Understanding *Trichoderma aggressivum* in Bulk Phase 3 compost

Mairéad Kilpatrick, Agri-Food and Biosciences Institute, Northern Ireland;
Caoimhe Fleming-Archibald and Helen Grogan, Teagasc, Ireland.

The MushTV project investigated the epidemiology of *T. aggressivum* in Phase 3 (Phase III) compost: growth in a Bulk Phase 3 system, distribution in the compost during tunnel emptying and transportation and how infected particles are distributed and detected during the bulk handling and growing operations. This factsheet is a summary of the outcomes.

MushTV

Factsheet **03/15**

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement No. 286836.

- Phase 3 compost fully colonised with mushroom mycelium is susceptible to *T. aggressivum* and infections can lead to 100% crop losses.
- *T. aggressivum* can readily infect and colonise otherwise healthy, highly productive mushroom compost.
- Any process that involves compost mixing and breaking up of the *Agaricus* mycelium can readily spread a small infection through larger volumes of compost.
- Contaminated equipment and machinery can infect clean Phase 3 compost.
- Stringent hygiene procedures are effective at decontaminating equipment/facilities.

1. *T. aggressivum* symptoms – extensive sporulation

Key recommendations

Compost facilities

- Stringent hygiene procedures must be implemented at all times.
- Where possible, the filling and emptying of Phase 3 tunnels should be done at different ends and with dedicated equipment for each activity to ensure no cross-contamination.
- Remove compost debris from, and thoroughly clean, disinfect and rinse winches, conveyors and all other compost handling equipment prior to use to prevent cross-contamination of tunnels.
- Steam cook-out or pasteurise tunnels between batches of Phase 3 compost.
- Implement routine monitoring procedures.

Haulage contractors

- All equipment that moves between facilities should be cleaned, disinfected and rinsed (inside and outside) frequently – as a minimum between every location.
- Be careful to remove all visible compost debris.
- Fill growing rooms load by load, ie do not mix compost loads.

Growing facilities

- Stringent hygiene procedures must be implemented at all times.
- Segregate all machinery, equipment and personnel used in filling Phase 3 compost.
- Thoroughly clean and remove any compost debris from all equipment and machinery, such as conveyors, filling heads and rufflers, prior to and after use.
- The filling of fresh Phase 3 compost should be done in isolation. Crops should not be emptied at the same time and growing rooms should be closed.
- Be vigilant in the critical 3-5 days post filling Phase 3 – actively look for symptoms.
- Steam cook-out at the end of every crop – 65-70˚C in the compost for a minimum of 8 hours.

Background

Trichoderma aggressivum f. europaeum (previously called *T. harzianum* type Th2) is the causative agent of compost green mould in *Agaricus* that has the potential to totally devastate crop production. Initially, it was associated with infections at spawning Phase 2 (Phase II) compost in traditional bag, block and tray (*in situ*) spawn run systems. Compost fully colonised with *Agaricus* mycelia was considered to be much less susceptible to infection. More recently, outbreaks of the disease across Europe where spawn run is performed in bulk systems at compost yards (Phase 3 compost), challenged this theory.

Infection can occur at compost facilities, growing facilities, during transportation or in filling/emptying operations. Therefore, it is the shared responsibility for all involved in the industry to prevent and reduce the impact of *T. aggressivum*.

Symptoms

T. aggressivum produces whitish mycelia indistinguishable from that of *Agaricus* during spawn run, therefore it is difficult to recognise infection at an early stage. Subsequently, and often on exposure to light, spore production begins on the *Trichoderma* mycelia. The spores often remain white for a day or so and within 3-5 days patches of compost rapidly turn dark green as extensive sporulation develops.

Where a *T. aggressivum* infection has occurred within a Phase 3 compost tunnel, there may be no overt visible signs at tunnel emptying or shelf filling. However, if closely examined, the *Agaricus* mycelium may appear retarded and less interwoven through the compost giving rise to a looser mass.

