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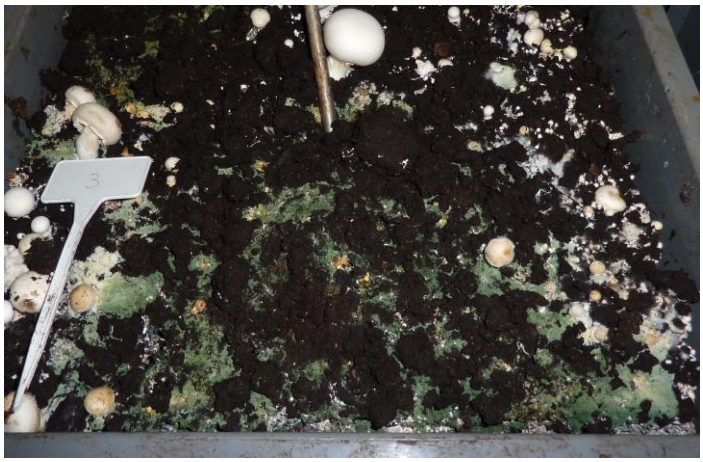
# Green mould disease in bulk phase 3 mushroom substrate



## Key external stakeholders:

Mushroom growers, mushroom substrate producers, mushroom allied trades

## Practical implications for stakeholders:

Green mould disease in mushroom substrate is caused by the fungus *Trichoderma aggressivum.* f *europaeum.* It can cause up to 100% loss of mushroom production and there are no approved plant protection products for its control. This project identifies new information on its epidemiology and detection.

- Phase 3 mushroom substrate that is fully colonised with the mycelium of *Agaricus bisporus* is still susceptible to infection by *T. aggressivum* highlighting the need for high levels of hygiene when handling bulk phase 3.
- The bulk handling process associated with bulk phase 3 mushroom substrate can exacerbate a *T. aggressivum* infection.
- *T. aggressivum* in substrate can be detected by several molecular and microbiological methods with quantitative PCR being the most reliable and least variable method.

## Main results:

- **Bulk phase 3 substrate is vulnerable to infection.** *T. aggressivum*-infected substrate fragments can cause an infection in mushroom substrate that is already fully colonised by *Agaricus bisporus* highlighting the need for high levels of hygiene when handling bulk phase 3.
- **Substrate mixing exacerbates a *T. aggressivum* infection**. The severity of a *T. aggressivum* infection event increases with increased levels of substrate mixing highlighting how the bulk handling process can aggravate a *T. aggressivum* infection
- **QPCR molecular detection method available.** *Trichoderma aggressivum* in substrate can be detected by several molecular and microbiological methods with quantitative PCR being the most reliable and least variable method
- ***Trichoderma aggressivum* proteins differentially expressed.** Preliminary proteomic analysis indicated that several *T. aggressivum* proteins are differentially expressed in the presence of *A. bisporus* and which may contribute to its virulence against it.

## Opportunity / Benefit:

Growers and substrate manufacturers should ensure that hygiene protocols are implemented and effective when handling bulk phase 3 substrate. Samples of substrate should be routinely monitored for presence of *T. aggressivum*.

## Collaborating Institutions:

Maynooth University



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Agriculture and Horticulture Development Board (AHDB), Stoneleigh Park, Kenilworth, Warwickshire, CV8 2TL, UK.

## 1. Project background:

Compost Green Mould Disease occurs when fast-growing *Trichoderma aggressivum* colonises the substrate on which the cultivated mushroom *Agaricus bisporus* is grown, resulting in severe or complete crop loss. Exclusionary methods and rigorous hygiene are employed on both mushroom farms and substrate facilities to prevent entry and spread of *Trichoderma* in the production system as there are no available plant protection products. The use of the 'Bulk Phase 3' system for mushroom production has become prevalent in Europe over the past two decades. In this system the substrate is inoculated with a pure culture of *A. bisporus* (spawn) and then incubated (spawn-run) in large tunnels (80 - 200 tonnes) under stringent hygiene and environmental conditions. There was a marked decrease in the occurrence of Compost Green Mould Disease in Europe with the introduction of this system, which was attributed to improved consistency of substrate, as well as improved, exclusionary and hygiene measures during the spawning process that minimised *Trichoderma* infection at spawning. More recently *T. aggressivum* infections have occurred sporadically in Bulk Phase 3 substrate with devastating consequences but there is little epidemiological information available in relation to how *T. aggressivum* infects the bulk system. Previously it was believed that substrate fully colonized by mushroom mycelium was immune to infection by *T. aggressivum.* This project conducted research to understand the epidemiology of *T. aggressivum* in the bulk system and to evaluate methods to detect it.

