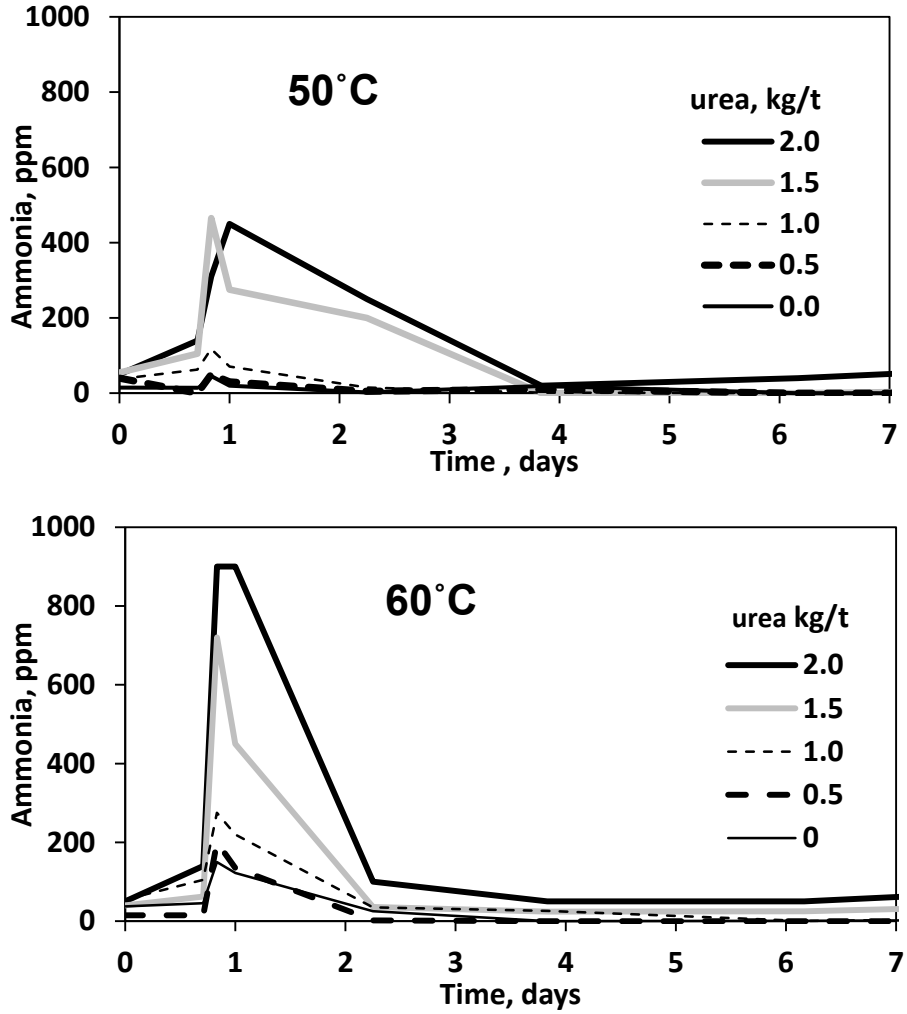

Fig. 3. Effect of urea addition and pasteurisation temperature on ammonia levels during flask-scale Phase II composting.

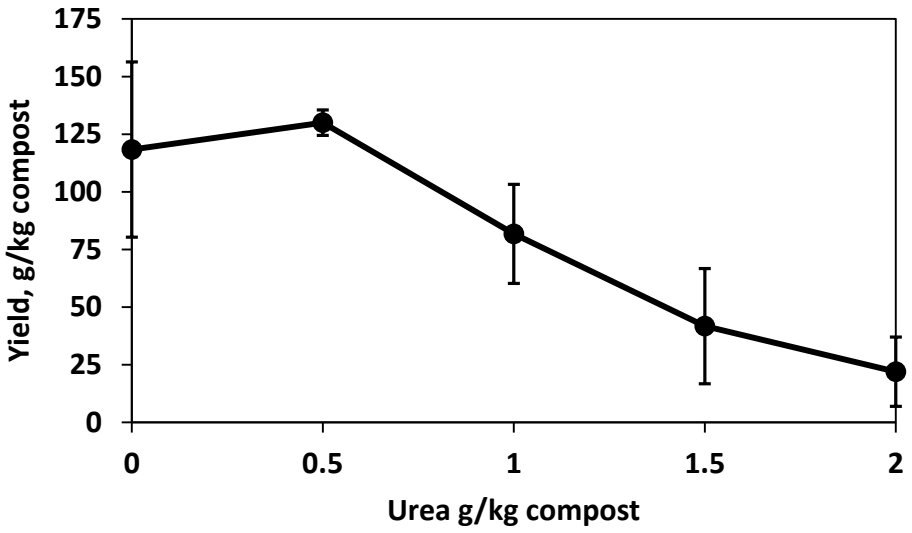

Fig. 4. Effect of adding urea to compost before Phase II on mushroom yield. Each value is the mean of two crops and two replicate pots

Effect of ammonia and pasteurisation time and temperature on Trichoderma

All of the positive control composts produced strong positive results with both the dilution plating and compost on agar detection methods (Figs. 5, 6 and 7). None of the negative uninoculated controls produced positive test results. Figures 5, 6 and 7 show the effects of pasteurisation temperature and time treatments on the survival of *Trichoderma aggressivum* spore, grain and compost inocula respectively. Compost samples analysed at spawning and after spawn-run (at application of casing) showed a similar trend, except for compost samples with spore inoculum pasteurised at 55°C for 12 hours where only the samples tested at spawning tested positive (Fig. 5a and 5b). Spore and grain inoculum required pasteurisation at 60°C for 12 hours to eradicate *T. aggressivum* (Figs. 5 and 6). However, this was not sufficient to eradicate compost inoculum of *T. aggressivum* (Fig. 7). Addition of urea at 0.5 g/kg compost slightly increased ammonia levels during pastuerisation but did not affect the survival of *T. aggressivum* inocula in compost (data not shown).