Ultimately, green *T. aggressivum* sporulation appears in large areas of the compost or casing layer, with the crop loss proportional to the area of compost infected; usually no mushrooms are produced in contaminated areas and if mushrooms do appear they are often unmarketable due to poor quality or the presence of red pepper mites on the cap surface.

Another consistent symptom of *T. aggressivum* is subtle increases in compost temperatures both during bulk spawn run and in the days immediately following the filling of growing rooms. A distinct smell is frequently detectable as the cycle progresses.

2 . *T. aggressivum* early symptoms – white strappy growth

Biology and epidemiology

Although a number of *Trichoderma* species have been isolated from mushroom compost, aggressive colonisation resulting in epidemic outbreaks in Europe is attributed to *Trichoderma aggressivum f. europaeum.* What confers this 'aggressiveness' is complex.

Numerous studies have shown:

- Interactions occur between *T. aggressivum* and *Agaricus bisporus,* yet the exact mechanisms of interaction are not fully understood.
- Many key features of mushroom cultivation – nutrition, warm temperatures and high relative humidity, absence of light in spawn run – are also ideal for *T. aggressivum.*

- Under optimum conditions, *T. aggressivum* shows rapid growth and, therefore, can compete for nutrients and space more effectively than the mushroom.
- *T. aggressivum* also produce extracellular enzymes, toxic secondary metabolites, as well as volatile organic compounds and can tolerate inhibitory effects of bacteria present in mushroom compost.
- All these characteristics result in saprotrophic growth on the fungal and bacterial compost microflora and intense competition, antagonism and/or parasitism that, ultimately, enables *T. aggressivum* to grow in otherwise, healthy productive mushroom compost.

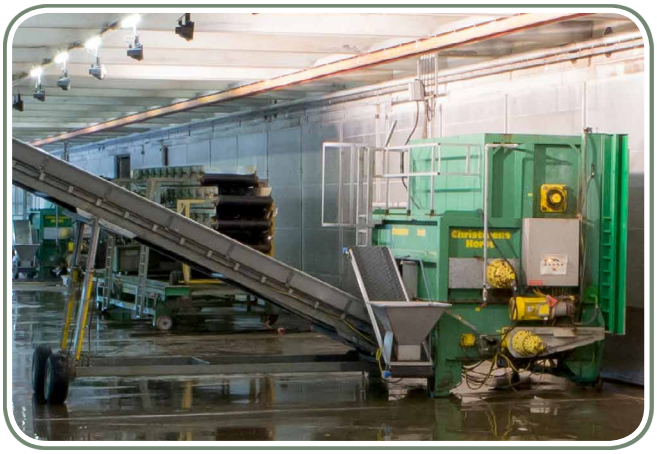
Trials as part of the MushTV project showed that when *T. aggressivum* was added to Phase 2 compost at filling, growth was restricted to an area *ca* 0.5-1.0m around the point of inoculation and was not generally visible at emptying. Using artificial procedures to limit compost mixing at tunnel emptying, mushroom yield from compost in the infected area was reduced by 100%. In contrast, compost from the remainder of the tunnel produced normal, high yielding mushroom crops. Crop loss was proportional to the area of compost infected with *T. aggressivum*. This new information indicates that, although large volumes of recirculated air are forced through the compost during bulk spawn run, there is only limited dispersal or spread within the tunnel. The fact that *T. aggressivum* does not sporulate readily in tunnels and that *Trichoderma* spores are not readily airborne may be limiting factors to its dispersal within a tunnel.

3. Experimental compost chambers at AFBI Loughgall

Subsequent trials were designed to recreate commercial bulk handling operations – compost mixing by the compost winch at emptying, layered filling of compost into transportation vehicles and shelf filling at grower holdings. Results showed that the previously small, contained *T. aggressivum* infection was spread through all compost from the tunnel; mushroom yield from the entire compost mass was reduced by 80-100%. The use of equipment that had handled *T. aggressivum* infected compost was also shown to infect clean compost from an adjacent newly opened tunnel, spreading the infection further. Consequentially, the location of an infection in a tunnel will have important ramifications; if near the front, all subsequent compost will come in contact with now infected machinery; if near the back then most of the compost should yield normally.

