Grower Summary

M 50

Trichoderma aggressivum f. europaeum (Th2): Epidemiology in bulk Phase III systems

Final Report 2011

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Further information

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Headline

- Compost pasteurisation conditions of 60°C for 12 hours are needed to eradicate *Trichoderma aggressivum*
- *Trichoderma*-infected compost mixed and diluted into healthy Phase III compost can cause from 5-100% crop reduction due to green mould
- A new molecular detection method for *Trichoderma aggressivum* in Phase III compost has been shown to be very sensitive and reliable

Background and expected deliverables

Trichoderma aggressivum f. europaeum (previously called *T. harzianum* type Th2) is an aggressive compost green mould that predominantly infects Phase II compost at spawning. It was a serious problem in the mid 1980's and 1990's but was largely controlled through improved hygiene at spawning, and fungicide treatment of spawn, when infection risks were high. It has started to appear in bulk Phase III facilities across Europe in recent years despite the fact that hygiene levels are generally considered to be much higher on Phase III facilities compared to smaller, less technologically advanced, facilities. These outbreaks of *T. aggressivum* in bulk Phase III raise the question as to whether or not *T. aggressivum (Th2)* behaves differently in the bulk Phase III system compared to in-situ spawn run systems.

There is no precise information on the conditions (temperature, time, compost moisture, ammonia) that are required to eradicate *Trichoderma aggressivum* (spores and mycelium) during compost pasteurisation. Current recommendations for eradication times, temperatures and ammonia concentrations are approximate. Previous work has shown that *Trichoderma aggressivum* (Th2) can be detected in chicken manure, on Phase II prefilters and in spawning halls. Other work has shown that *Trichoderma aggressivum* (Th4) spores can withstand up to 200 ppm gaseous ammonia and 60°C for 9 hours under laboratory conditions, and 60°C for 10 hours under simulated Phase II conditions. However experiments combining both factors in compost have not been done and combined effects of these factors are likely to be more efficacious than either factor alone. However, commercial pasteurisation tunnels may have numerous 'cool zones' in which *Trichoderma aggressivum* inoculum can survive.

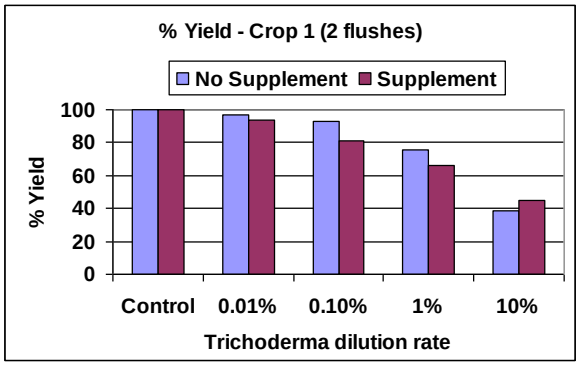
Trichoderma aggressivum (Th2) is known to derive nutrition from the starchy grains used in the manufacture of mushroom spawn. Some level of control was achieved in Canada and the USA when compost spawn and non-grain spawn products were used during spawning rather than standard grain based spawn. Similarly, some facilities are now mixing bulk Phase II with bulk Phase III and results from projects M47 and M49 indicate that this may reduce the incidence of *T. aggressivum* (Th2), providing the bulk Phase III is *Trichoderma*-free. With the increased dependence on bulk Phase III there is a need to determine if non-grain mushroom spawn products offer a control strategy for bulk Phase III systems of production.

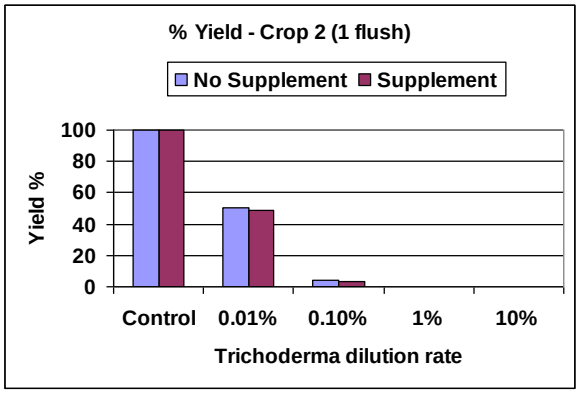
A recently completed HDC-funded project (M 48) successfully developed (a) a method to extract *Trichoderma* DNA directly from Phase III compost in conjunction with (b) a molecular test using "real-time TaqMan PCR" technology to detect *T.aggressivum* in Phase III compost. On a small scale, the *T.aggressivum* assay was found to be very sensitive and it was equal to microbiology-based tests but the PCR test result can be achieved within a working day as opposed to the two stage microbiological method that takes at least 8 days. The new assay needs to be verified with samples that are representative of commercial scale production.

