CONTRACT REPORT MIA

Control of *Trichoderma harzianum* in compost using fungicides

by

Helen Grogan
Horticulture Research International, Littlehampton
West Sussex, BN17 6LP

and

J T Fletcher
ADAS Boxworth, Boxworth, Cambridge, CB3 8NN

Work done jointly by ADAS and HRI at Littlehampton, West Sussex

ADAS, HRI/HDC CONTRACT

Final Report:	November 1993
Project Number:	MIA
Project Title:	Control of *Trichoderma* in relation to compost and fungicides
Project Leader:	Helen Grogan - experiment leader and author of the research report, and J T Fletcher
Location of Project:	Work done jointly by ADAS and HRI at HRI Littlehampton, West Sussex
Project Co-ordinator:	G Pointing
Date Project Commenced:	November 1992
Date Project Completed:	September 1993
Key Words:	Mushroom (*Agaricus bisporus*) *Trichoderma harzianum* Fungicides Thiabendazole Carbendazim Benomyl

CONTENTS

Relevance to growers and practical application

Application

Carbendazim (Bavistin) or thiabendazole (H250) applied to spawn at the rate of 1.1 g per tonne of compost equivalent successfully reduced the effects of *Trichoderma harzianum* on yield. Fungicide residue levels in first flush mushrooms were within acceptable limits. Subject to further residue data and off label approval being obtained, compost *Trichoderma* can be successfully controlled by a single fungicide application to spawn.

Background

A large number of mushroom growers have had direct experience of *Trichoderma* compost mould since it was first seen in 1985. Effects on yield have varied from slight to devastating. The problem has been most severe for bag and block growers but severe problems have occurred, albeit less frequently, in all systems of growing. The only control available to the industry at present is strict hygiene and particular attention to all phases of crop culture from the cool down of phase II compost until the application of the casing layer. Application of fungicides to control other diseases has had little or no effect on the development of *Trichoderma*. This is not surprising as the fungus colonises the compost and surface application of fungicides is unlikely to have any effect.

Understanding how and why *Trichoderma* develops has been a major objective of MAFF funded research at ADAS Wye and HRI Littlehampton. Early experiments aimed at

reproducing the problem failed even when high levels of *Trichoderma* inoculum were added to the compost. But progress has been made since it was realised that the grain of the spawn is an important food base for the *Trichoderma*. When spawn grains were inoculated with *Trichoderma* spores or when spawned compost was exposed to high concentrations of spores at spawning, the compost mould problem was consistently reproduced with some strains of *T. harzianum*. This led on to the work requested here. The question was asked, could *Trichoderma* be controlled by protecting the grain with a small amount of fungicide for a sufficiently long period to allow the *Agaricus* to grow away from the grain? The results of the experiment summarised below show clearly that the answer to this question is yes.

Summary

Phase II mushroom compost was inoculated with a high concentration of *T. harzianum* spores (a known aggressive strain). Fungicides were applied to the inoculated compost or to mushroom spawn at rates equivalent to 70 g and 1.1 g per tonne of compost respectively. Three similar benzimidazole fungicides were used, Benlate, Bavistin and H250 (a new formulation of Hymush) to evaluate their effectiveness in controlling the effects of *Trichoderma* in compost.

The yield of mushrooms from *Trichoderma*-inoculated compost with no fungicide treatments was reduced by 62% in comparison with the healthy uninoculated compost (Figure 1). The Bavistin spawn treatment gave 84% control, i.e. reducing the yield loss to only 16%. Good control was also achieved by the H250 spawn treatment and the Bavistin and Benlate compost treatments. Residue levels in first flush mushrooms from all the treatments were low.

Generally *Trichoderma* populations in the compost were highest where yield reductions were greatest (Figure 5), but during the course of this experiment very little visible green mould was seen although abundant red pepper mites were observed. Improvements in control may be obtained by a more even distribution of fungicide on the spawn. Furthermore, only one rate of fungicide application was examined and it may not represent the optimum rate for effective control.

Action Points for Growers

1. Until further residue analysis results and off label approval are obtained the fungicidal spawn treatment cannot be used.

2. Growers with *Trichoderma* problems should be aware of the importance of the spawn grains as food sources for *Trichoderma*. Other similar concentrated carbohydrate foods in the compost could act similarly.

3. The presence of red pepper mites on the casing surface is a very good indication of the presence of *Trichoderma*. These mites only reproduce when they are feeding on *Trichoderma* spores.

4. The absence of green mould on the casing surface or in the compost does not necessarily mean that *Trichoderma* is not present. If the yield has been reduced and areas of compost are poorly colonised by *Agaricus* it is worthwhile having the compost checked for *Trichoderma* or other weed moulds.