Therefore, all compost mixing operations – bulk handling at the compost facilities, loading and unloading for transportation and shelf filling at grower units are high risk activities. They are very efficient at mixing small *T. aggressivum* infections through large compost volumes. It is feasible that immediately after a mixing procedure, the *Agaricus* mycelium in spawn run compost is in a weakened and vulnerable state that *T. aggressivum* can readily exploit.

Cropping trials indicated that handling *Trichoderma* infected Phase 3 compost readily dispersed propagules of the disease (spores and infected compost fragments) around the growing unit. Detection studies identified that the corridor, canteen area, pack-shed, growing room floor, matting, shelves, tunnel doors, door handles, computer interface panels, keyboards, telephones and even noticeboards all harboured viable *T. aggressivum.* This indicates that cross contamination on a growing unit from one infected crop to newly filled crops is very likely to occur. This is particularly relevant at any point in the process where compost is mixed, ie filling, ruffling or emptying.



4 & 5 above. Research indicates that all compost mixing operations are high risk activities with the potential to spread *T. aggressivum* infections

Stringent hygiene procedures and cleaning up of infected materials/facilities following crop termination were effective at containing the disease. Effective post-crop steam sterilisation (65-70ºC in the compost for a minimum of 8 hours applied both with compost in the growing room and again when compost was removed) was very effective. This should be coupled with rigorous procedures to remove all sources of organic matter from equipment, machinery and work areas followed by effective clean, disinfect and rinse programmes, as outlined in MushTV Factsheet 01/15: Use of chemical disinfectants in mushroom production.

This new information is summarised in five key points highlighted at the start of the factsheet and may help explain previous anomalies, where several growers getting compost from the same tunnel reported completely different levels of infection, ranging from none to total crop wipe-out.

Typically, control measures for some mushroom pests and diseases are implemented just before they reach a level that causes unacceptable economic damage, otherwise known as the economic threshold. However, with *T. aggressivum* and especially for bulk Phase 3 facilities, there is no acceptable threshold. Constant vigilance is important and any incidence of the disease, no matter how small, is cause for significant concern.

Early detection and correct identification of *Trichoderma* infections are key to enabling fast action by composters and growers to contain/prevent significant outbreaks.

There is a range of reliable detection methods available, from simple present/absent tests of air fall out plates/swabs and compost fragment plates, to sophisticated real-time PCR molecular methods developed to quantify *T. aggressivum* that only relatively recently could be used for detection in compost.

For compost testing, it is important that a representative compost sample is obtained and critical that this is thoroughly mixed before sub-sampling for testing. Air fall out plates positioned close to the tunnel doors during emptying provide a cheap and reliable detection method, as *Trichoderma* spores, though not readily airborne, are easily transmitted on dust particles and compost fragments. Any initial positive *Trichoderma* result should be supplemented with real-time PCR to confirm *T. aggressivum*. The quicker sample turn-around time and quantification of the level of *Trichoderma* infection of real-time PCR make it the preferred detection method for high risk situations.

Agaricus and *Trichoderma* produce metabolites that affect each other's growth. New diagnostic techniques, used in the MushTV project and still undergoing development, have compared volatiles produced in non-infected and infected compost. This showed different patterns of volatile emissions after 12 days of spawn run between healthy *A. bisporus* compost and those infected with *T. aggressivum.* This could lead to a sophisticated non-invasive detection method for *T. aggressivum* during bulk spawn run as an early warning system for composters.

6. New volatile diagnostic techniques under development

Pilot studies and surveys were undertaken at both compost and growing facilities to identify critical control points for optimum sampling locations to detect *T. aggressivum*.

