Project title	*Trichoderma* green mould – determining diversity and highlighting risks
Project number:	M46
Project leader:	Charles Lane, Central Science Laboratory
Report:	Final report May 2008
Previous report	
Key staff:	Dr Charles Lane Prof Peter Mills Dr John Burden
Location of project:	Central Science Laboratory, York
Project coordinator:	Richard Gaze
Date project commenced:	01/12/2007
Date project completed (or expected completion date):	31/05/2008
Key words:	Trichoderma compost mould

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

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.

Nicola Spence
Head of Plant Health Group
Central Science Laboratory

Signature ..... Date .....30/05/2008.....

[Name]
[Position]
[Organisation]

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

Report authorised by:

[Name]
[Position]
[Organisation]

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

[Name]
[Position]
[Organisation]

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

CONTENTS

Grower Summary

Headline

- *Trichoderma aggressivum f. europaeum* (formerly known as *Trichoderma harzianum* Th2) remains the dominant cause of Trichoderma compost mould in UK mushroom production.
- The North American strain of Trichoderma compost mould (*Trichoderma aggressivum f. aggressivum*; Th4) was not detected in a UK survey.

Background and expected deliverables

The fungus Trichoderma is comprised of numerous species of which only some cause economic losses to the Mushroom industry. At the onset of the problem in the mid 1980s Trichoderma (green) compost mould and cap spotting was attributed primarily to *T. harzianum,* leading to between 30 and 100% losses. However, several studies that investigated Trichoderma outbreaks in Europe and North America (Fletcher, 1986; Seaby, 1996; Muthumeenashi et al. 1998) showed the situation to be much more complex with a number of biotypes, subsequently reclassified as species, as summarised below:

Biotype	Current Name	Present in UK	Economically damaging
Th1	*Trichoderma harzianum*	yes	no
Th2	*Trichoderma aggressivum f. europaeum*	yes	yes
Th3	*Trichoderma atroviride*	yes	no
Th4	*Trichoderma aggressivum f. aggressivum*	no	yes

Defra's Commodity Pest Risk Assessment highlighted the potential risks from the North American strain (Th4) and the lack of information about the current diversity of Trichoderma in UK production. Recent samples sent to the Mushroom Clinic at the Central Science Laboratory initially appeared to be *Trichoderma aggressivum f. aggressivum* (Th4), further highlighting the need to study Trichoderma diversity in the UK.

A targeted survey of UK growers requesting samples of Trichoderma causing economic damage was initiated in December 2007 and lasted for six months. Isolates were obtained and analysed using molecular techniques that could reliably discriminate the different species as well as the subspecies of *T. aggressivum*. Information concerning each farm outbreak was collected and analysed with respect to the type of Trichoderma found.

Summary of the project and main conclusions

Samples of green mould were obtained from 15 farms resulting in 28 isolates of Trichoderma for analysis. Samples were primarily obtained from the UK but also isolates from Ireland and South Africa. *Trichoderma aggressivum f. europaeum* (Th2) the economically damaging type was found on seven farms (17 isolates) whilst *Trichoderma harzianum* (Th1) was found on six farms (8 isolates).

Three other species of Trichoderma (e.g. *Trichoderma atroviride* (Th3) were found on late flushes causing minor damage. *Trichoderma aggressivum f aggressivum* (Th4) was not detected.

In summary, *Trichoderma aggressivum f. europaeum* was found most commonly on pre-first flush and first flush causing bare areas, with losses up to 50% over limited periods (less than one year); whilst *Trichoderma harzianum* occurred on later flushes, causing less damage but being present on farms for longer periods.

Although based on a limited survey (six months duration) *Trichoderma aggressivum f europaeum* (Th2) was primarily found on premises who bought in compost (six farms) as opposed to home produced compost (1 farm) whilst the opposite situation was observed for *Trichoderma harzianum* (Th1).