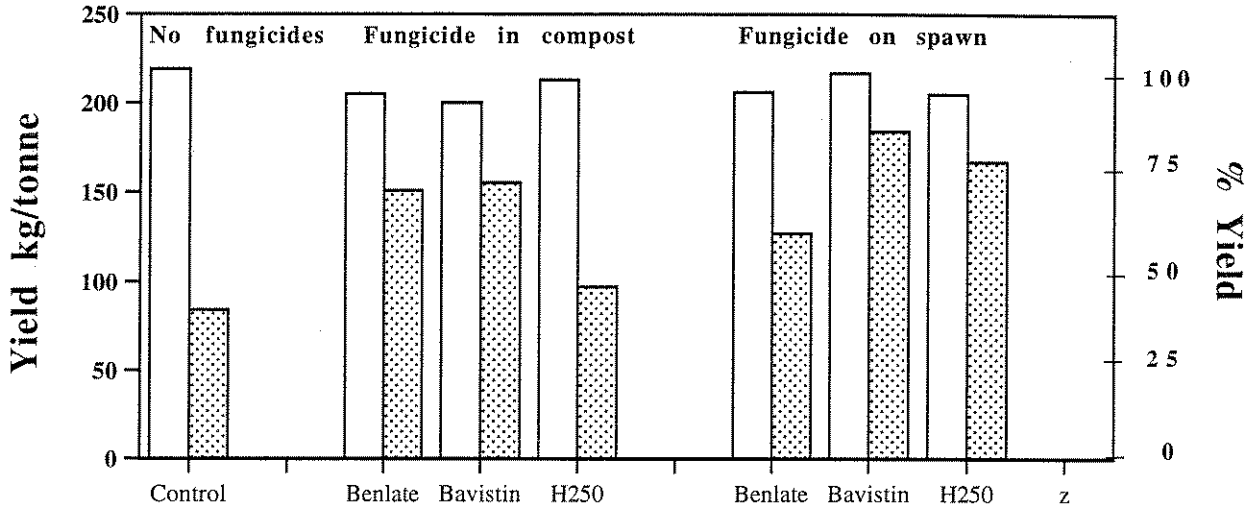

Figure 1. Yield of mushrooms obtained from *Trichoderma inoculated* ⊡, or uninoculated □ compost. Fungicides were either incorporated into compost or applied to spawn. Least significant difference (5%) = 32 kg. Percentage yield scale based on yield from uninoculated control with no fungicides.

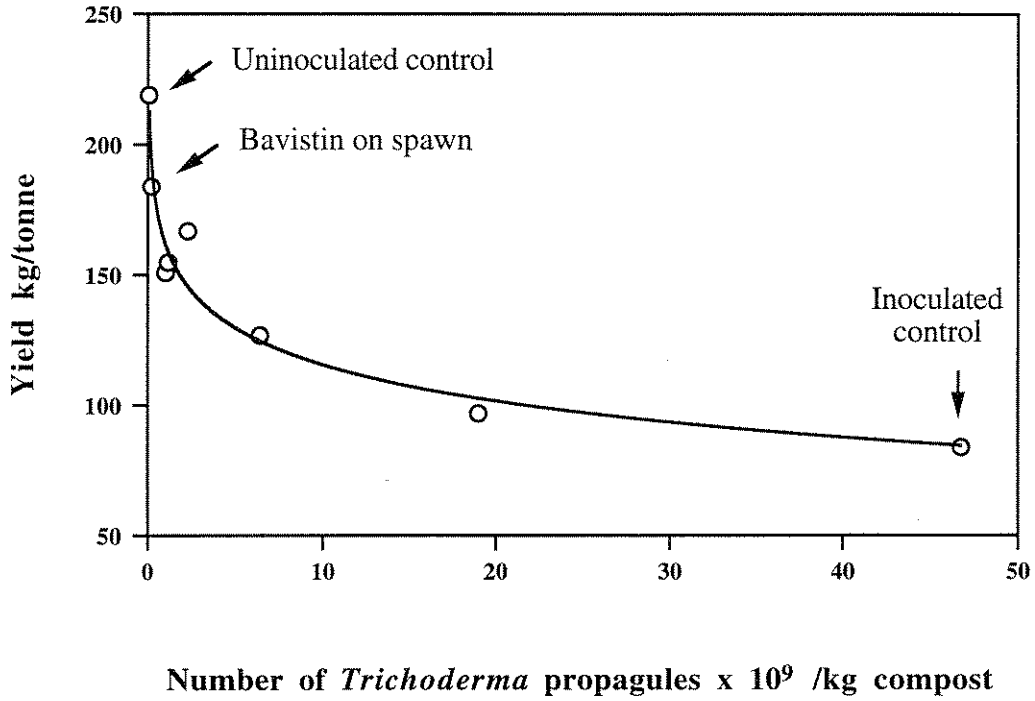

Figure 5. Relationship between yield and the number of *Trichoderma* propagules isolated from compost after cropping.