Summary of the project and main conclusions

T. aggressivum in Phase III compost

When *Trichoderma* infected compost was mixed and diluted with healthy (uninfected) Phase III compost, there was a significant correlation between the extent of yield loss recorded and how diluted the *Trichoderma*-infected compost was. The more dilute the compost the less the effect on yield loss but a dilution rate of 0.01% caused a 3-6% yield reduction in one experiment and a 50% yield reduction in another. At a 10% dilution rate the yield loss was 40-50% in one crop and 100% in a second crop. Thus the extent of yield loss can be affected by other factors. How heavily colonised the initial *Trichoderma*-infected compost is one factor and how well the infected compost is mixed

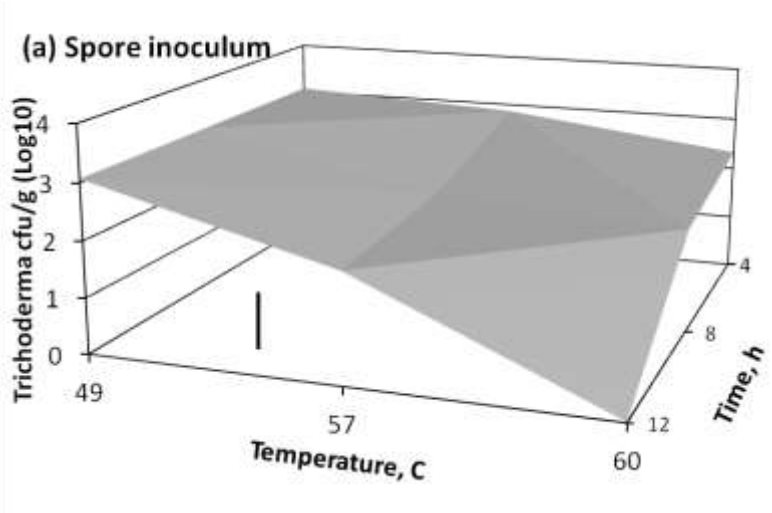




through the healthy compost is likely to be a second factor. Bulk Phase III compost goes through several mixing stages so it is possible to envisage how a small localised patch of *Trichoderma*-infected compost in a Phase III tunnel could be diluted quite efficiently throughout a sizeable proportion of the compost from the tunnel. Supplementing the Phase III compost as it was being emptied did not have any effect on the severity of the *Trichoderma* which developed although only one supplement was evaluated. *Trichoderma*-infected Phase III compost can infect Phase II compost at spawning.

Trichoderma aggressivum was shown to sporulate sooner and more heavily when it was exposed to the light, as might happen in tray, bag and block spawn-runs in situ, where a grower may visit spawn-running rooms from time to time or rooms may not be totally light-sealed. Thus, *Trichoderma* infections within a Phase III tunnel are likely to be less "visible" although they do contain many spores. In one experiment compost that had been infected with *Trichoderma* and incubated in the light had double the number of *Trichoderma* propagules than compost that was incubated in the dark.

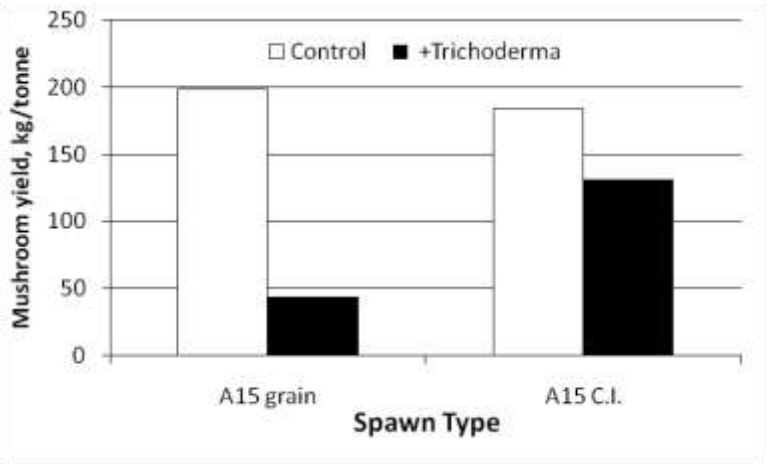
T. aggressivum and pasteurisation conditions