There appeared to be no relationship between the type of growing system, compost type, use and timing of supplementation with respect to the type of Trichoderma present.

Financial benefits

- A reliable molecular diagnostic test for Trichoderma species and sub-species identification is now available.

Action points for growers

- Aggressive forms of Trichoderma compost mould (*Trichoderma aggressivum f. europaeum,* Th2) can cause significant yield losses (in excess of 50%) due primarily to bare areas and cap spotting.
- *Trichoderma harzianum* (Th1) appears to be a more inherent problem causing minor losses but can be associated with bare areas and cap spotting.
- The source of compost can influence the type of Trichoderma problems encountered.
- The type of growing system, compost type and supplementation type and timing did not appear to influence the prevalence of aggressive forms of Trichoderma compost mould.

- Early detection and rigorous hygiene measures are key to successful Trichoderma management.

Science Section

Introduction

The fungus Trichoderma is comprised of numerous species of which only some cause economic losses to the Mushroom Industry. At the onset of the problem in the mid 1980s Trichoderma (green) compost mould and cap spotting was attributed primarily to *T. harzianum,* leading to between 30-100% losses. However, several studies that investigated *Trichoderma* outbreaks in Europe and North America (Fletcher, 1986; Seaby, 1996; Muthumeenashi et al. 1998) showed the situation to be much more complex. Differences in the morphology and pathogenicity of these fungi and more recently molecular analysis has lead to different groups being referred to as initially biotypes of *Trichoderma harzianum* (Th1, Th2, Th3 and Th4) but since 2002 the most damaging strains of *T. harzianum* Th 2 and 4 have been raised to species level and should be referred to as *Trichoderma aggressivum* f. *europaeum* and *T.a*.f. *aggressivum* (Samuels et al., 2002) as summarised below:

Biotype	Current Name	Present in UK	Economically damaging
Th1	*Trichoderma harzianum*	yes	no
Th2	*Trichoderma aggressivum f. europaeum*	yes	yes
Th3	*Trichoderma atroviride*	yes	no
Th4	*Trichoderma aggressivum f. aggressivum*	no	yes

Many other species of Trichoderma have been reported in association with mushroom production with varying degrees of significance with respect to economic damage.

Previous HDC projects (e.g. M1, M10, M13, M14) have investigated aspects of Trichoderma including control and rapid detection, however, there has been no recent work on *T. aggressivum f. aggressivum*. There have been other recent studies looking at the identity of Trichoderma in the mushroom industry both in America and Europe (Ospina-Giraldo et al., 1998; Samuels et al., 2002) in addition to worrying reports of fungicide resistance to carbendazim (thiophanate methyl) in strains of *Ta.f. aggressivum* (Romaine et al., 2005).

Recent samples tested by the Central Science Laboratory on behalf of Dr John Burden from two separate locations initially revealed the possible occurrence of *T. aggressivum f. aggressivum* based on DNA sequence of the ITS region and morphological features. However, following further work by comparison to reference isolates and additional more discriminatory molecular analysis (sequencing analysis of the translocation elongation factor gene 1 alpha [EF-1α]) their identity was resolved as *Trichoderma aggressivum f. europaeum* (Th2). This work, and the Commodity Pest Risk Assessment, highlighted a need to review

the current diversity of Trichoderma causing economic damage to the UK industry. That need was addressed by the project covered by this report, in which the work was conducted on behalf of the industry by the collaboration of mushroom pathologists at the Central Science Laboratory and Warwick HRI. Samples were obtained from all sectors of the industry and fungal cultures produced. Isolates were identified quickly using cost effective molecular profiling tools (based on DNA sequencing) to determine species diversity.