Research Report

Introduction

Trichoderma harzianum remains a serious weed mould of mushroom cultivation ever since the initial devastating outbreaks in 1985/86. Until now control has relied heavily on good hygiene practices as once green mould has been observed on compost significant yield reductions may have already occurred and the potential for reinoculation of clean compost is high (Seaby 1987; Doyle 1991). Understanding how and why *Trichoderma* develops in compost has been a major objective of MAFF funded research at ADAS Wye and HRI Littlehampton. Early small scale experiments which tried to reproduce the problem failed even when high levels of *Trichoderma* were added to compost. It was only when *Trichoderma* spores were placed directly on spawn grains that the compost mould problem was consistently reproduced. However, large scale experiments at HRI Littlehampton showed that it was not essential for the *Trichoderma* spores to contaminate spawn in order for the compost mould problem to develop. It sufficed for the spawned compost to be exposed to *Trichoderma* spores at spawning. Devastating crop losses were achieved when compost was inoculated with a concentrated spore suspension during the spawning process using perfectly clean spawn. There is no doubt, however, that the spawn grain is an important food base for *Trichoderma*. In the absence of spawn, *Trichoderma* spores alone never colonise compost. The question to be asked then is can *Trichoderma* outbreaks be controlled by simply protecting the grain with a small amount of fungicide or is it necessary to treat all the compost? An HDC-funded experiment was carried out at HRI Littlehampton to examine this hypothesis. Three benzimidazole fungicides were tested for their effectiveness to control the development of *Trichoderma* green mould in mushroom compost following inoculation with a concentrated suspension of *Trichoderma* spores. The spawn used was perfectly clean and free from any *Trichoderma* contamination.

Materials and Methods

Fungus

An aggressive Th2 strain of *T. harzianum* was used for the experiment. Cultures were grown on malt agar at 25° for four days after which time abundant green spores were produced. These were washed off with sterile distilled water and diluted to give a spore suspension with 2.8×10^6 spores per ml. One hundred ml of this suspension were sprayed into mushroom trays as they were being filled with 45 kg of spawned compost. The concentration of *Trichoderma* spores in the compost was approximately 6 million per kg.

Compost

Six tonnes of Phase II compost made from straw, horse manure and poultry manure were brought in from a nearby commercial farm. Analyses of the compost are presented in Table 1.

Table 1. Analyses of mushroom compost (ADAS, Starcross).

Dry Mass (%)	28.4
pH Fresh	7.5
pH Dry	7.3
Total Nitrogen (% dm)	2.04
Ammonia -NH_4 (% dm)	0.227
Ash (% dm)	17.6
Starch (% dm)	2.3
Sugar (% dm)	1.0

Fungicides

Three benzimidazole fungicides, Benlate (active ingredient: benomyl), Bavistin DF (active ingredient: carbendazim) and H250 (active ingredient: thiabendazole), a new liquid formulation of Hymush from Agrichem (not yet approved for mushrooms in UK), were tested for their ability to control *T. harzianum* colonisation of compost. Fungicides were applied either into the compost at the rate of 70 g of product/tonne of compost or onto spawn at the rate of 0.23 g/kg of spawn. Assuming a 0.5% spawning rate, 1.1 g of fungicide would be required to treat the spawn for 1 tonne of compost. The Benlate and Bavistin were bulked up in chalk for ease of handling and the liquid thiabendazole (H250) was diluted in sterile distilled water (Table 2). The bulked up fungicides were sprinkled or sprayed (a) into the compost at spawning (compost treatment) or (b) onto the spawn which was then thoroughly shaken to achieve an even distribution of product (spawn treatment).

Table 2. Rates of fungicide application to compost and spawn for the control of *Trichoderma harzianum*.

	Compost (per tonne)	Spawn (per kg)
Benlate	70 g in 2.2 kg Chalk	0.23 g in 9.77 g Chalk
Bavistin DF	70 g in 2.2 kg Chalk	0.23 g in 9.77 g Chalk
H250 (liquid)	70 g in 800 mls H_2O	0.23 g in 10 mls H_20

Mushroom Cultivation

The trays of spawned compost (Somycel 609) were placed in a spawn-running chamber and allowed to spawn run for 18 days with compost temperatures on average 24-25°C at which time a standard peat/chalk casing (7:1 v/v) was applied to each tray to a depth of 45 mm. The trays were placed in a mushroom cropping house and allowed to case run for 6 days with compost temperatures at 22-24°C. The trays were watered on days 1, 2 and 3 after casing, and on day 6 the cropping house was aired and the air temperature brought down to 17.6°C to induce pinning. The first mushrooms were picked on day 19 and 5 flushes were harvested over 6 weeks. Mushrooms were harvested predominantly as closed cups or buttons.

Assessment of *Trichoderma* during and after Cropping

The compost and casing were visually assessed during the cropping cycle for any manifestations of *T. harzianum* and/or associated red pepper mites (*Pygmephorus* spp.). Both organisms were scored as the % of surface area which they covered at a given time.