The conditions needed during pasteurisation of Phase I compost to eradicate inoculum of *Trichoderma aggressivum* (Th2) to below a detectable limit were determined to be 60°C for 12 hours. The results showed that both *Trichoderma* spores and *Trichoderma*-infected compost were highly temperature tolerant and survived 57°C for 8 hours. They could also survive in moderately high ammonia concentrations of 300 ppm for several hours. The pasteurisation requirement was not increased for dry (69% moisture) Phase I compost compared with normal (74% moisture) compost. Three types of *Trichoderma* viability testing were used at casing. The detection limit using dilution plating was 10 cfu/g compost. This

corresponded with visible *Trichoderma* growth from compost on semi-selective agar, and severe or even complete mushroom yield loss compared with a non-infected control compost.

T. aggressivum and alternative mushroom spawns



The effect of spawning composts with different types of mushroom spawn (grain, Speedy Inoculum™ and commercial casing inoculum (CI) on vulnerability to green mould infection was investigated. Grain spawn and Speedy Inoculum™ were equally susceptible to green mould infection and mushroom yield loss, but casing inoculum was significantly less susceptible.

T. aggressivum and compost analyses

The relationship between compost analysis and susceptibility to green mould infection was investigated. No relationships were found between green mould susceptibility and compost moisture, ash content or pH but compost ammonium nitrogen contents above 0.02% of dry matter increased the likelihood of severe mushroom yield loss resulting from green mould infection.

T. aggressivum detection in Phase III and Phase II compost

Detection of *T. aggressivum* in Phase III compost using the molecular-based "real time Taqman PCR" test was very sensitive and reliable. It was capable of detecting propagules when *Trichoderma*-infected compost was diluted into healthy compost up to a dilution factor of 0.01% (10^{-4}). Microbiological tests (Most Probable Number - MPN and Weed Mould Analysis - WMA) were effective and reliable at detecting *Trichoderma* propagules in Phase III compost when propagule counts were relatively high - 100-1000 propagules per gram fresh weight (gfw). When counts were lower, these methods were variable and false negatives

were obtained. This is likely to reflect the difficulty in sampling and subsampling compost that contains a low population of *T. aggressivum* propagules.

Molecular detection of *T. aggressivum* in pasteurised Phase II compost using a real time TaqMan PCR test was inconclusive due to the small number of samples tested. Microbiological methods were relatively reliable for the detection of *T. aggressivum* in Phase II compost.

Financial benefits

Trichoderma aggressivum can cause yield reductions of up to 100% and although outbreaks can be very sporadic, the impact of an outbreak is very severe. Individual Phase III compost producers in Europe have indicated that compensation to growers for crop loss due to *T. aggressivum* has been in the order of millions of Euro. Thus it makes sense that Phase III compost producers exercise extreme vigilance in the prevention, and early detection of, *T. aggressivum*. This can be achieved through routine testing for *T. aggressivum* and ensuring that Phase II pasteurisation conditions are set at levels known to eradicate *T. aggressivum*, especially in the event of a green mould outbreak.

Action points for growers

Phase I, Phase II

- All pasteurisation tunnels should be examined for cool zones in the compost; tunnel insulation and airflow should be modified accordingly.

- In the event of a green mould outbreak, a pasteurisation temperature of 60°C should be maintained for 10 hours; the effect of a longer (12 hour) pasteurisation on compost quality should also be examined.

- During green mould outbreaks, the use of commercial Casing Inoculum in place of grain spawn should be investigated.

Phase III

- Phase III compost should be monitored for presence of *T. aggressivum* during tunnel emptying. This could be done by placing Petri dishes at strategic locations during emptying and getting any “green mould” cultures identified by FERA or Teagasc.

Alternatively, a representative sample of the Phase III compost should be sent to FERA for testing using the sensitive Real Time PCR method.

- Unexpected reductions in yield from bulk Phase III compost should prompt a closer look at the compost for *Trichoderma* green mould. If compost green mould is present, the identity should be confirmed and the Phase III supplier should be notified.