Overall Aim

To determine the current diversity of Trichoderma green mould species in the UK Mushroom industry

Objectives

1. Collect samples, isolate Trichoderma into pure culture and determine the occurrence of species using molecular sequencing and morphological techniques

Materials and methods

1. Sample collection

A questionnaire (Appendix 1) was produced in consultation with the consultants Richard Gaze and John Burden, who contacted a range of growers across the UK to obtain samples. A sampling pack, questionnaire and project background letter were sent, either speculatively to growers, or as a result of verbal confirmation of a Trichoderma green mould problem. Further samples were requested in a short news item in HDC News. Small quantities of compost, casing or mushrooms were sent to the Mushroom Clinic at The Central Science Laboratory for analysis.

2. Sample analysis

Samples were allocated a unique CSL reference number on receipt in order to retain the confidentiality of participating farms. Samples were examined for the presence of any Trichoderma green mould and isolations onto potato dextrose agar (PDA) were made from any sporulation observed. In the absence of any green mould, samples were incubated in a damp chamber to encourage sporulation. Cultures were incubated at laboratory conditions (20 to 22°C: 12 hours light/12 hours dark) and checked after three to five days for growth and purity. Isolates were sub-cultured as necessary to obtain pure cultures required for molecular testing. Once pure, a small piece of the colony (0.5 x 1 centimetre) was removed for DNA analysis. DNA was extracted from cultures using the Nucleospin Plant Kit (Abgene)

and amplified by PCR with primers EF-1 and TEF-1 for the elongation factor gene as recommended by Dr Samuels as the most discriminatory region for *Trichoderma aggressivum*. PCR products were sequenced by Central Science Laboratory's Molecular Technology Unit using the BigDye Terminator kit (Applied Biosystems) on an ABI Prism 3130xl Sequencer. Resulting DNA sequences were aligned using the ClustalW algorithm within the Lasergene software suite (DNAStar) to reference sequences obtained from Genbank described in the paper by Samuels *et al* (2002). The alignment was checked manually for any discrepancies. The sequences were then subjected to neighbour-joining analysis and a tree produced for species identification.

3. Sample provenance

Data on the origin of samples was collated and tabulated.

4. Reporting results

Following receipt of samples a preliminary report was sent thanking growers for their assistance (Appendix 2), informing them of their unique reference number, describing the diagnostic process and advising them that a final report would be sent in due course. Following analysis, a final report was sent to all growers advising them of their results in addition to providing background information in order to help them to interpret these results (Appendix 3).

Results

Samples were received from 15 farms as presented in Table 1.

Geographical distribution

The majority of growers were distributed around England (11 farms) but also included two farms in Ireland (farms 12 and 13), one farm in Scotland (farm 15) and one farm in South Africa (farm 7). In total, 28 isolates of Trichoderma were obtained and analysed. Although it was only a limited survey due to the six-month duration, an acceptable geographical distribution was achieved. The findings of *Trichoderma aggressivum* f. *europaeum* (Th2) in South Africa is the first report of this particular species for this country.

Trichoderma diversity

Trichoderma aggressivum f. europaeum (Th2) was found on seven farms (farms 1, 7, 9, 10, 11, 14, 15) comprising 17 isolates whilst *Trichoderma harzianum* (Th1) was found on six farms (farms 2, 3, 4, 5, 6, 12) comprising eight isolates. *Trichoderma atroviride* (Th3) was

found on one farm (farm 8), two other species of Trichoderma were found (farms 8, 13) that did not fit within the *Trichoderma harzianum* group. No other species of Trichoderma, including *Trichoderma aggressivum f. aggressivum (Th4),* were found. Multiple samples were analysed for seven farms (farms 1, 2, 3, 7, 8, 9, 11) but on all occasions the same type of Trichoderma was found. On two farms (farms 7 and 9) isolates were obtained from casing, compost and mushrooms but again there was no variation in the type of Trichoderma identified. On farm 11 *Trichoderma aggressivum f. europaeum* (Th2) was found, not only on the surface of blocks, but also within a block but with no symptoms of Trichoderma on the surface of that block.