Prior to cook-out five sub-samples of compost were taken from every inoculated tray, as well as all uninoculated control trays. The sub-samples for each tray were well mixed together in a plastic bag. A 20 g sample was then taken from each bag and used to estimate the number of *T. harzianum* propagules present in the compost by means of a dilution series. Each 20 g sample was placed in a 500 gauge 18 x 26 cm plastic bag, covered with 360 ml of sterile distilled water (sdw), and allowed to stand for 1 hour. The compost + sdw was then agitated for 1 minute using a Stomacher Lab-Blender 400, allowed to stand for 5

minutes and then agitated again for another minute. One ml of the resulting compost suspension was added to 99 ml of sdw to give a 10^{-2} dilution. Similarly, 1 ml of this dilution was added to a further 99 ml of sdw to give a 10^{-4} dilution from which a 10^{-6} dilution was also prepared in the same way. One ml from each of the three dilutions 10^{-2}, 10^{-4} and 10^{-6} was pipetted into a sterile Petri dish to which was added 10-15 ml of molten Ohio State Experimental Station (OSES) agar with streptomycin (Appendix I). When the agar had set, the dishes were inverted and incubated at 27°C for 3 days. The number of *Trichoderma* colonies growing on each dish was then counted and a mean value was calculated for each treatment. The mean value was put into the following equation to give the number of *Trichoderma* propagules (T) present per gram fresh weight of compost.

$$T = \frac{\bar{X} \text{ x dilution factor x } 360}{20}$$

Residue analysis

One to two hundred grams of first flush mushrooms were randomly selected from the eight replicate trays for each of the six fungicide treatments for fungicide residue analysis. First flush mushrooms from the uninoculated control treatment which had received no fungicides were also analysed for fungicide residues. The eight replicate samples for each treatment were bulked together and a sub-sample was taken from each to give a single residue level per treatment. The analyses were done to "Good Laboratory Practice" (GLP) standards by Aspland and James of Chatteris, Cambridgeshire.

Experimental Design and Statistical Analyses

A randomized block experimental design was set up consisting of 14 treatments which are summarised in Table 3. Each treatment was replicated eight times.

Table 3. Summary of fungicide and *Trichoderma* treatments used.

	Trichoderma treatments	
	Inoculated	Uninoculated
Fungicide treatments		
Fungicide on compost	Benlate Bavistin H250	Benlate Bavistin H250
Fungicide on spawn	Benlate Bavistin H250	Benlate Bavistin H250
No fungicides	Control	Control

The raw yield data were subjected to randomized block analysis of variance (anova) and the means for each treatment were then presented as histograms along with the least significant difference value (l.s.d.). Percentage red pepper mite cover was logit transformed and an anova was carried out on the transformed data for days 5, 12 and 19 after casing. Mean values were then back-transformed into % values and presented in a graph format. The number of *Trichoderma* propagules isolated from the compost after cropping was log transformed and an anova was carried out on the transformed data. The mean values were then back-transformed and presented as histograms.

Results and Discussion

Yield

The Bavistin spawn treatment gave the best control of *Trichoderma* overall with mushroom yields of 84% of control being recorded compared with 38% for inoculated compost with no fungicide treatment (Figure 1). The control yield from uninoculated compost with no fungicide treatment was 219 kg mushrooms per tonne compost. The H250 spawn treatment also gave very good control with a yield of 77% of control being obtained. The Benlate spawn treatment was one of the least successful giving a yield of 58%. Two of the compost fungicide treatments, Bavistin and Benlate, gave good levels of control with yields of 71 and 69% of control being achieved. The H250 compost treatment gave a yield of only 44% of control which was not significantly different from inoculated compost with no fungicides. None of the fungicides used had any effect on yield in the absence of *Trichoderma*.

Assuming a compost spawning rate of 0.5%, 5 kg of spawn are required to spawn 1 tonne of compost. Based on the rate of fungicide used during this experiment, 1.15 g of fungicide is needed to treat the spawn for 1 tonne of compost. This is considerably less than the 70 g of product required to treat the compost itself so that spawn treatment represents a much more cost effective use of these chemicals. In addition, better yields were obtained from spawn treatments compared with compost treatments for two of the three chemicals used (H250 and Bavistin) indicating that spawn treatment is more effective at controlling the effects of *Trichoderma*.

