Brown Cap Mushroom Virus (associated with Mushroom Virus X) prevention

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Brown Cap Mushroom Disease is the occurrence of ‘brown’ or ‘off-white’ mushrooms in white strain mushroom crops. This browning symptom has been the most prominent symptom associated with Mushroom Virus X (MVX) in recent years and is mainly associated with bulk Phase 3 (Phase III) compost. MVX disease describes a range of symptoms which include pinning delay, crop loss, distortions and cap browning. Brown Cap Mushroom Disease is associated with what we now know as ‘Agaricus bisporus Virus 16 (AbV16)’ or ‘Brown Cap Mushroom Virus (BCMV)’. This factsheet summarises the biology, causes and control of this disease.

Key recommendations

**Compost facilities**

- Steam cook-out or pasteurise tunnels between every batch of Phase 3 compost.
- 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.

- Ensure the highest levels of hygiene during spawning and complete isolation of the spawning process.
- Remove compost debris from and thoroughly clean, floors, winches, conveyors and all other compost handling equipment prior to use to prevent cross-contamination of tunnels.
- Periodically, test Phase 3 compost for the presence of viruses.
Outbreaks of Brown Cap Mushroom Disease occur sporadically every year, predominately associated with bulk Phase 3 compost. Outbreaks have been linked to breakdowns in hygiene procedures such as compost debris being carried over on machinery, equipment and transport vehicles, as well as the absence of end of crop steam cook-out. Increased attention to hygiene has been proven to alleviate symptoms on affected facilities and is an effective means of controlling the disease.

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 likelihood of contamination.

**Background**

Outbreaks of Brown Cap Mushroom Disease occur sporadically every year, predominately associated with bulk Phase 3 compost. Outbreaks have been linked to breakdowns in hygiene procedures such as compost debris being carried over on machinery, equipment and transport vehicles, as well as the absence of end of crop steam cook-out. Increased attention to hygiene has been proven to alleviate symptoms on affected facilities and is an effective means of controlling the disease.

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**Symptoms**

The symptoms of Brown Cap Mushroom Disease are complex and show great variability in expression within and between crops.

- The browning symptoms may occur in only one flush (often the first flush), they may be visible on the bed before harvest or they may not manifest themselves until after the mushrooms have been picked and cold-stored.
- Symptoms may consist of an ‘off-white’ or ‘cream’ cap colour, which can be very difficult to spot.
- Sometimes, mushrooms develop a more obvious colouration, from pale cream through to a deep brown coffee colour on just a few mushrooms or up to as much as 80% of a flush.
- The symptom of just a few brown mushrooms is sometimes ignored. A few brown mushrooms can mean that the crop and/or compost is highly infected with the Brown Cap Mushroom Virus. This compost could be the source of further outbreaks and it is important to maintain high standards of hygiene, including steam cook-out.
- Sometimes, crops may just appear to be of poor quality with a tendency to open prematurely.
Biology and epidemiology

Nineteen viruses have now been identified in mushrooms and these are listed in Table 1. Many of these are found in healthy crops and have not been associated with disease. *Agaricus bisporus* Virus 1 (AbV1) is responsible for La France virus disease. This is not very common any more, due to improved hygiene standards across the industry, especially steam cook-out. Mushroom cap browning is largely caused by the newly described virus AbV16, also known as Brown Cap Mushroom Virus (BCMV). Other viruses, AbV6 and Mushroom Bacilliform Virus (MBV), may also contribute to browning disease.

When AbV16 is present at low levels, the crop undergoes a subtle colour change from ‘pure-white’ to ‘off-white’, which is not detectable by eye. These mushrooms may be of good quality and fully acceptable. However, if AbV16 increases to very high levels, mushrooms start to show obvious signs of being ‘off-colour’, with some showing varying levels of brown colour.

Some of the other new viruses recently identified may be responsible for the pinning delay and patch symptoms of MVX but further scientific study is required to confirm this.