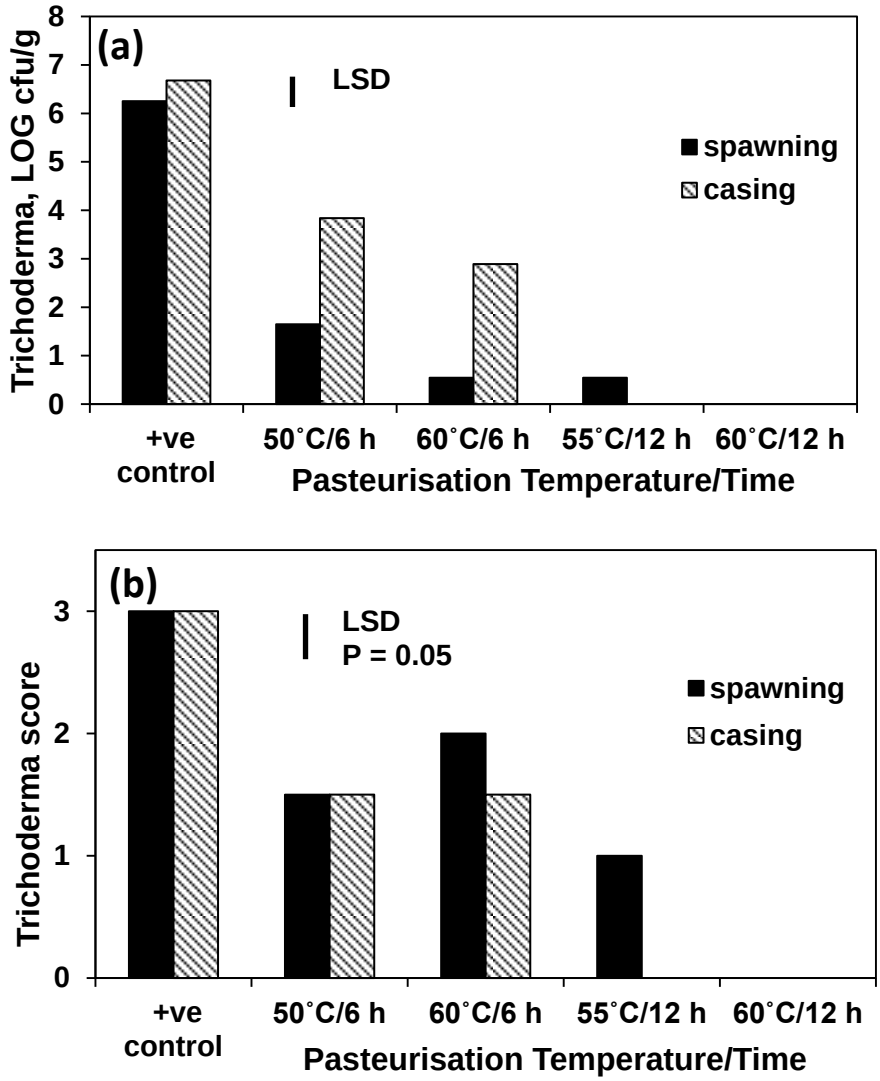

Fig. 5. Effect of pasteurisation temperature and time on survival of spore inoculum of *Trichoderma aggressivum* determined from (a) dilution plating (b) compost on agar at spawning and at casing.

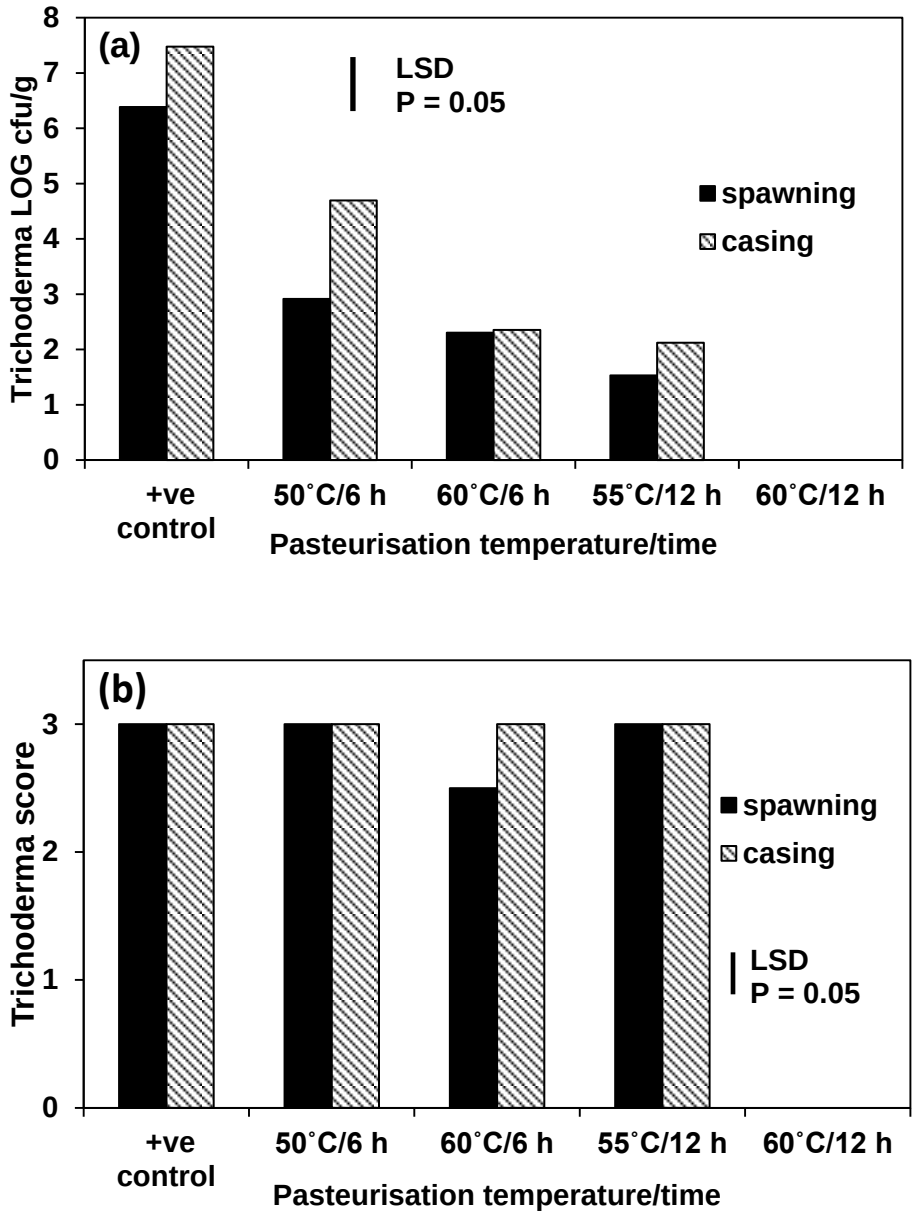

Fig. 6. Effect of pasteurisation temperature and time on survival of grain inoculum of *Trichoderma aggressivum* determined from (a) dilution plating (b) compost on agar at spawning and at casing.