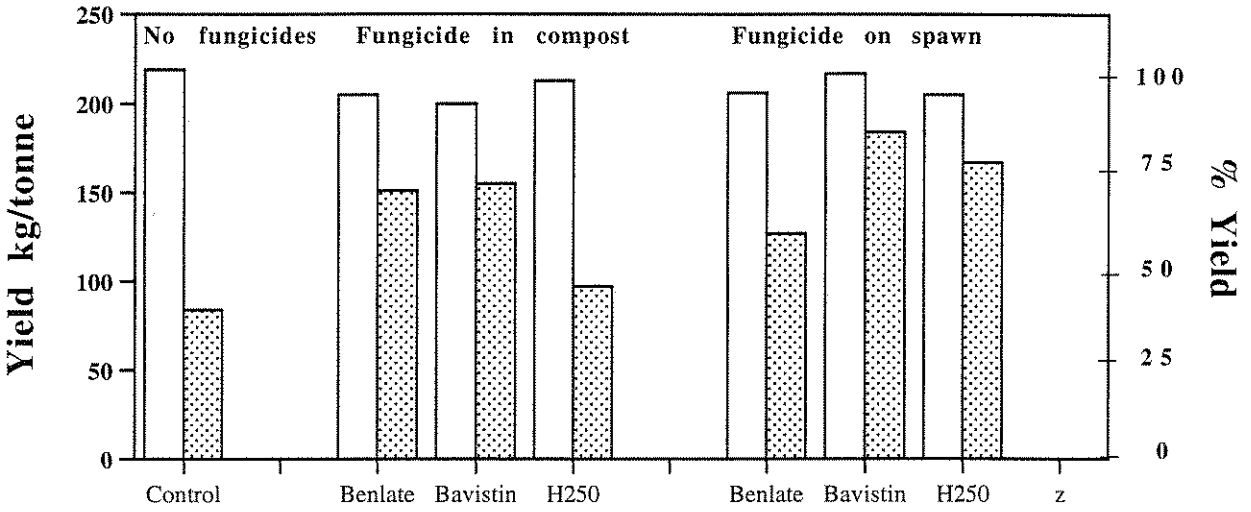

Figure 1. Yield of mushrooms obtained from *Trichoderma inoculated* ⊡ , or uninoculated □ compost. Fungicides were either incorporated into compost or applied to spawn. Least significant difference (5%) = 32 kg. Percentage yield scale based on yield from uninoculated control with no fungicides.

The fungicides H250 and Bavistin applied to spawn resulted in a significant improvement in yield compared with *Trichoderma* inoculated compost with no fungicide-treatments however total control of *Trichoderma* was not achieved. There are three possible explanations for this. The first is the very high number of *Trichoderma* spores inoculated into the compost (8 million/kg). MAFF funded work carried out at HRI indicates that a spore concentration of 80,000 spores/kg is sufficient to severely reduce crop yields. The *Trichoderma* inoculum used in the fungicide experiment was 100 times more concentrated than what is required to seriously affect *Agaricus* production and therefore probably represents an unrealistically high level of inoculation. If that is the case then it is probably not surprising that 100% control was not achieved. Thus the degree of control obtained during this experiment where a very high *Trichoderma* inoculation level was used looks very promising.

A second possible explanation for not achieving 100% control may have been due to an uneven distribution of fungicides in the compost or on the spawn. When some of the fungicide-treated spawn grains were incubated on *Trichoderma*-inoculated plates, some grains appeared to have received little or no fungicides (Plate 1, Appendix II). It is quite possible therefore that pockets of compost or individual spawn grains which did not receive any fungicide as a result of incomplete mixing could serve as safe havens for the growth of *Trichoderma*. Thirdly, the fungicides themselves are broken down by various microbial and environmental factors so that after a period of time there is no longer any fungicidal activity. Incubation of treated spawn grains on *Trichoderma*-inoculated plates showed how H250 treated spawn grains still suppressed *Trichoderma* growth six weeks after treatment (Plate 2, Appendix II). Although control of *Trichoderma* colonisation of compost is probably most critical during the two weeks of spawn running, the longer lasting effect of the thiabendazole

fungicide H250 warrants further investigation, particularly since it is unclear whether *T. harzianum* can colonise spawn-run or cased compost. Unfortunately, the liquid formulation of H250 was more difficult to apply on an experimental basis than either of the two powder formulations, Benlate or Bavistin. Nonetheless, the H250 spawn treatment gave a good measure of control in terms of yield and refinement of the application method could improve yields even further.

Incidence of *Trichoderma*

At the end of the spawn-run only inoculated, non-fungicide-treated controls showed any visible signs of *Trichoderma* and then only at low levels occurring on about 11% of the surface area. After casing, and during cropping, no *Trichoderma* was seen but by the end of the crop very small areas (<1%) of *Trichoderma* green mould were observed on a small number of trays. A few days after casing, however, large numbers of red pepper mites (*Pygmephorus* spp.) appeared on the casing surface. These were considered to reflect the presence of *Trichoderma* within the compost. Populations increased in all inoculated treatments between day 5 and day 12 after casing and then subsequently declined. The *Trichoderma*-inoculated controls with no fungicides had the highest % red pepper mite cover than either the Bavistin- or Benlate-treated compost (Figure 2). Similarly, when Bavistin- or Benlate-treated spawn was used fewer, red pepper mites were recorded (Figure 3).

Trichoderma harzianum propagules were isolated from the compost from all the inoculated treatments with the two least successful fungicide treatments (H250 in compost and Benlate on spawn) having the highest numbers of propagules after the inoculated controls (Figure 4).