## 2. Questions addressed by the project:

- Can *T. aggressivum* infect mushroom substrate that is fully colonized with *A. bisporus*?
- Does the substrate mixing associated with bulk phase 3 substrate affect *T. aggressivum* infection?
- Does supplementation of bulk phase 3 substrate affect *T. aggressivum* infection?
- Can *T. aggressivum* be detected in phase 3 substrate?
- Can proteomic studies provide insight into the mechanism by which *T. aggressivum* antagonizes *A. bisporus?*

## 3. The experimental studies:

Mushroom substrate that was fully colonised with *A. bisporus* mycelium was infected with different rates of *T. aggressivum*. The inoculum that was used consisted of mushroom substrate that was already colonized by *T. aggressivum* as this would reflect a localized pocket of infection within a bulk incubation tunnel. Rates of infection ranged from 0.01% to 1%. Two levels of mixing were evaluated - a low level and a high level - as well as supplementation with a standard commercial supplement. Four methods of detection were evaluated: *Trichoderma* propagule numbers were determined by (1) quantitative polymerase chain reaction (qPCR), (2) weed mould analysis (WMA), (3) most probable number (MPN) determination and (4) direct plating assessment (DPA). Reverse transcription PCR (RT-PCR) was used to verify the identity of selected *Trichoderma* cultures from the WMA, MPN and DPA tests. Inoculated crops were grown under commercial conditions in the environmentally controlled growing rooms at the Kinsealy Mushroom unit. Mushrooms were harvested over two flushes and data were subjected to ANOVA to identify any significant treatment and/or interaction effects. Proteomic analysis was employed to study the response of *T. aggressivum* to mushroom compost and *A. bisporus* tissue in vitro. Previous studies have described the importance of proteins secreted by *T. aggressivum* in its interaction with *A. bisporus*, however there is little information published on the intracellular protein fraction of *T. aggressivum,* which was the focus of this study.

## 4. Main results:

- **Bulk phase 3 substrate is vulnerable to infection.** When *T. aggressivum*-infected substrate fragments were incorporated into fully spawn-run Phase 3 substrate, subsequent crop yields were reduced and the degree of crop loss correlated with the rate of infection, with the highest rate of infection ($10^{-1}$ g/kg substrate) causing the highest crop loss (see figure on Page 3). This result demonstrates for the first time that *T. aggressivum* can cause an infection in mushroom substrate that is already fully colonised by *A. bisporus,* and under normal conditions would crop well, highlighting the need for high levels of



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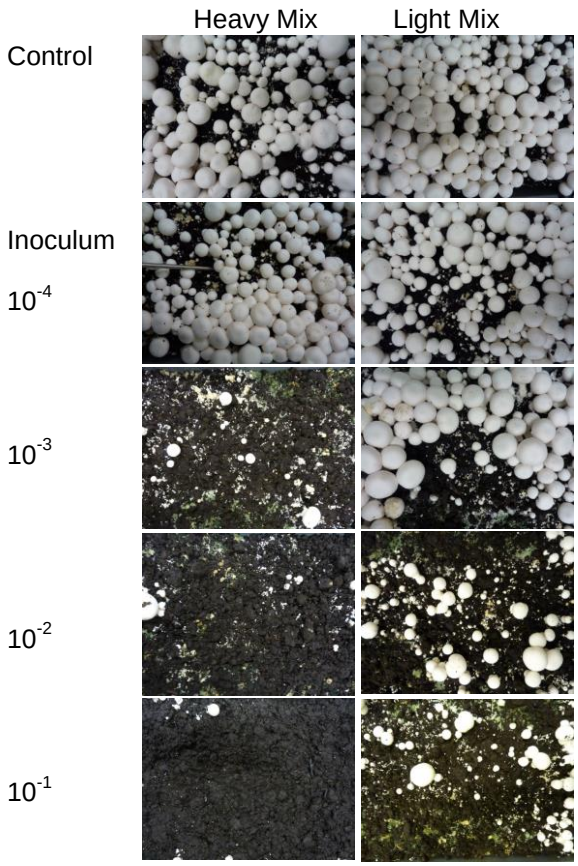
hygiene when handling bulk phase 3. Supplementation with one commercial supplement had no effect on either yield or on severity of *T. aggressivum*.