Trichoderma diversity and provenance of isolates

Trichoderma aggressivum f. europaeum (Th2) was found more commonly on pre-first flush and first flush samples than on later flushes, where *Trichoderma harzianum* (Th1) was more prevalent. There appeared to be no relationship between the type of growing system (bag, block, tray, shelf, block/shelf) and the Trichoderma present. *Trichoderma aggressivum f. europaeum* (Th2) was primarily found on premises that had bought in compost (six farms) as opposed to home produced compost (one farm). The opposite situation was observed for *Trichoderma harzianum* (Th1), which was predominantly found on home produced compost (five farms) as opposed to bought in compost (one farm). The type of compost (phase II, III, or II1/2) did not seem to influence the prevalence of *Trichoderma aggressivum f. europaeum* (Th2) but *Trichoderma harzianum* (Th1) was predominantly found on the Phase II compost (five farms) as opposed to phase III (one farm), which is more a reflection on production techniques than Trichoderma prevalence. The use and timing of supplementation seemed to have no influence on Trichoderma diversity. *Trichoderma aggressivum f. europaeum* (Th2) was predominantly responsible for causing bare areas (six farms) as opposed to cap spotting (one farm) whilst *Trichoderma harzianum* (Th1) was also associated with bare areas (three farms), cap spotting (two farms) or few/no symptoms (one farm). Determining the pathogenicity of isolates was not within the scope of this project. Three other species of Trichoderma were associated with bare areas and cap spotting. One isolate was *Trichoderma atroviride* (Th3) whilst the other two did not fall within the *Trichoderma harzianum* group. In general, farms that had problems with Trichoderma green mould for more than one year had *Trichoderma harzianum* (Th1) with less than 20% losses, whilst more recent problems were attributed to *Trichoderma aggressivum f. europaeum* (Th2), causing greater losses of up to 50%.

Table 1. Provenance of isolates and Trichoderma diversity.

Farm	CSL ref	Origin	Symptoms	Losses (%)	Time (years)	Growing System	Compost (source)	Phase	Supple-mentation	Isolates	Identification (EF-1α)
1	20723443	First flush	Bare areas	90	0.25	Block Shelf	Bought in	II	None	A compost B compost	Tafe (Th2) Tafe (Th2)
2	20724651	Third flush	Few/none	-	-	Tray	Home	II	None	A casing B casing	Thz (Th1) Thz (Th1)
3	20800144	Third flush	Bare areas	1-2	1	Tray	Home	II	Spawning	A casing B casing	Thz (Th1) Thz (Th1)
4	20800156	Spawn run compost	Bare areas	5	10	Bag	Home	II	None	A compost	Thz (Th1)
5	20800374	Third flush	Cap spotting	<1	2	Tray	Home	II	Spawning	A casing	Thz (Th1)
6	20800377	Second flush	Cap spotting	Up to 20	Many	Tray	Home	II	None	A2 casing	Thz (Th1)
7	20800731	Pre-first flush and caps	Bare areas Cap spotting	>10	0.1	Shelf	Home	II	Spawning	A compost B caps spotting C mushroom	Tafe (Th2) Tafe (Th2) Tafe (Th2)
8	20800806	Third flush	Bare areas Cap spotting	25	1	Block	Bought in	II	None	A casing B compost	Unknown Trichoderma sp. T.atroviride (Th3)
9	20800906	Spawn run compost Second flush Spawn run compost Third flush	Bare areas Spotting	<25	0.7	Block	Bought in	II1/2	None	1 casing 2A casing 2B compost 3 casing 4 compost	Tafe (Th2) Tafe (Th2) Tafe (Th2) Tafe (Th2) Tafe (Th2)
10	20802812	Compost	Bare areas	Up to 40	<1	Blocks on shelf	Bought in	III	None	Compost	Tafe (Th2)
11	20803127 20803128 20803129 20803130	Spawn run compost	Bare areas	25	0.4	Block	Bought in	II	None	Compost	Tafe (Th2) Tafe (Th2) Tafe (Th2) Tafe (Th2)
12	20803377	First flush	Bare areas	10	-	Shelf	Bought in	III	Casing	Casing	Thz (Th1)
13	20803378	Fourth flush	Bare areas	<1	-	Shelf	Bought in	III	Casing	Casing	Trichoderma sp. (not Th 1-4)
14	20803847	Spawn run compost/Pre-first flush	Bare areas	25	-	Shelf	Bought in	III	Spawning	Compost	Tafe (Th2)
15	20804008	Spawn run compost	Bare areas	-	0.1	Shelf	Bought in	III	Spawning	Compost	Tafe (Th2)