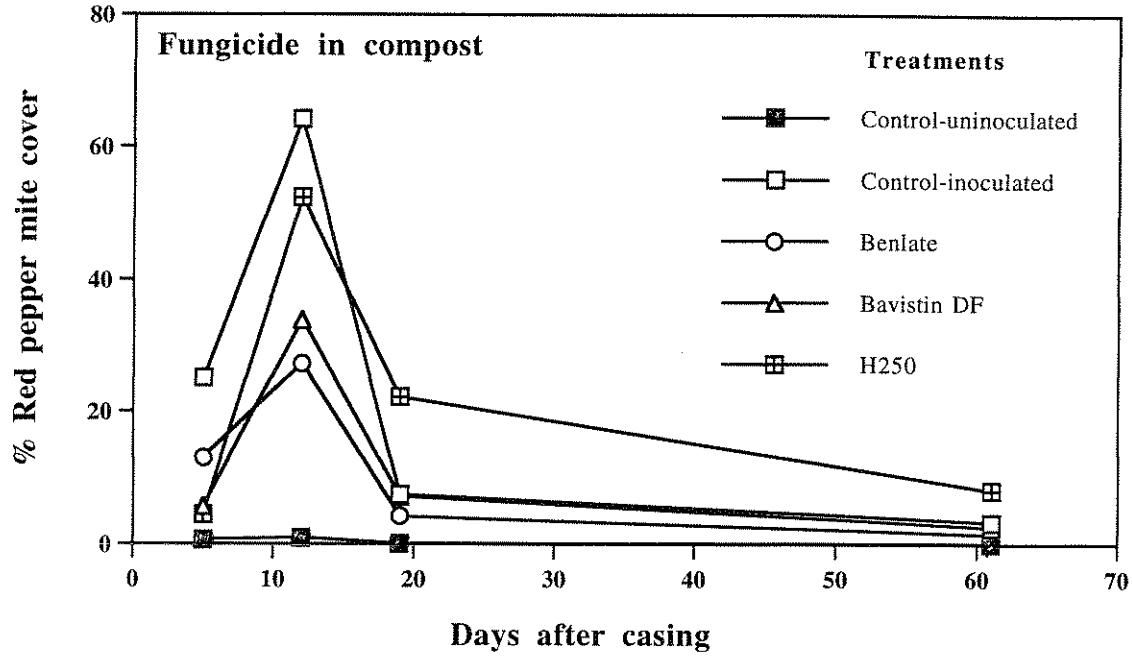

Figure 2. Percentage of casing surface with red pepper mites following *Trichoderma* inoculation of fungicide-treated and untreated compost.

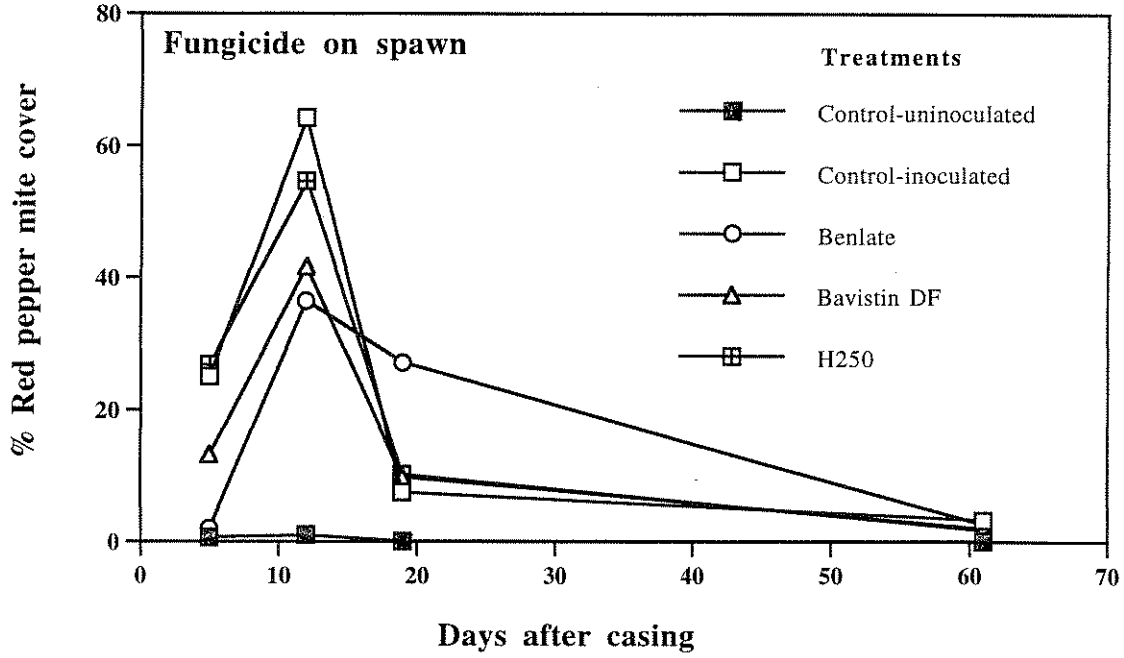

Figure 3. Percentage of casing surface with red pepper mites following *Trichoderma* inoculation of compost using fungicide-treated and untreated spawn.