- **Substrate mixing exacerbates a *T. aggressivum* infection**. During the emptying of a Phase 3 tunnel of fully spawn-run substrate, the block of semi-solid substrate is broken up using a mechanised tunnel winch. The now friable substrate is moved by conveyor and filled into a transport vehicle and delivered to the farm, where it is once again moved by conveyor and filled onto shelves. All this moving and mixing ensures the substrate batch is homogeneous. Our research has shown however that increased mixing of substrate exacerbates the effect of a *T. aggressivum* infection highlighting how the bulk handling process can aggravate a *T. aggressivum* infection
- **QPCR molecular detection method available.** Once a batch of Phase 3 substrate is delivered to a grower, if *T. aggressivum* is present then the grower will sustain a crop loss. Thus substrate suppliers need to monitor for *T. aggressivum* presence in batches of substrate. Simple 'direct plating assessments', whereby 10 fragments of Phase 3 substrate from a batch of mixed substrate, was found to detect *T. aggressivum* if present, representing a cheap and effective diagnostic method. Any *Trichoderma*-positives have to be verified by PCR. A commercial test is offered by FERA, UK and uses a qPCR method to detect and quantify *T. aggressivum* in substrate. It was demonstrated to be the most reliable and least variable method out of four methods evaluated.




- ***Trichoderma aggressivum* proteins differentially expressed.** Preliminary proteomic analysis indicated that several *T. aggressivum* proteins are differentially expressed in the presence of *A. bisporus* and which may contribute to its virulence against it.

## 5. Opportunity/Benefit:

Producers of bulk Phase 3 mushroom substrate have benefited from this research as they are now aware of the vulnerability of the Phase 3 system to infection by T. aggressivum. Many substrate producers around Europe have implemented routine *Trichoderma* monitoring regimes. Currently there is limited availability of laboratories who can do a *T. aggressivum* diagnostic test, other than FERA, UK. This research provided the basis for larger scale research into *T. aggressivum* epidemiology as part of EU-funded (FP7-SME-2011) project n°286836 (MushTV) (2012-2015).

## 6. Dissemination:

### Main publications:

O'Brien, M., Kavanagh, K. & Grogan, H. (2016). Detection of *Trichoderma aggressivum* in bulk phase III substrate and the effect of *T. aggressivum* inoculum, supplementation and substrate-mixing on *Agaricus bisporus* yields *European Journal of Plant Pathology,* ( in press), http://link.springer.com/article/10.1007/s10658-016-0992-9

O'Brien, M., Grogan, HM and Kavanagh, K. (2014). Proteomic response of *Trichoderma aggressivum f. europaeum* to *Agaricus bisporus* tissue and mushroom compost. *Fungal Biology* 118 (9-10): 785-791.

Grogan, H, O'Brien M, Kavanagh, K, Dobrovin-Pennington A, Nixon, T, Lane, C and Noble R (2012). Epidemiology of *Trichoderma aggressivum* in bulk Phase 3 compost for *Agaricus bisporus* production. 18th Congress of the International Society for Mushroom Science, Beijing, China, Aug. 26th-30th 2012.

O'Brien, M., Kavanagh, K. & Grogan H. (2011). Characterisation of *Trichoderma aggressivum* infection in bulk Phase III mushroom systems. Proc. 7th Int. Conf. Mush Biol Mush Prod, Arcachon, France. P 225.

O'Brien, M., Kavanagh,K. & Grogan H. (2011). Compost Green Mould Disease can Spread in Bulk Phase III



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compost. All Ireland Mushroom Conference and Trade Show, Monaghan, Co Monaghan, 20-21 October 2011, P 54

**Popular publications:**

Grogan, H. Noble, R, Lane, C. Dobrovin Pennington, A, Nixon, T. O Brien, M. (2012). Green for Danger – (Progress in Understanding *Trichoderma* Compost Green Mould in mushroom compost). HDC News 179, pp28-29.

**7. Compiled by:** Helen Grogan



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