Figure 1. Phylogenetic relationship of Trichoderma - isolates analysis of the translocation elongation factor gene 1 alpha. 207 and 208 isolates used in this study and known reference isolates AF.

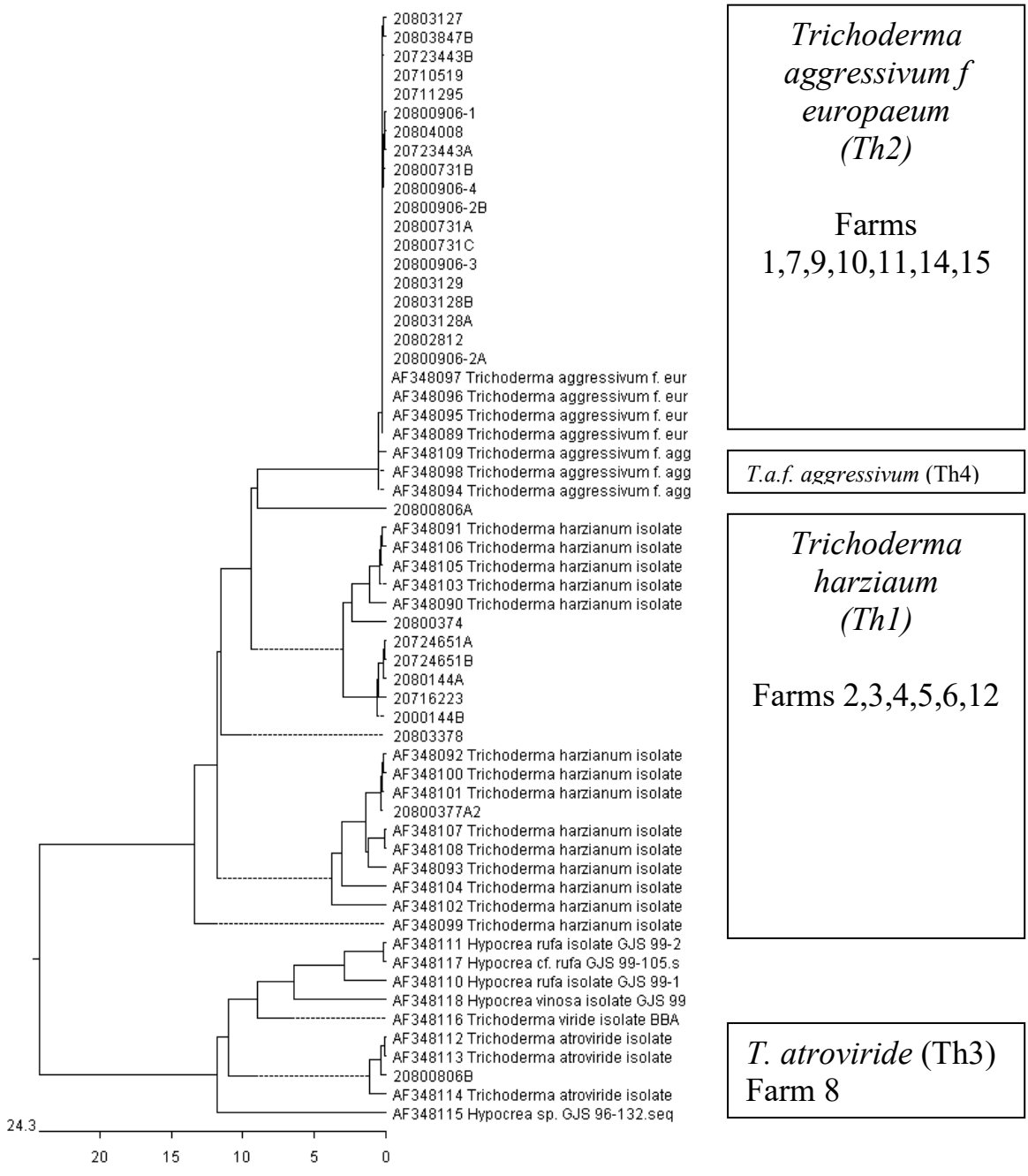

Table 2. Growing conditions and Trichoderma diversity

			T. aggressivum f. europaeum (Th2)	*T. harzianum* (Th1)	Trichoderma sp.
1.	Origin of Sample	Spawn run /1st flush	6½	2	0
		≥ 2 flush	½	4	2
2.	Growing System	Bag	-	1	-
		Block	2	0	1
		Tray	0	4	0
		Shelf	3	1	1
		Block/Shelf	2	-	-
3.	Compost Source	Home	1	5	2
		Bought-in	6	1	2
4.	Type of Compost	II	3	5	1
		III	3	1	1
		II½	1	0	1
5.	Supplementation	Spawn	3	2	0
		Casing	0	1	1
		None	4	3	1
6.	Crop	Bare	6	3	1½
		Cap Spotting	1	2	½
		Few/None	0	1	0
7.	Duration	0-6 months	2	1	0
		6-12 months	2	1	1
		>1 year	0	5	1
		Not stated	2	0	0
8.	Losses	0-5%	0	3	1
		6-20%	2	2	0
		20-50%	3	0	1
		>50%	1	0	0
		Not stated	2	-	-

Where farms reported more than one answer for a category a score of a ½ was allocated.

Discussion

The study, targetting growers experiencing economically damaging green mould problems, analysed 28 isolates of Trichoderma from 14 farms around primarily England but also Scotland, Northern Ireland and the Republic of Ireland. Although the project was of a short duration this represents a reasonable cross-section of the industry, but is clearly not as comprehensive as previous studies that analysed a greater number of isolates. The diversity of isolates encountered in this study is not as extensive as in earlier studies, however it highlights the dominance of *Trichoderma aggressivum f. europaeum* (Th2). This species primarily caused bare areas, seen early in the crop and causing economic losses of up to 50% within the past year. Although based on a small sample size, this appeared to be related to the source of the compost, with a much higher incidence for this pathogen on bought in compost. As expected, *Trichoderma harzianum* (Th1) was found later on in the crop, causing less damage and was more of an inherent, ongoing problem. The incidence of this species was greatest in home produced phase II compost. It was interesting to note that it was also present on bare areas (three farms) in addition to producing cap spotting, with only one farm reporting few or no symptoms. The role of these isolates of *Trichoderma harzianum* in bare areas was not within the scope of this project. It is unknown whether they are the cause of the bare areas or another factor is involved and they are merely growing saprophytically on these areas. *Trichoderma atroviride* (Th3), another saprophytic species, was found on one occasion causing cap spotting on a third flush, as would be expected. On the same farm, another isolate of Trichoderma was obtained that did not fit within an existing clade but was most closely associated with *Trichoderma atroviride* (Th3). One further isolate (farm 14) did not fit within an existing clade and so could only be identified as *Trichoderma* sp.. It would be recommended to establish the aggressiveness of these isolates to determine the risks that they pose.