Table 1. A list of viruses which may be present in mushrooms

<table>
<thead>
<tr>
<th>Viruses previously identified:</th>
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<tbody>
<tr>
<td>AbV1</td>
<td>Responsible for La France disease (also known as LIV)</td>
</tr>
<tr>
<td>MBV</td>
<td>Mushroom Bacilliform Virus – linked with other viral diseases including La France</td>
</tr>
<tr>
<td>AbEV1</td>
<td><em>A. bisporus</em> Endornavirus 1 – previously associated with MVX complex</td>
</tr>
<tr>
<td>AbSV</td>
<td><em>A. bisporus</em> Spherical Virus</td>
</tr>
<tr>
<td>AbMV1</td>
<td><em>A. bisporus</em> Mitovirus 1</td>
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<table>
<thead>
<tr>
<th>New viruses identified in MushTV:</th>
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<tbody>
<tr>
<td>AbV16 (BCMV)</td>
<td>Responsible for Brown Cap Mushroom Disease</td>
</tr>
<tr>
<td>AbV6</td>
<td>Also associated with Brown Cap Mushroom Disease</td>
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<th>Other viruses of unknown pathology:</th>
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<tbody>
<tr>
<td>AbV2, AbV3, AbV5, AbV7, AbV8, AbV9, AbV10, AbV11, AbV12, AbV13, AbV14, AbV15</td>
<td></td>
</tr>
</tbody>
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Sources of infection

The main source of Brown Cap Mushroom Virus is contamination by infected mycelium or compost, or casing fragments. Mushroom waste or mushroom spores from infected crops are other possible sources. The most vulnerable times for infection are during tunnel emptying and room filling, when the compost is exposed.

Approximately 3% of Phase 3 samples from compost facilities, taken at tunnel emptying, tested positive for AbV16 during the course of the MushTV project. Mushroom and/or compost samples tested positive for AbV16 on 9 out of 17 grower facilities. Table 2 provides a summary of facilities testing positive for AbV16.

Table 2. Results of tests for AbV16 on facilities across Europe

<table>
<thead>
<tr>
<th>Facilities tested between 2012-2014</th>
<th>Presence of AbV16</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 compost facilities</td>
<td>3 facilities were positive for AbV16</td>
</tr>
<tr>
<td>11 out of 351 samples (3%) of Phase 3 compost tested positive for AbV16</td>
<td></td>
</tr>
<tr>
<td>17 grower facilities</td>
<td>9 facilities were positive for AbV16</td>
</tr>
<tr>
<td>10 out of 63 samples (16%) were positive for AbV16 (mushrooms and/or compost)</td>
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</table>
If spent compost and casing are effectively cooked-out by steam sterilisation, then the mushroom mycelium, which carries the viruses, will be killed and cannot act as a source of infection.

Routes of contamination by infected compost (or casing) fragments can occur as follows:

- Infected compost debris can infiltrate Phase 3 tunnels at spawning or during the spawn run.
- Infected Phase 3 compost can infect transport vehicles and filling equipment, especially conveyors and filling heads that are difficult to clean.
- Infected crops that are not steam sterilised will generate a high load of contaminated compost and casing fragments. These will be deposited throughout the farm and on filling and casing machinery, equipment and haulage trucks.
- Infected compost and casing fragments deposited around the farm or compost facility can be blown around on windy days to reinfect cleaned conveyors, machinery, equipment and haulage trucks.

Research has shown that only a tiny amount of infected material is required to spread infection. In experimental trials, a pinch of infected compost incorporated into Phase 3 compost or casing during filling operations, consistently caused brown and off-white symptoms. Viruses can move rapidly in Phase 3 compost, for example, through the depth of compost on a shelf in less than two days. A small amount of infection will be transmitted throughout the crop very quickly. The higher the number of infection points, the greater the severity of the disease. Thus, heavily infected filling heads, conveyors or rufflers will result in a heavily infected crop.

5. Compost debris left on the filling head after cleaning
6. Debris build-up on conveyor during Phase 3 emptying
7. Debris on ruffling machine after use
8. The small amount of compost used to produce symptoms in experimental trials

**Prevention**

Hygiene is the key method to prevent infection. Research has shown that disinfection does not completely kill mushroom mycelium that is protected in a compost fragment. It is vitally important to ensure that equipment, common areas and transport vehicles are thoroughly cleaned to remove all compost debris; disinfected and rinsed after all activities.

Cleaning should take place after all compost handling activities, such as filling, ruffling or emptying.