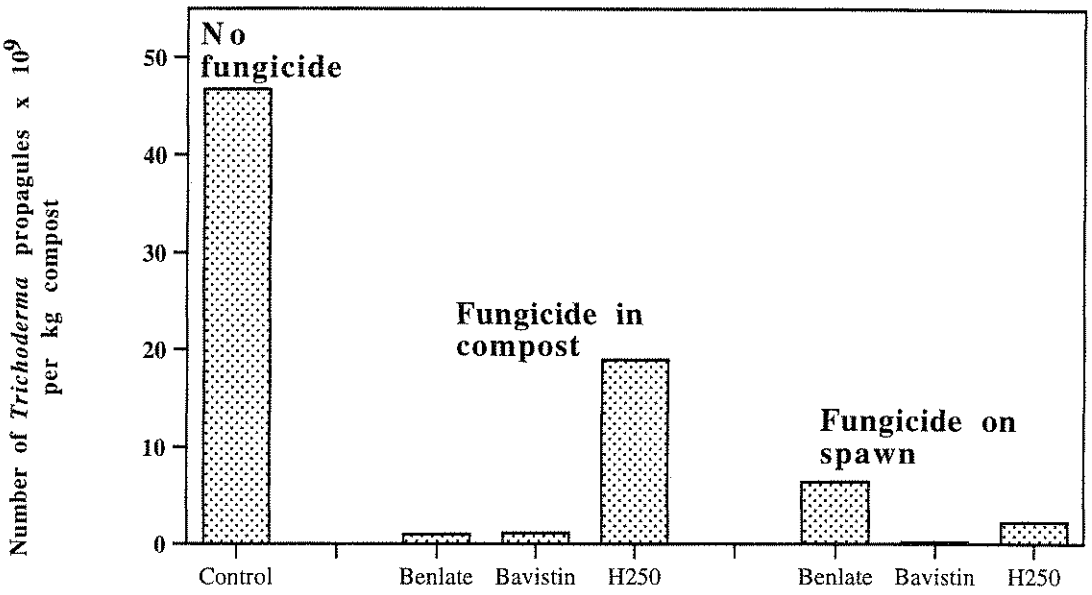

Figure 4. Number of *Trichoderma* propagules present in inoculated compost after cropping. Fungicides had been incorporated into compost, applied to spawn or not applied.

There was a significant correlation between the number of *Trichoderma* propagules present in compost samples at the end of cropping and the yield of mushrooms obtained (Figure 5).

In view of the very high inoculation rate it was quite surprising that so little *Trichoderma* green mould was observed during spawn running and cropping. *Trichoderma* was clearly present in the compost as reflected by yield reductions, large populations of red pepper mites during case-running and numbers of propagules isolated from compost samples after cropping. This absence of visible signs of *Trichoderma* has been recorded before at HRI during MAFF-funded experimental work and the absence of visible *Trichoderma* appeared to be related to some unidentified compost factor. The observation that *T. harzianum* can be present in compost, severely reducing yields, and yet still not manifest itself very significantly on compost and/or casing surfaces as green mould, is a major cause of concern. Unless compost samples are analysed for *Trichoderma* propagules the cause of the yield losses may be attributed to some other factor allowing a build-up of *Trichoderma* to occur on the farm unchecked. The presence of red pepper mites in large numbers is a good indicator of the presence of *Trichoderma* in the compost but they do not always occur and one species can feed on other weed moulds (Gurney & Hussey 1967).

Residue Analyses

The results of the residue analyses (Table 4) show acceptable or zero levels for all treatments. Benomyl, the active ingredient in Benlate, was detected as carbendazim - a natural breakdown product of benomyl. The levels of carbendazim which were detected in mushrooms from the Benlate and Bavistin spawn treatments were higher than those from the