The project also received isolates from a farm in South Africa that was experiencing significant economic losses prior to the first flush and cap spotting. All three South African isolates were identified as *Trichoderma aggressivum f. europaeum* (Th2). Following consultation of the published literature and in discussion with Dr Gary Samuels this is believed to be the first report of *Trichoderma aggressivum f. europaeum* (Th2*)* in South Africa, which would explain why they grower was encountering so many difficulties in controlling this new problem.

Conclusions

In a targeted survey of economically damaging Trichoderma compost mould, *Trichoderma aggressivum f. europaeum* (Th2) was the dominant species causing significant economic damage early in the crop cycle. The incidence of this pathogenic species was greater on farms that bought in compost as opposed to those who produced their own. *Trichoderma harzianum* (Th1) was primarily found on later flushes but was also associated with bare areas (investigating pathogenicity of these isolates was not part of this project's scope). *Trichoderma harzianum* appeared to be a more inherent problem on farms with symptoms seen for over a year and was more common on farms using home produced compost (phase II). Three other isolates of Trichoderma were encountered on later flushes. No evidence was detected in this study of the economically damaging strain, *Trichoderma aggressivum f. aggressivum* (Th4), prevalent in North America.

Technology transfer

- Preliminary and final report to individual growers submitting samples
- Interim report to HDC Mushroom panel (February 2008)
- HDC News - short news item on project initiation
- HDC News - feature article (in press)
- Defra, PHSI Technical Conference, platform presentation (January 2008)
- European Mycological Network meeting, platform presentation (April 2008)
- Final report to HDC Mushroom panel (July 2008)
- Results supplied to Defra to update Commodity Pest Risk Assessment

Glossary

Biotype	Current Name	Present in UK	Economically Damaging
Th1	*Trichoderma harzianum*	yes	no
Th2	*Trichoderma aggressivum f. europaeum*	yes	yes
Th3	*Trichoderma atroviride*	yes	no
Th4	*Trichoderma aggressivum f. aggressivum*	no	yes

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Appendices

Appendix 1 – Letter sent to participants requesting samples and sample information sheet

Introduction
Trichoderma is comprised of numerous species of which only some cause economic losses to the mushroom industry. Since the mid 1980s Trichoderma compost mould and cap spotting has been attributed to primarily *Trichoderma harzianum* biotype 2 (Th2). Other biotypes and species of Trichoderma may also colonise casing and compost but not result in serious economic losses.

Recent reports of Trichoderma outbreaks have resulted in the HDC agreeing to fund an exploratory project to determine the current position within the industry. Therefore, we need to collect samples of Trichoderma green mould from both compost and casing to determine the diversity of this fungus in the UK. Isolates will then be chosen for detailed characterisation using molecular and morphological techniques to determine their identity.

The project involves collaboration of mycologists at the Central Science Laboratory and Warwick HRI and technical advice from Richard Gaze and John Burden.

The success of the project depends, of course, on the provision of samples from the industry and we are grateful to you for providing samples of Trichoderma from your farm.

Samples should be sent to: Dr Charles Lane at the Central Science Laboratory's Mushroom Clinic in York.

Sampling
Please place a small piece of infected compost or casing showing green spores, preferably in a sealed tube or a small punnet packed with clean paper to prevent the sample moving about in transit. Infected mushrooms can be wrapped individually in clean paper, packed in a punnet so they will not be shaken during transit, and sent by next day delivery to:
Dr Charles Lane, Central Science Laboratory, Sand Hutton, York, YO41 1LZ.
A sampling kit is available and can be provided from CSL if required.

All samples will be allocated a unique reference number, and the source of samples will not be made available outside the project partners and will remain confidential. Once a range of samples has been collected and cultured they then will be analysed together in the Spring next year to determine their identity. Participants will be provided with the results and information about the strain present in their sample at the end of the project in May 2008.

It would be very helpful if you could complete the attached form when submitting samples.