The lists below should be used as a guide for sampling, with the frequency determined by a standard risk assessment:

Sampling locations for compost facilities

Compost/debris samples:

- Sample of Phase 3 compost from tunnel being emptied, taken off the conveyor.
- Accumulated debris on the conveyor structure.
- Debris taken from the back and sides of the emptying winch.
- Debris material from around the supplement mixer (if appropriate).
- Debris from the ground adjacent to truck-filling/dispatch area.
- Debris from the back of the truck being filled.
- Accumulated debris from the general dispatch area.
- Debris from a second (returning) truck waiting to be refilled/ dispatched (if appropriate).

Air plate locations:

- Placed beneath the entrance to the tunnel being emptied.
- Placed on the emptying winch.
- Placed close to where supplement is being added (if appropriate).
- Placed in the dispatch hall close to the truck-filling area.

In an industry survey of the five compost facilities, no *T. aggressivum* was detected in any of the critical control point samples, either around the facility or in fresh Phase 3 compost (Table 1). However, other species of *Trichoderma* were detected in a small percentage of the Phase 3 samples (< 6%) and other moulds (*Penicillium, Aspergillus*, *Mucor*) also occurred on occasion.

Table 1. Survey results from Phase 3 Compost Facilities

Facility	*T. aggressivum* on facility	No of Tunnels sampled	*T. aggressivum* in Phase 3	No of Tunnels with high levels** of other moulds detected
1	No	30	No	0
2	No	24	No	5
3	No	32	No	3
4	No	26	No	12 (7 *Trichoderma* spp)
5	No	30	No	2 (1 *Trichoderma* spp)

** Mould growth on more than 5 of the 10 compost fragments plated

Sampling locations for grower facilities

When sampling at grower facilities, a certain degree of flexibility in the sample locations will be necessary, depending on the individual farm. Samples should be taken in all growing rooms with symptoms and any freshly filled room. More than one sample may be taken in the office or canteen, depending on the size or contents:

- Swab of interior door and floor in growing room.
- Swab of shelving.
- Debris in growing room.
- Sample of compost at any visible infection.
- Swabs of canteen and office areas.
- Swab of equipment (forklift, casing mixer, filling head).
- Debris on equipment and in the yard.
- Swabs of staff hands/clothes/boots.

Four grower facilities with recent or ongoing green mould issues were targeted for survey (Table 2). None of these had steam sterilisation ability and all relied on cleaning and disinfection in post-crop termination procedures. *Trichoderma* species were detected on all of the farms and *T. aggressivum* confirmed on three. Sampling at these grower facilities, it became apparent that if *Trichoderma* is present in crops, the likelihood is that it is easily transmitted via contaminated equipment and/or staff and spread across many locations in the facility. This again highlights the need for stringent hygiene procedures as well as awareness and vigilance by all staff as to how easy it is to transmit *Trichoderma* from one location to another.

7 & 8 above. Routine monitoring procedures using simple compost fragment plate tests

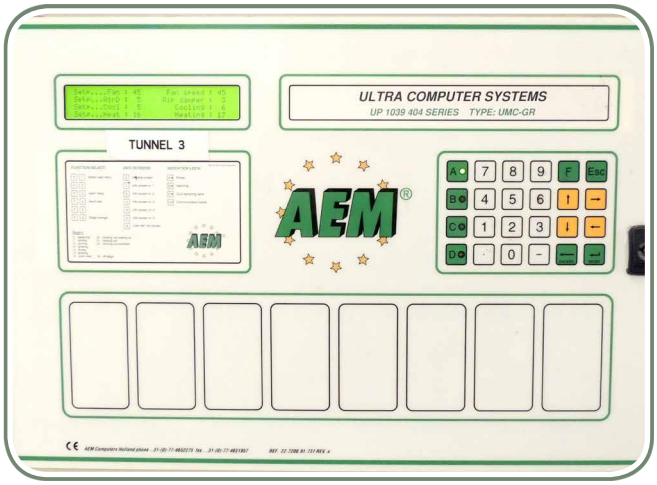
Table 2. Survey results from European Mushroom Grower Facilities (2012-2013)