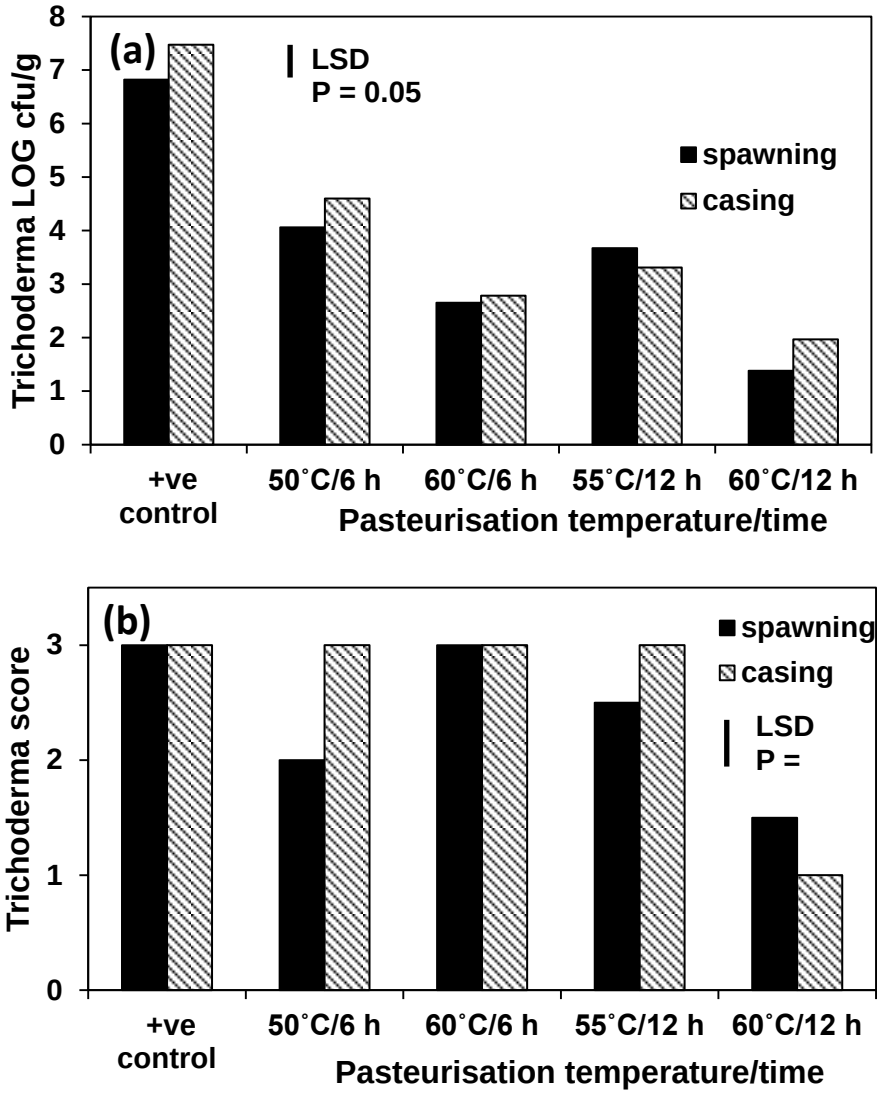

Fig. 7. Effect of pasteurisation temperature and time on survival of compost inoculum of *Trichoderma aggressivum* determined from (a) dilution plating (b) compost on agar at spawning and at casing

Detection methods for Trichoderma aggressivum

Tables 2 and 3 show the detection of Trichoderma in selected compost samples from the flask experiments at spawning and casing. Samples were selected which were close to the limit of detection from the dilution plating and compost on agar methods. Of 13 compost samples at spawning, 9 samples tested positive or negative with all three detection methods (dilution plating, compost on agar and RT-PCR). Of the 11 compost samples tested at casing, 7 tested positive or negative with all three detection methods. Four samples at spawning and three at casing tested positive with the compost on agar detection method but negative with the dilution plating and/or RT-PCR methods. One sample at casing tested negative with compost on agar method but positive with dilution plating and RT-PCR. The

difference between the dilution plating or compost on agar test results and those obtained using RT-PCR may have been due to the presence of *Trichoderma* species other than *T. aggressivum*. These would have given a positive test result with plating but a negative with RT-PCR. Variability in Trichoderma inoculum, particularly at low levels of infection, could also have caused variability in test results.

The RT-PCR test results at spawning and casing confirm that *T. aggressivum* was eradicated from spore and grain inoculum after pasteurisation at 60°C for 12 hours but survived this treatment as compost inoculum.

Table 2. Trichoderma detection in compost samples at spawning, and mushroom yields from each sample (g/kg spawned compost). Shading indicates samples where the test results differed between plating and RT-PCR detection methods.

Tafe Inoculum	**Pasteurisation Temp/Time**	**Dilution plating, cfu/g***	**Compost on agar score****	**RT-PCR *****	**Yield g/kg**
spores	50°C/ 6 h	65	1	+ve	105
spores	60°C/6 h	0	2	-ve	134
spores	55°C/12 h	7	1	-ve	205
spores	55°C/12 h	0	1	-ve	125
spores	60°C/12 h	0	0	-ve	150
spores	60°C/12 h	0	0	-ve	213
grain	60°C/6 h	50	2	+ve	102
grain	60°C/12 h	0	0	-ve	128
grain	60°C/12 h	0	0	-ve	120
compost	60°C/12 h	26	1	+ve	109
compost	60°C/12 h	22	2	+ve	23
none	50°C/6 h	0	1	-ve	114
none	55°C/12 h	0	0	-ve	178

* mean of 3 replicate plates

** 0= not detected, 1=detectable level; 2=moderate infection; 3=severe infection

*** +ve positive test result; -ve negative test result; following duplicate RT-PCR tests.

Table 3. Trichoderma detection in compost samples at casing and mushroom yield from each sample (g/kg spawned compost). Shading indicates samples where the test results differed between plating and RT-PCR detection methods.

Tafe Inoculum	Pasteurisation Temp/Time	Dilution plating, cfu/g	Compost on agar score	RT-PCR ***	Yield, g/kg
spores	60°C/6 h	20	0	+ve	98
spores	55°C/12 h	0	0	-ve	205
spores	60°C/12 h	0	0	-ve	213
grain	55°C/12 h	33	3	+ve	37
grain	60°C/12 h	0	0	-ve	120
compost	50°C/6 h	2540	3	+ve	18
compost	55°C/12 h	73	3	+ve	0
compost	50°C/6 h	7	1	+ve	109
none	50°C/6 h	22	1	-ve	169
none	55°C/12 h	7	2	-ve	178
none	60°C/6 h	0	1	-ve	125

* mean of 3 replicate plates

** 0= not detected, 1=detectable level; 2=moderate infection;3=severe infection

*** +ve positive test result; -ve negative test result; following duplicate RT-PCR tests.

There was a relationship between RT-PCR results and the mushroom yield obtained from different compost samples (Fig. 8). Samples that produced more than 110 g/kg were negative whereas those that produced a lower yield were positive. It is possible that quantified RT-PCR results would give a more precise relationship between RT-PCR test results and mushroom yield.