Futher information
If you are require any further information please contact the project leader:
Dr Charles Lane at CSL, York
Phone: 01904 462326
Fax: 01904 462147
E-mail: mushroomclinic@csl.gov.uk

Many thanks for your assistance

Sample information (please answer as many questions as possible when sending samples by ticking the appropriate boxes)

Your contact details:

Name: Company:

Address:

Phone number:

E mail:

1. Origin of the sample	Spawn run compost	☐
	Casing surface	pre-1st ☐ 1st ☐ 2nd ☐ 3rd flush ☐
	Chogs	☐
	Mushroom caps (spotting)	☐
2. Growing a system used	Bag ☐ Block ☐ Tray ☐ Shelf ☐	
3. The source of compost	Home produced	☐
	Bought in	☐
4. Type of compost filled	Phase II	☐
	Phase III	☐
5. Supplementation	At spawning	☐
	At casing	☐
	None	☐
6. Crop symptoms	Bare areas	☐
	Cap spotting	☐
	Few/none	☐
7. Duration of Trichoderma presence (specify number)	Months	……months
	Years	……years
8. Estimate of crop loss (0-100%)		……%

The source of samples and related information will not be made available outside the project partners and your responses will remain confidential.

Samples to: Dr Charles Lane, Central Science Laboratory, Sand Hutton, York, YO41 1LZ.

Appendix 2 - Sample receipt letter

HDC Research Project (M46)

Trichoderma green mould - determining diversity and highlighting risks

Thank you for your sample, that arrived safely.

It has been allocated CSL reference number ……

Analysis has begun and will involve isolating the fungus to obtain a pure culture to permit detailed morphological and molecular analysis as necessary. Analysis will be performed on batches of isolates and will take several weeks to complete.

As soon as the results are available I will be in contact again.

The results will be used to help determine the diversity of Trichoderma green mould within the industry and will be a key part of the final report that will be submitted to the HDC in June 2008.

The source of isolates will remain confidential to the project partners and your sample will be referred to by a unique reference number.

It would be very helpful if you could complete the attached 'Sample Information' sheet.

Once again many thanks for your support

Yours sincerely

Dr Charles Lane
Senior Plant Pathologist

mushroomclinic@csl.gov.uk
Phone 01904 462326
Fax 01904 462147

Appendix 3 – Sample final report letter

Horticultural Development Council Research Project (M46)

Trichoderma green mould - determining diversity and highlighting risks

CSL reference 2080
Farm Number

We have now completed molecular analysis of the samples you kindly submitted and the results are detailed below.

Trichoderma diversity
As I am sure you are aware there are numerous species of Trichoderma reported in association with mushroom production including *Trichoderma aggressivum, T. atroviride, T. koningii, T. harzianum, T. pseudokoningii, T. virens, T. viride*. The most commonly encountered isolates belong to the *Trichoderma harzianum* complex previously described as for biotypes (e.g. Th1) but now referred to as discrete species.

Biotype	**Current Name**	**Present in UK in UK**	**Economically damaging**
Th1	*Trichoderma harzianum*	yes	no
Th2	*Trichoderma aggressivum f. europaeum*	yes	yes
Th3	*Trichoderma atroviride*	yes	no
Th4	*Trichoderma aggressivum f. aggressivum*	no	yes

DNA molecular analysis (sequencing of the elongation factor gene) has been completed for seven farms. To date we have found *Trichoderma aggressivum f. europaeum* (Th2) on three farms primarily associated with pre-first flush and first flush. *Trichoderma harzianum* (Th1) has been found on a further three farms all on later flushes. There has been one occurrence of *Trichoderma atroviride* (Th3) on a late flush in addition to one isolate from a late flush similar to but not identical to the molecular profile for *Trichoderma atroviride*. It is interesting to note that no other species have been found to date.

We are still interested in collecting samples from new farms so if any colleagues have Trichoderma green mould could you please ask them to contact me to arrange sample submission and analysis.

Once again many thanks for your support and assistance.

Yours sincerely

Charles Lane
mushroomclinic@csl.gov.uk
01904 462326