Mushroom Farms	*Trichoderma* Problems	*T. aggressivum* Present	Key locations *Trichoderma* detected
1	Yes	Yes	Growing rooms, doors of growing rooms, equipment, canteen, office, yard
2	Yes	Yes	Growing rooms, equipment, forklift, canteen
3	Yes	Yes	Growing rooms, yard, pick trolleys
4	Yes	No (other *Trichoderma* species)	Growing rooms, office, forklift

Disease management strategies

It is important that stringent routine hygiene procedures are in place and adhered to by composters, haulage contractors and growers at all times. This is a basic prerequisite of any control programme. Certain common measures are crucial:

- Implement routine detection/monitoring procedures for *T. aggressivum*.
- Monitor compost temperatures and/or increased cooling demand.
- Continuously inspect compost for the presence of the disease.
- Sensibly allocate staff, mindful of hygiene requirements.
- Pay additional attention in any situation that involves breaking up or mixing compost.
- In case of infection – immediately implement specific precautions.
- Communicate immediately with other parts of the production chain.
- Use effective end of process steam sterilisation and verify its effectiveness.



9, 10 & 11 above. Detection studies identified less obvious key areas that harbour *T. aggressivum* infection

Additional specific measures tailored for each stage of the process include:

Compost facilities

Stringent hygiene procedures must be implemented at all times to include regular routine cleaning, disinfection and rinse programmes. Careful management in the handling and storage of mushroom spawn is essential.

Using the critical sampling point guidelines, implement routine monitoring procedures and take immediate action if the results dictate. Air plate and swab sampling, together with regular compost tests will validate the efficacy of the hygiene procedures. The optimum sample frequency will vary and ought to be determined by a standard risk assessment.

Where possible, the filling and emptying of Phase 3 tunnels should be done at different ends, and with dedicated equipment for each activity to ensure no cross-contamination. In all situations, machinery and equipment used in Phase 2 and 3 productions should be segregated. Never use Phase 3 equipment for Phase 2 processes.

Remove all visible compost debris before cleaning equipment and machinery. Be mindful of and regularly tackle greasy residues and biofilms that naturally build up on equipment. In particular, regularly clean, disinfect and rinse the emptying winch.

During the bulk spawn run process, examine both the compost temperature and cooling demand data. Look for subtle unexplained increases and heighten precautionary measures as appropriate.

It is absolutely critical to steam cook-out or pasteurise tunnels between batches of Phase 3 compost.

If *T. aggressivum* is detected:

- Communicate with appropriate haulage contractors and growers.
- Check companion loads for commonality.
- Use critical control point sampling to locate and isolate all infection points.
- Heighten compost sampling frequency and test sophistication, eg real-time PCR.
- Clean, disinfect and rinse the emptying winch after each tunnel.
- Load compost truck by truck, ie do not mix compost loads.
- Monitor loading the trucks by air plate exposure.

12 & 13 above. Stringent hygiene in evidence at compost and growing facilities

Haulage contractors

This is a critical link in the production chain where regular movement to and from compost and growing facilities occurs. Stringent hygiene procedures must be implemented at all times. All equipment that moves between facilities should be cleaned, disinfected and rinsed (inside and out) frequently – as a minimum requirement between every location. Be particularly careful to remove all visible compost debris.

Growing rooms should be filled in sequence load by load – do not mix compost loads. MushTV trials have shown that any process that involves compost mixing and breaking up of the *Agaricus* mycelium can readily spread a small infection through larger volumes of compost. The possible benefit of homogenising distinct compost loads at filling is far outweighed by the potential risk of spreading a limited *T. aggressivum* infection.

Implement monitoring procedures at filling, ie compost temperatures and air plates.

Grower facilities

Filling Phase 3 compost should be a high priority activity carried out only after a rigorous cleaning, disinfection and rinse programme has been implemented for the receiving growing room and adjacent concrete aprons. It should be done in isolation – crops should not be emptied at the same time and all growing rooms should be closed.