Relationships were identified between the dilution plating and compost on agar scoring of Trichoderma of compost samples and the subsequent mushroom yield (Figs. 9 and 10). The relationships were closer on samples analysed at spawning that at casing. The relationships were less clear cut than those between RT-PCR and mushroom yield. This may have been

due to the presence of background *Trichoderma* species in the compost, other than the *T. aggressivum* inoculum that was added to the compost.

Since the compost on agar method is relatively cheap compared with the dilution plating and RT-PCR methods, it is probably best if compost on agar is used for detecting low levels of Trichoderma on large numbers of samples (baiting) and testing any positive samples with RT-PCR to confirm *T. aggressivum* or other *Trichoderma* species.

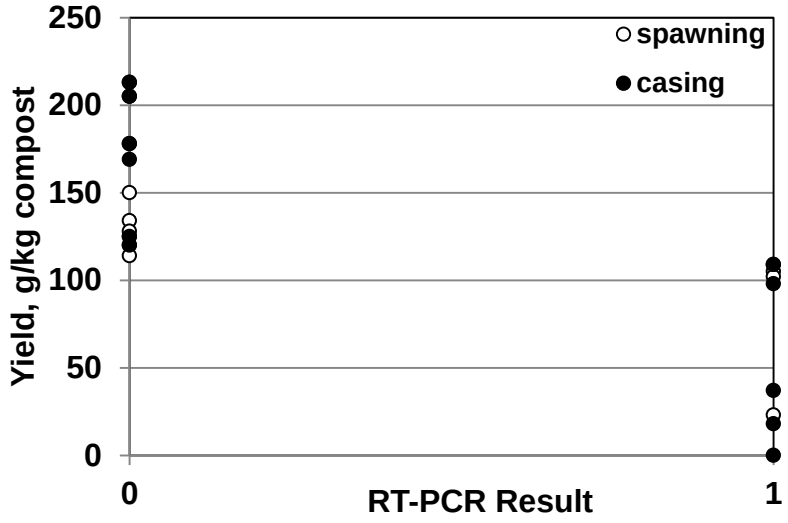

Fig. 8. Relationship between RT-PCR results at spawning and casing and mushroom yields from compost samples with different levels of Trichoderma inoculum, urea addition, and pasteurisation treatment.

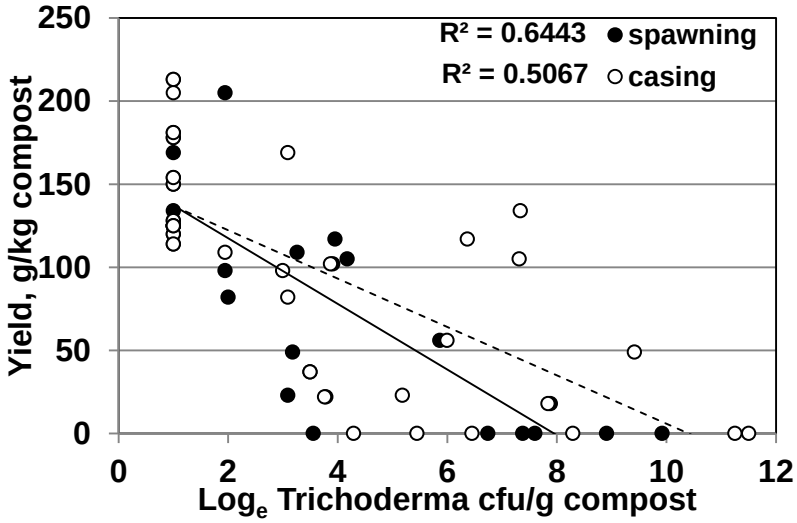

Fig. 9. Relationship between dilution plate counting of Trichoderma propagules at spawning and casing and mushroom yield from compost samples with different levels of Trichoderma inoculum, urea addition, and pasteurisation treatment.

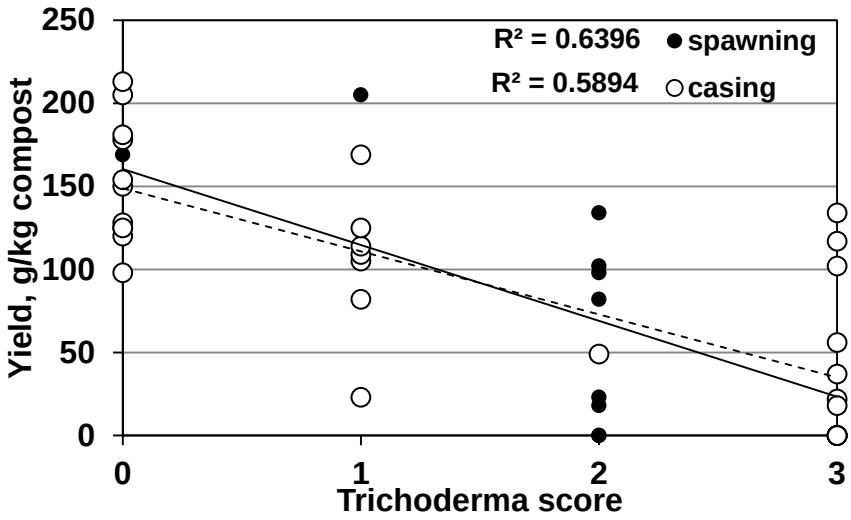

Fig. 10. Relationship between Trichoderma score from compost on agar cultures at spawning and casing and mushroom yield from compost samples with different levels of Trichoderma inoculum, urea addition, and pasteurisation treatment.

Effect of Trichoderma, urea and pasteurisation temperature and time on mushroom yield

On average, compost and grain Trichoderma inoculum reduced mushroom yield by more than 50% (Table 4). The effect of spore inoculum on mushroom yield was not significant. The addition of urea at 0.5 g/kg compost reduced mushroom yield when compost inoculum was used or when compost was pasteurised for 6 hours instead of 12 hours (Table 5). Pasteurisation at 60°C for 12 hours resulted in a higher mushroom yield than pasteurisation at for 6 hours or at lower temperatures (Table 5). This was due to the eradication or substantial reduction of *T. aggressivum* inoculum added to the compost.