Airborne sources of contamination, such as mushroom spores and microscopic compost fragments, can be minimised by using high-grade air filtration.
Monitoring and detection

Grower awareness of the symptoms of Brown Cap Mushroom Disease is very important, as these can be confused with, or attributed to, other causes such as abiotic factors. Picking staff should be trained to identify symptoms. ‘Off-white’ mushrooms can be readily detected by sensitive colour-measuring devices (although expensive). If any mushrooms exhibit the classic Brown Cap Mushroom Disease symptoms or if mushrooms seem ‘off-white’ or creamy or are of poor quality, then growers should adopt the precautionary principle, assume they have Brown Cap Mushroom Disease and take appropriate actions (see below).

A new highly sensitive molecular test has been developed as part of the MushTV project to detect the levels of AbV16, AbV6 and MBV. This is an ‘Early Warning’ test, as it can measure virus levels in Phase 3 compost before mushrooms are produced. The test could be performed on freshly emptied Phase 3 compost from a compost facility and also on mushrooms or compost from a growing facility. Virus levels are correlated with disease severity. Therefore, obtaining information on background levels of virus that occur in Phase 3 compost during routine monitoring will provide composters and growers with the facts to know when these levels increase above normal.

Disease management strategies

The most effective strategy for the control of Brown Cap Mushroom Disease is very stringent hygiene. The viruses are transmitted in infected mycelium, usually within compost; therefore, a focus on removal of compost and other debris prior to disinfection is a key control measure. Surfaces should be rinsed extensively.

Disease management strategies should be tailored for each stage of the process.

Compost facilities

Tunnels should be steam cooked-out or pasteurised between every batch of Phase 3 compost. Where possible, fill spawned Phase 2 (Phase II) and empty Phase 3 at different ends of the tunnel and with dedicated machinery for each activity. The spawning process should be completely isolated.

Debris should be removed from floors, winches, conveyors and all other equipment and should be thoroughly cleaned, disinfected and rinsed.

Haulage trucks delivering to farms with Brown Cap Mushroom Disease may return contaminated with infected material on wheels, truck covers and personnel, so strict hygiene levels should be in place.

Growing facilities

All equipment and machinery such as filling heads, conveyors and rufflers should be thoroughly cleaned and disinfected prior to and after use. Waste mushroom material, such as butts and stalks, should be disposed of appropriately. Concrete areas and corridors should be washed and disinfected at the end of every day.

Concrete aprons adjacent to bulk-filling operations should be cleaned and disinfected before operations begin and kept damped down to reduce wind-blown dust. Filling should be done in isolation, with no emptying, picking or cleaning occurring at the same time. Growing rooms containing mushrooms should be closed while filling is taking place. Debris on the floor after rooms are filled and cased should be cleaned away carefully using low pressure water and a floor scraper.

Personnel involved in filling and casing operations should have no contact with the rest of the farm before operations start, to prevent picking up any contamination.

Steam cook-out of spent compost and casing at the end of every crop is the most effective measure growers 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 prior to emptying the spent compost and casing.

After emptying, growing room shelves and netting should be cleaned thoroughly to remove debris and disinfected. Where there have been serious levels of Brown Cap Mushroom Disease, cleaned nets should be laid out onto shelves and the empty growing room and nets should be re-steamed at 65°C for 2 to 8 hours to ensure that any potential residual disease contamination is killed.

Where Brown Cap Mushroom Disease is present, there is an extremely high risk of reinfection as the
virus-infected compost will not be killed off with disinfectant, because the disinfectant will not penetrate into the bulk of the compost. Therefore, steam cook-out is essential to control Brown Cap Mushroom Disease.

After emptying of spent compost, the floors, shelving and walls of the room must be washed and disinfected with an approved disinfectant. The concrete areas outside the growing room must also be washed and disinfected. Spent compost must be removed off-site as soon as possible.

Maintain growing rooms in good condition as smooth, sealed surfaces and rooms are easier to clean and disinfect. The surroundings of the mushroom farm should also be kept in good condition, avoiding the accumulation of organic matter (compost, casing soil, mushroom stalks), which can act as a source of infection.

10. Debris in a freshly filled growing room which needs thorough post-fill cleaning

Further information

MushTV Factsheets

01/15: Use of chemical disinfectants in mushroom production
03/15: Understanding *Trichoderma aggressivum* in Bulk Phase 3 compost
04/15: Fungal diseases of mushrooms and their control

HDC Factsheets and publications

HDC Factsheet 11/07: Mushroom Virus X (MVX) prevention

HDC Grower summaries and reports

See the HDC website (www.hdc.org.uk) for copies of M 58, 51, 39a, 39b, 39c, 39d and 07.

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