All machinery, equipment and personnel used in filling Phase 3 compost should be segregated. Thoroughly clean and remove any compost debris from equipment and machinery such as conveyors, filling heads and rufflers, prior to and immediately after use. Be mindful of and regularly tackle greasy residues and biofilms that naturally build up on equipment. Implement routine monitoring procedures with air plate and swab sampling to validate the hygiene procedures.

Monitor compost temperature on arrival and regularly in the days immediately after filling. Be vigilant in the critical 3-5 days post filling Phase 3 compost. Actively look for symptoms – white strappy mycelial growth, green sporulation (Figures 1 and 2), increases in compost temperature, distinct non *Agaricus* smells, etc.

Steam cook-out at the end of every crop is the most effective measure a grower can take to minimise the spread of disease. Steam cook-out should be to a temperature of 65-70˚C in the compost for a minimum of 8 hours.

14. Compost dust on a growing tunnel roof after emptying reinforces the importance of effective end of crop steam cook-out

If *T. aggressivum* is detected:

- Communicate with appropriate composters and haulage contractors.
- Restrict all but essential access to the farm.
- Use critical control point sampling to locate and isolate all infection points.
- Increase the cleaning and hygiene programme.
- Pay attention to common areas (canteens, toilets, offices, pack-sheds) and equipment (interface panels, computer keyboards, telephones, etc.).
- Be mindful of potential cross contamination especially when compost is mixed or *Agaricus* mycelium is broken up, eg reconsider second ruffle (if appropriate).
- Pay particular attention at filling – do not mix loads, fill compost straightaway, reconsider the addition of water.
- Efficient end of crop steam sterilisation (65-70ºC in the compost for a minimum of 8 hours) applied both with compost in the growing room and when it is removed.

Further information

MushTV factsheets

01/15: Use of chemical disinfectants in mushroom production

02/15: Brown Cap Mushroom Virus (associated with Mushroom Virus X) prevention

04/15: Fungal diseases of mushrooms and their control

HDC Grower summaries and reports

See the HDC website (www.hdc.org.uk) for copies of M 57 and 50.

Acknowledgements

Thanks go to Caroline van der Horst, C4C Holdings BV, Netherlands; Krystian Szudyga SGBU, Poland; Patrick Delva, NV Karel Sterckx, Belgium; and Piet Liempens, Hooymans Compost BV, Netherlands, for helpful commercial insight and comments during the preparation of this factsheet.

Image Copyright

Images are courtesy and copyright of Mairéad Kilpatrick, AFBI; Johan Baars, C4C Holding; Piet Liempens, Hooymans Compost BV; Brendan Burns, Sylvan; and Roland Mumm, Wageningen UR.

While the Agriculture and Horticulture Development Board, 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.

© Agriculture and Horticulture Development Board 2015. No part of this publication may be reproduced in any material form (including by photocopy or storage in any medium by electronic means) or any copy or adaptation stored, published or distributed (by physical, electronic or other means) without the prior permission in writing of the Agriculture and Horticulture Development Board, other than by reproduction in an unmodified form for the sole purpose of use as an information resource when the Agriculture and Horticulture Development Board is clearly acknowledged as the source, or in accordance with the provisions of the Copyright, Designs and Patents Act 1988. All rights reserved.

Contact us

Project Co-ordinator

Dr. Helen Grogan

Teagasc, Ashtown Research Centre,
Dublin 15, Ireland

Tel: 00 353 1 8059780
Email: helen.grogan@teagasc.ie

Communications Co-ordinator

Ruth Ashfield

HDC, Stoneleigh Park, Warwickshire, UK

Tel: 00 44 (0) 247 647 8663
Email: ruth.ashfield@hdc.ahdb.org.uk

AHDB Agriculture & Horticulture DEVELOPMENT BOARD

For more information visit
www.mushtv.eu