Table 4. Effect of different types of Trichoderma inoculum and addition of urea (0.5 g/kg) on mushroom yield. Each value is the mean of four pasteurisation treatments and two replicate flasks per treatment. LSD (*P* = 0.05) between mean values = 15.

	Control	Spores	Grain	Compost	Mean
Control	147	149	58	61	104
+ Urea	136	121	59	6	80
Mean	141	135	58	33	

Table 5. Effect of pasteurisation regime and addition of urea (0.5 g/kg compost) on mushroom yield (g/kg compost). Each value is the mean of four types of Trichoderma inoculum and two replicate flasks. LSD (*P* = 0.5) between mean values = 15.

	50°C /6 h	55°C /12 h	60°C /6 h	60°C /12 h	Mean
Control	62	96	120	139	104
+ Urea	38	79	70	134	80
Mean	50	87	95	137	

Ammonia levels in commercial pasteurisation tunnels

Ammonia concentration measured during pasteurisation of commercial Phase II tunnels ranged from 110 to 425 ppm (Table 6). These concentrations were equivalent to those obtained in the flask experiments with 0 – 1 g urea added to 1 kg compost (Fig. 3).

Table 6. Ammonia levels in tunnel compost pasteurisation on commercial composting sites.

Composting Site	Peak ammonia, ppm
A	110-150
B	250-425
C	150-200
D	200-250

Efficacy of disinfectants against Trichoderma inoculum

(a) Liquid disinfectants against Trichoderma spores

The effect of exposing *T. aggressivum* spores to higher and lower concentrations of a range of disinfectants for set time periods is shown in Tables 7 and 8. The Tables show the longest durations the spores could withstand at each concentration of disinfectant (positive test result +ve, more than 300 spores surviving per test plate) and the shortest duration needed to achieve eradication (negative test result -ve). The column headed ‘Counts’ refers to an intermediate treatment effect which resulted in a reduction of surviving spores to between 1 and 300 spores per plate.

The control spores remained viable at high numbers after immersion in water and then centrifuging. Sporekill and activated Purogene were not effective in killing spores at dilutions

of 1:100 and 1:20 respectively. The time required to eradicate spores was greater with the higher initial level of Trichoderma spores. Making the disinfectant more dilute either increased the length of the exposure time needed to kill the spores or made the disinfectant ineffective (Environ, Prophyl, Omnicide M and Jet 5). Jet 5 only eradicated the lower concentration of spores, and only if it was used at the higher concentration (1:100) for the longest time period tested (14 minutes). It is possible that exposure to longer time periods than 14 minutes may have had an effect; the maximum exposure period of spores to disinfectants examined by Romaine et al (2003) was 4 minutes.

Disolite and Omnicide M were more effective than Environ and Prophyl at the same dilutions in killing Trichoderma spores, with Disolite being the most effective. Bleach also showed efficacy in killing spores, but it needed to be used at a dilution of 1:5 to eradicate the higher concentration of spores.

Table 7. Effect of higher dose of disinfectants on high and low concentrations of *Trichoderma aggressivum* spores. Times shown are the maximum number of minutes exposure which resulted in survival of more than 300 spores per test plate (positive test result, +ve) or the minimum number of minutes needed for eradication (negative test result, -ve). 'Counts' refers to an intermediate effect whereby surviving spores were reduced to between 1 and 300 spores per plate. The maximum exposure duration was 14 minutes.

Disinfectant	**Rate**	**Trich**	**+ ve**	**Counts**	**- ve**
			Time, min	**Time, min**	**Time, min**
Bleach	1:5	LOW	0.5	-	2
	1:5	HIGH	0.5	2	8
Disolite	1:100	LOW	0.5	-	2
	1:100	HIGH	2	-	8
Environ	1:100	LOW	2	8	14
	1:100	HIGH	8	14	-
Jet five	1:100	LOW	8	-	14
	1:100	HIGH	14	-	-
Omnicide M	1:100	LOW	0.5	2	8
	1:100	HIGH	2	-	8
Prophyl	1:100	LOW	2	8	14
	1:100	HIGH	8	-	14
Purogene active.	1:20	LOW	14	-	-
	1:20	HIGH	14	-	-
Sporekill	1:100	LOW	14	-	-
	1:100	HIGH	14	-	-
Control	-	LOW	14	-	-
	-	HIGH	14	-	-

LOW =2.3 x 10^7 spores or 5.2 x 10^8 cfu/ml

HIGH = 2.2 x 10^8 spores or cfu 1.7 x 10^9 cfu/ml

Table 8. Effect of lower dose of disinfectants on high and low concentrations of *Trichoderma aggressivum* spores. Times shown are the maximum number of minutes exposure which resulted in survival of more than 300 spores per test plate (positive test result, +ve) or the minimum number of minutes needed for eradication (negative test result, -ve). 'Counts' refers to an intermediate effect whereby surviving spores were reduced to between 1 and 300 spores per plate. The maximum exposure duration was 14 minutes.

Disinfectant	**Rate**	**Trich**	**+ ve**	**Counts**	**- ve**
			Time, min	**Time, min**	**Time, min**
Bleach	1:9	LOW	0.5	-	2
	1:9	HIGH	8	-	14
Disolite	1:250	LOW	8	-	14
	1:250	HIGH	8	14	-
Environ	1:250	LOW	14	-	-
	1:250	HIGH	14	-	-
Jet 5	1:250	LOW	14	-	-
	1:250	HIGH	14	-	-
Omnicide M	1:250	LOW	8	14	-
	1:250	HIGH	14	-	-
Prophyl	1:250	LOW	14	-	-
	1:250	HIGH	14	-	-
Sporekill	1:150	LOW	14	-	-
	1:150	HIGH	14	-	-
Control	-	LOW	14	-	-
	-	HIGH	14	-	-

LOW =2.3 x 10^7 spores or 5.2 x 10^8 cfu/ml

HIGH = 2.2 x 10^8 spores or cfu 1.7 x 10^9 cfu/ml

(b) Liquid disinfectants against Trichoderma mycelial growth

The radial growth rates of *Trichoderma aggressivum* 23443B and *Trichoderma harzianum* on PDA were 11.1 and 11.6 mm/day respectively. When Disolite, Environ or Prophyl were added in dilutions of 1:750 (or more concentrated) to the PDA medium, no mycelial growth of either *Trichoderma* species occurred. Bleach totally suppressed mycelial growth when added to PDA at a 1:5 dilution (Fig. 11). Purogene only reduced mycelial growth of *T. aggressivum* when used at a dilution of 1:50 although it was more inhibitory to the growth rate of *T. harzianum*.

