Grower Summary

M 057

Mushrooms: Influence of ammonia during compost pasteurisation and disinfectants on eradication of Trichoderma aggressivum (Th2)

Final 2013

Disclaimer

AHDB, operating through its HDC division seeks to ensure that the information contained within this document is accurate at the time of printing. No warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.

Before using all pesticides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the HDC office (hdc@hdc.ahdb.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

HDC
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel – 0247 669 2051

HDC is a division of the Agriculture and Horticulture Development Board.

Project Number:	M 057
Project Title:	Mushrooms: Influence of ammonia during compost pasteurisation and disinfectants on eradication of Trichoderma aggressivum (Th2)
Project Leader:	Dr Ralph Noble
Contractor:	East Malling Research
Industry Representative:	N/A
Report:	Final Report 2013
Publication Date:	16 July 2013
Previous report/(s):	N/A
Start Date:	01 April 2013
End Date:	31 March 2013
Project Cost:	£33,572

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).

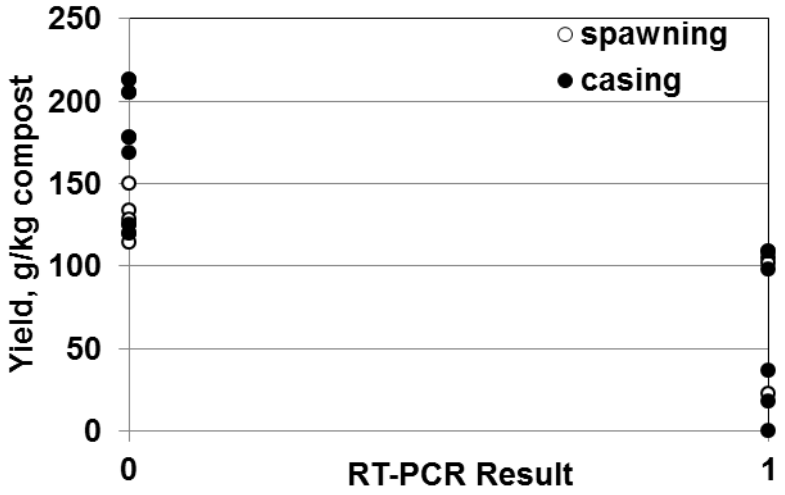

Fig. 1. Relationship between RT-PCR results at spawning and casing and mushroom yields from compost samples with different levels of Trichoderma inoculum, urea addition, and pasteurisation treatment.

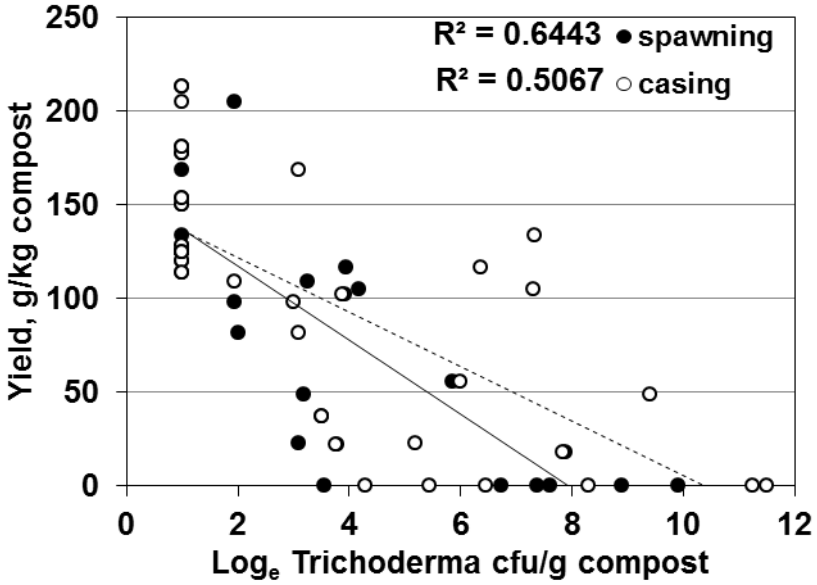

Fig. 2. Relationship between dilution plate counting of Trichoderma propagules at spawning and casing and mushroom yield from compost samples with different levels of Trichoderma inoculum, urea addition, and pasteurisation treatment.

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.