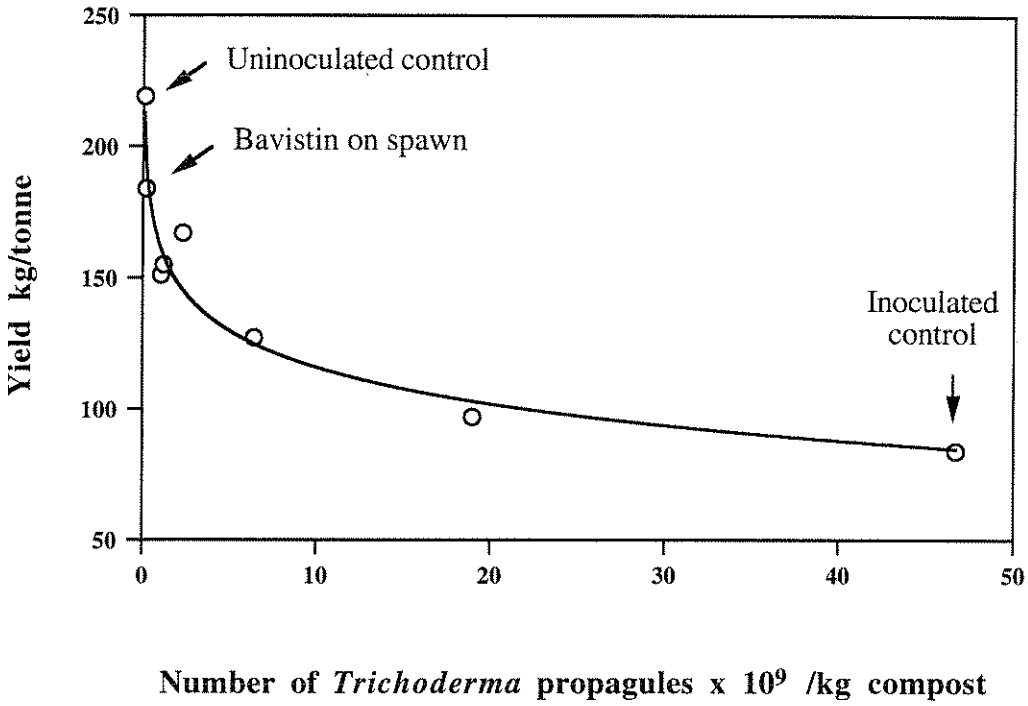

Figure 5. Relationship between yield and the number of *Trichoderma* propagules isolated from compost after cropping.

Table 4. Residue levels in first flush mushrooms following fungicide treatments of spawn and compost.

Treatment	Residue level (mg/kg)	
	Carbendazim (M.R.L.* = 1 mg/kg)	Thiabendazole (No M.R.L.)
Control	ND**	D
Benomyl in compost	0.31	-
Carbendazim in compost	0.73	-
Thiabendazole in compost	-	D
Benomyl on spawn	0.62	-
Carbendazim on spawn	0.93	-
Thiabendazole on spawn	-	ND

* M.R.L. = Maximum Residue Level accepted by Pesticide & Safety Division

** ND = Not detected
D = Detected but below the limit of quantification
\- = Not tested

compost treatments despite the fact that almost 70 times less fungicides were used. This probably reflects the more accurate targeting of the fungicides. The carbendazim levels recorded were below the maximum residue levels (M.R.L.) accepted by MAFF Pesticide and Safety Division (HMSO 1988). There is currently no M.R.L. for thiabendazole residues in mushrooms. In the experiment reported on here, thiabendazole was detected at levels which were below the limit of quantification. In contrast to the carbendazim results more thiabendazole was detected in mushrooms from the compost treatment compared with those from the spawn treatment. In addition, thiabendazole was detected in mushrooms from the

control treatment which is an unexpected and unexplained result. Further residue data are required to clarify these findings. The level of carbendazim recorded for first flush mushrooms following the application of Bavistin to spawn was very close to the maximum residue level allowed in the U.K. of 1 mg/kg. It is therefore important to examine the effectiveness of lower fungicide application rates in addition to maximising the distribution of fungicides on the spawn. However, until further residue analysis results and off label approval are obtained, fungicidal spawn treatment cannot be used.

Conclusions

Trichoderma harzianum in compost can be controlled by a single application of carbendazim (Bavistin DF) or thiabendazole (H250) to the spawn. Applying the fungicides to the spawn is as effective or more effective than incorporating them into the compost but requires far less product for similar or better results, thereby being more economical. Residue analyses of first flush mushrooms from this experiment were within acceptable limits. The presence of *T. harzianum* in the compost is not always accompanied by visible green sporulation on the compost or casing. In the absence of visible *Trichoderma* large populations of red pepper mites are an indication of *Trichoderma* in the compost.

References

Doyle, O. (1991). *Trichoderma* green mould - Update. **Irish Mushroom Review** 3(12): 13-17.

Gurney, B. & Hussey, N.W. (1967). *Pygmephorus* species (Acarina: Pyemetodidae) associated with cultivated mushrooms. **Acarologia** 9(2): 353-358.

HMSO (1988). Statutory Instruments 1988 No. 1378. Pesticides. The Pesticide (maximum residue levels in food) regulations 1988, 9 pages.

Seaby, D. (1987). Infection of mushroom compost by *Trichoderma* species. **The Mushroom Journal** 179: 355-361.

Appendix I

Ohio State Experimental Station (OSES) Agar Medium

Glucose	5.0 g
Yeast Extract	2.0 g
Sodium nitrate	1.0 g
Magnesium sulphate (hydrated)	0.5 g
Potassium dihydrogen phosphate	1.0 g
Ox-bile	1.0 g
Sodium propionate	1.0 g
Agar (Technical Grade N° 3)	15.0 g

Made up to 1 litre with distilled water

Streptomycin: 1 g in 100 ml sterile distilled water.
Add 10 ml of this solution to the molten agar before pouring to give 100 ppm.