Sporekill totally suppressed mycelial growth of both *Trichoderma* species at a dilution of 1:250, whereas Omnicide M required a higher concentration (1:150) to achieve the same effect (Fig. 10). Jet 5 was more inhibitory to the growth of *T. harzianum* than to that of *T. aggressivum* and required a concentration of 1:100 to completely suppress the growth of *T. aggressivum* (Fig. 12).

(c) Liquid disinfectants against Trichoderma in infected mushroom compost

None of the disinfectants used at a dilution of 1:100 completely eradicated *T. aggressivum* from compost inoculum, although Disolite and Environ reduced the colony count by nearly 2 log compared with the initial propagule count (Table 9).

Table 9. Effect of disinfectants on compost inoculum of *Trichoderma aggressivum*

Disinfectant	**Dilution**	**cfu/g**
Disolite	1:100	7.1×10^7
Environ	1:100	5.5×10^7
Jet 5	1:100	2.4×10^8
Prophyl	1:100	1.2×10^8
Purogene activated	1:20	3.9×10^8
Sporekill	1:100	4.8×10^9
Control (water)	-	1.2×10^9

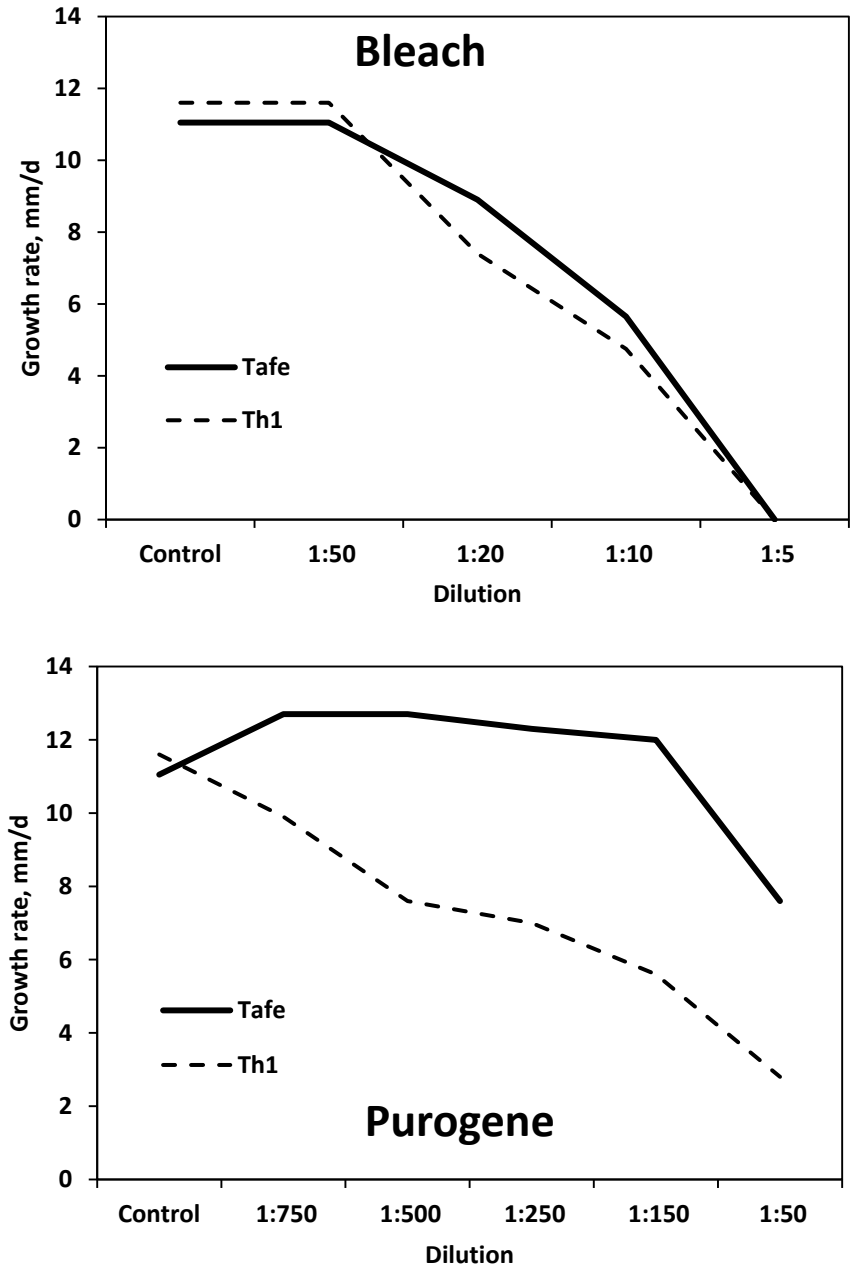

Fig. 11. Effect of bleach and activated Purogene in agar on the mycelial growth rate of *Trichoderma aggressivum* (Tafe) and *Trichoderma harzianum* (Th1). Each value is the mean of three replicate plates.

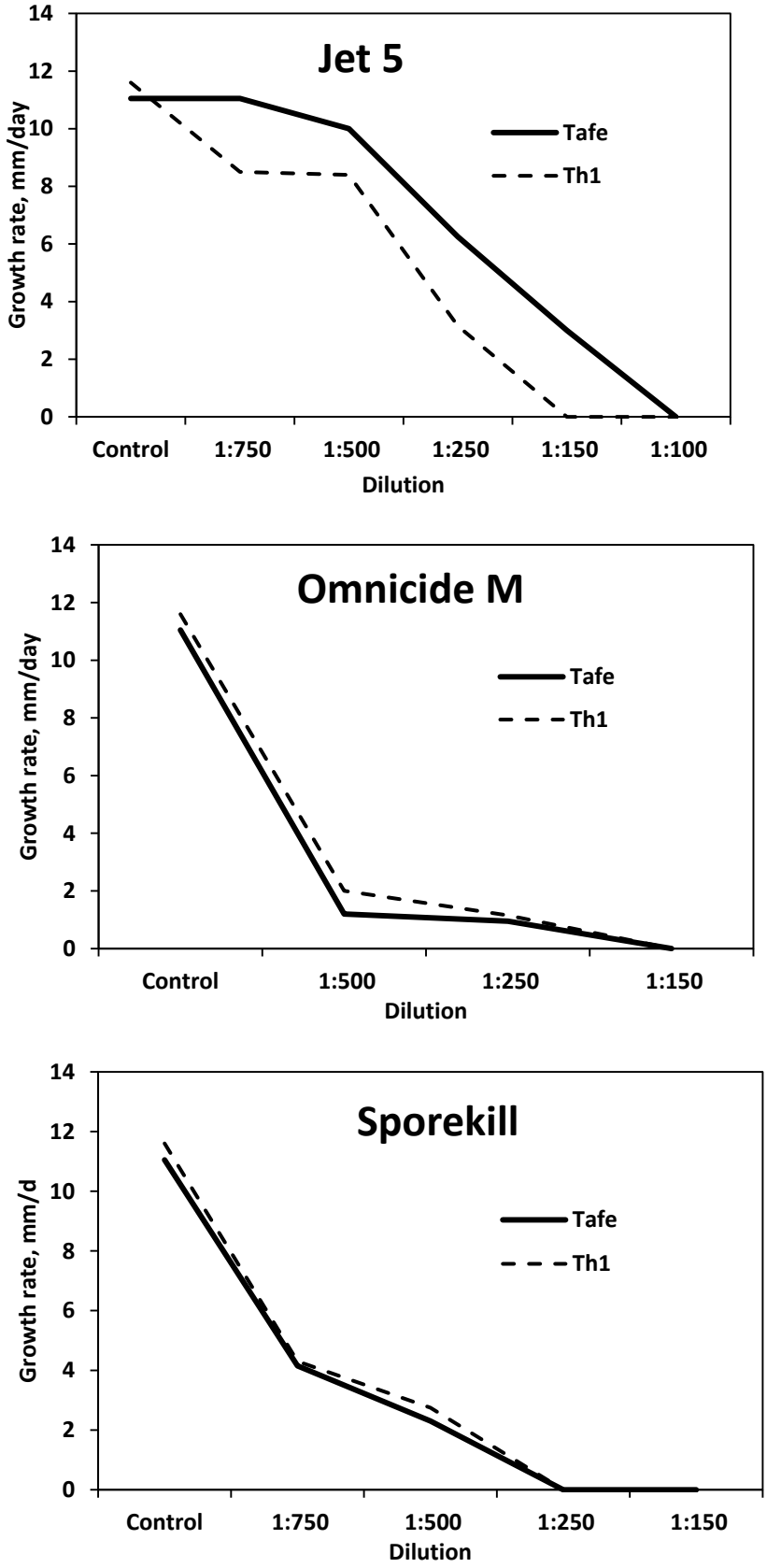

Fig.12. Effect of Jet 5, Omnicide M and Sporekill in agar on the mycelial growth rate of *Trichoderma aggressivum* (Tafe) and *Trichoderma harzianum* (Th1). Each value is the mean of three replicate plates.

(d) Gaseous disinfectants against Trichoderma aggressivum spores

The concentrations of ammonia, chlorine dioxide and ozone declined during the 17 hours after the flasks were filled (Table 10). This was due to the decomposition of the compounds and/or adsorption on to the glass walls of the flask. This decline was proportionately greater with chlorine dioxide than with ammonia or ozone.

Viable spores from the control (5 ml water) flasks were retrieved at a level of 60% of those which were inserted into the flasks at the start. Gaseous ammonia did not kill all *T. aggressivum* spores at an initial concentration of 6000 ppm. Spores survived an initial ozone concentration of 240 ppm but were killed after exposure to an initial 400 ppm of ozone. Activated Purogene at 1:33 resulted in an initial gaseous chlorine dioxide concentration of 12 ppm which killed all spores but a 1:100 dilution resulted in 2 ppm and was not effective.
The vapour from Disolite at 1:250 dilution killed all spores, as did Prophyl at a 1:100 dilution.

Table 10. Effect of gaseous disinfectants applied for 17 hours on survival of *Trichoderma aggressivum* spores.

Disinfectant	Dilution	Volume, ml	Gaseous concentration		Counts cfu/ml
			Initial	Final	
Ammonia	1:140	2	6000 ppm	5000 ppm	3.0×10^4
Ammonia	1:140	0.05	160 ppm	50 ppm	4.5×10^7
Disolite	1:100	5	2.1%	n.d.	0
Disolite	1:250	5	0.8%	n.d.	0
Prophyl	1:100	5	2.1%	n.d.	0
Prophyl	1:250	5	0.8%	n.d.	4.5×10^7
Ozone	-	-	400 ppm	300 ppm	0
Ozone	-	-	240 ppm	70 ppm	3.6×10^6
Purogene (ClO_2)	1:33	5	12 ppm	0.5 ppm	0
Purogene (ClO_2)	1:100	5	2 ppm	0 ppm	8.7×10^5
Control water	-	5	-	-	4.5×10^7

Residues of phenolic disinfectants applied to cropping tray wood

The wood blocks absorbed about 30 ml of water or disinfectant solution (0.12 ml disinfectant) before the simulated cook-out treatment (Table 11). Analysis of the disinfectant solutions at 1:250 dilution is shown in Table 12. The main phenolic compound detected in Disolite and Environ solutions was 2-phenylphenol and the main phenolic compound detected in Prophyl solution was 4-chloro-3-methylphenol.

Low background levels of phenols were detected in the water and water treated wood blocks (Tables 12 and 13). In washings from Disolite and Environ treated blocks, the main phenolic compound detected was 2-phenylphenol. In washings from Prophyl treated blocks, 4-chloro-3-methylphenol was the main phenolic compound detected (Table 13). The levels of 2-benzyl-4-chlorophenol in washings from the Disolite and Prophyl treated blocks were also higher than that in the background level in the water treated wood blocks (Table 13). The high levels of phenolic compounds detected in washings from disinfected treated wood blocks (>100 µg/L) indicates that it would still be possible to detect these disinfectants if they were used at much lower concentrations than used here (1:250 dilution).

Table 11. Quantity of disinfectants at 1:250 dilution absorbed by wood blocks before simulated cook-out treatment.

	Water	**Disolite**	**Environ**	**Prophyl**
Disinfectant, ml	0	0.12 ml	0.116 ml	0.124 ml
Total solution, ml	33 ml	30 ml	29 ml	31 ml

Table 12. Analysis of disinfectants at 1:250 dilution, µg/L.

Compound	**Water**	**Disolite**	**Environ**	**Prophyl**
2-phenylphenol	2.20	76000	43000	6.40
4-chloro-3-methylphenol	<0.20	2.20	<0.20	2800
2-benzyl-4-chlorophenol	<0.20	270	1700	670

Table 13. Residues of phenolic compounds detected in washings from cooked-out wood blocks, µg/L.

Compound	**Water**	**Disolite**	**Environ**	**Prophyl**
2-phenylphenol	<1.00	2600	490	<1.0
4-chloro-3-methylphenol	0.21	0.22	0.21	120
2-benzyl-4-chlorophenol	10.0	18.0	10.0	14.0

Conclusions

Compost pastuerisation treatments

- Spore and grain inoculum of *Trichoderma aggressivum* required compost pasteurisation at 60°C for 12 hours to achieve eradication although this treatment was not sufficient to completely eradicate compost inoculum containing a high level of Trichoderma.
- Addition of urea to compost increased the ammonia concentration during pasteurisation but did not affect *Trichoderma aggressivum*, the spores of which were able to withstand 5000 to 6000 ppm ammonia for 17 hours.
- Mushroom yield was reduced by the addition of urea at 1 g/kg Phase I compost or at 0.5 g/kg compost if Trichoderma compost inoculum was also present.
- Ammonia concentration measured during pasteurisation of commercial Phase II tunnels ranged from 110 to 425 ppm.

Trichoderma detection methods

- There was general agreement between the results of RT-PCR, dilution plating and compost on agar methods for detecting low levels of *Trichoderma aggressivum* inoculum in compost samples. Some discrepancies between the detection methods may have been due to the presence of other background *Trichoderma* species in the compost not detected by RT-PCR or the heterogeneity of the Trichoderma inoculum in the compost samples.
- There were relationships between the levels of Trichoderma detected in compost samples using RT-PCR, dilution plating or compost on agar methods and the subsequent mushroom yield from the compost samples.

Effect of disinfectants on Trichoderma inoculum

- Disolite and Omnicide M were more effective than Environ and Prophyl at the same dilutions in killing Trichoderma spores, with Disolite being the most effective. Bleach also showed efficacy in killing spores, but it needed to be used at a dilution of 1:5 to eradicate the higher concentration of spores.
- Jet 5 at 1:100 was not very effective in killing Trichoderma spores and Sporekill at 1:100 or activated Purogene at 1:20 were ineffective.
- Disolite, Environ or Prophyl added in dilutions of 1:750 (or more concentrated) to PDA medium completely suppressed the mycelial growth of *Trichoderma aggressivum* and *T. harzianum.*

- Sporekill suppressed mycelial growth of both *Trichoderma* species at a dilution of 1:250, whereas Omnicide M required a higher concentration (1:150) to achieve the same effect. Jet 5 was more inhibitory to the growth of *T. harzianum* than to that of *T. aggressivum* and required a concentration of 1:100 to completely suppress the growth of *T. aggressivum*.
- Bleach suppressed Trichoderma mycelial growth when added to PDA at a 1:5 dilution. Mycelial growth rate was reduced by activated Purogene at a concentration of 1:33.
- The vapour from Disolite at 1:250 dilution killed all Trichoderma spores, as did Prophyl at a 1:100 dilution after 17 hours exposure.
- Trichoderma spores survived 70-240 ppm ozone but were killed after exposure to 300-400 ppm ozone for 17 hours. Activated Purogene at 1:33 resulted in an initial gaseous chlorine dioxide concentration of 12 ppm which killed all Trichoderma spores after 17 hour exposure.
- The residues of phenolic disinfectants were detected on blocks of wood which had been dipped in 1:250 dilutions and then subjected to a simulated cook-out treatment.

Recommendations for Compost Pasteurisation Treatment, Trichoderma detection and the Use of Disinfectants

1. In the event of a *Trichoderma aggressivum* outbreak, compost pasteurisation at 60°C for 12 hours is necessary. Shorter periods at 60°C should only be considered if *T. aggressivum* is not a problem.
2. Urea should not be added to compost immediately before filling of Phase II; *Agaricus bisporus* is more sensitive to ammonia than *Trichoderma aggressivum*.
3. Detection of Tafe in compost samples should be determined by RT-PCR; however, positive baiting of a larger number compost samples on agar followed by RT-PCR analysis of Trichoderma positive samples will improve the detection limit of Trichoderma whilst reducing the cost of conducting a large number of RT-PCR analyses.
4. Disolite was the most effective disinfectant against Trichoderma spores and was equally effective against Trichoderma mycelium as Environ and Prophyl. The vapour from Disolite in a 1:250 dilution is also toxic to spores.
5. Omnicide M was the most effective non-phenolic disinfectant against Trichoderma spores and Sporekill was the most effective in inhibiting Trichoderma mycelial growth.
6. Bleach needs to be used at 1:9 or more concentrated to kill Trichoderma spores and inhibit mycelial growth.

7. Residues of phenolic disinfectants (Disolite, Environ and Prophyl) could be detected at high levels on treated and cooked-out wood after dipping in 1:250 dilutions. They should not be used where they are likely to come into contact with the crop or substrates.

Recommendations for Further Work

1. Test efficacy of other non-phenolic disinfectants against Trichoderma spores and mycelium (e.g. quaternary ammonium chlorides such as Virocid, Verticide, Tri-Clean and Tri-Fog; iodine based disinfectants such as Rapidyne and Tricide, and gluteraldehyde products such as Viroshield and Cidex Plus).
2. The effect of shorter periods than 17 hours of vapour and fogging disinfectant treatments (e.g. Disolite, activated Purogene) should be established.
3. Effective disinfectants for Trichoderma in infected compost are needed.

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Appendix

Manufacturers and UK Suppliers of Disinfectants Used in the Tests

(a) Progress Products, Queenswood House, 70 Durleigh Road, Bridgwater, Somerset, TA6 7JE.

(b) Steris Ltd, Chancery House, 190 Waterside Road, Hamilton Industrial Park, Leicester, LE5 1QZ.

(c) Certis, 1b Mills Way, Boscombe Down Busioness Park, Amesbury, Wiltshire, SP4 7RX.

(d) IPP Ltd, Unit 13 Bollington Lane, Nether Alderley, Cheshire, SK10 4TB.

(e) J.F. McKenna Ltd, 66 Cathedral Road, Armagh, BT61 8AE, Northern Ireland.

(f) Onnic International Ltd, Unit 12, St Tristan Close, Locks Heath, Southampton, Hampshire, SO31 6XR.

(g) Tristel Technologies Ltd, Lynx Business Park, Fordham Road, Snailwell, Cambridgeshire, CB8 7NY.

(h) Staples Disposables Ltd, Hurlingham Business Park, Fulbeck Heath, Grantham, Lincolnshire, NG32 3HL.

(i) Sigma-Aldrich Company Ltd, The Old Brickyard, New Road, Gillingham, Dorset, SP8 4XT.