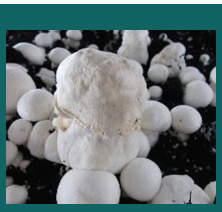

MUSHROOM PEST & DISEASE

MU16003 **fact sheet #6**

Lecanicillium fungicola – Dry Bubble disease

GROWERS' NOTES

- Dry Bubble infection is characterised by a range of symptoms: undifferentiated masses of tissue forming on the bed, cap spotting, stipe blowout, warty cap and fluid production.
- The initial route of entry of Dry Bubble infection onto the farm is unclear, but the main source of successive infections is on-farm reservoirs.
- Infection at casing leads to symptoms expressing in 1st flush resulting in the greatest reduction in yield and quality.
- The form of symptoms expressed is determined by the time of infection.
- The main methods of spread are by vectoring of sticky spores, water splash and by airborne dust.
- Effective fly and mite control is a key to disease management.
- During an outbreak, ALL crops must be monitored for infection. Well-trained harvesters are critical to ensure constant surveillance.
- During an outbreak, crops should be checked on non-picking days as watering onto untreated disease is a common method for the disease to spread more widely.
- The recommended method for spot treatment is to carefully remove the affected mushroom by picking up the bubble firmly with a gloved hand inside a plastic bag, pulling the bubble off the bed, then pulling the plastic bag over the hand and tying the bag closed. The bag must be disposed of carefully.
- In a diseased room, spot treatment must be carried out up until the day of crop termination.
- Fungicides with the active ingredient Prochloraz-manganese can be legally used against Dry Bubble according to the label directions by incorporating into the casing or by surface spray following harvest of the first flush.
- Apply fungicides only if Dry Bubble is evident. Routine application as a preventative measure will promote development of fungicide resistance in the pathogen.

To control Dry Bubble, an extremely rigorous and holistic hygiene programme must be implemented across the entire farm.

1. INTRODUCTION

Dry Bubble is the common name given to a serious fungal disease affecting cultivated *Agaricus bisporus* crops. It is caused by the soil-borne mycoparasites *Lecanicillium fungicola* var *fungicola* (syn: *Verticillium fungicola*; *Verticillium malthousei*) which is found in Europe and *Lecanicillium fungicola* var *aleophilum* which is more common in North American *Agaricus* crops, including *Agaricus bitorquis*. Dry Bubble is consistently the most significant problem facing growers wherever button mushrooms are grown, including Australia.

Lecanicillium fungicola was first identified as a mushroom pathogen in France in 1892 and was described as the causal agent of 'La mole' disease, the French term for what we now refer to as Dry Bubble. But despite more than 130 years since its first appearance, Dry Bubble continues to have a detrimental impact on mushroom production. The disease causes significant losses estimated at 2-4% of total revenue annually and poor control of the disease may result in losses approaching 20-25% or more, while uncontrolled disease can result in farm closure.

Lecanicillium fungicola causes a range of symptoms on *A. bisporus* depending on when in the mushroom development cycle infection occurs. Symptoms expressed include small necrotic lesions on fully formed mushroom caps which progress to extensive areas of necrosis, warty outgrowths developing on caps, stipe distortion and disintegration (stipe blow-out) and the characteristic bubble, an amorphous mass of undifferentiated mushroom tissue.

While the infection severely reduces marketable yield if not managed correctly, the actual overall yield does not decline and ironically, may exceed yields of healthy crops. In fact, Dry Bubble disease is known to *increase* pinning rates thought to be due to aborted pins leaving gaps and nutrients for successive pins to develop.

With the overall reduction of pesticides available in horticulture due primarily to consumer concerns, effective disease management is increasingly becoming reliant on disease prevention. Australian growers only have two chemical control options available, one of which will soon be likely withdrawn from the market.

Dry Bubble cannot be controlled by relying solely on a single fungicide to 'do the job' as the inability to rotate fungicides of different active ingredients will likely result in resistant pathogens. Effective management of Dry Bubble in Australia therefore depends on a back-to-basics IPM approach of strict hygiene, exclusion and containment.

2. DRY BUBBLE SYMPTOMS

The symptoms expressed by Dry Bubble disease are determined by the stage of mushroom development at which infection occurs. Infection of young primordia with *L. fungicola* results in the formation of the characteristic 'Dry Bubble', an amorphous mass of undifferentiated tissue (Fig. 1a).

Often, numerous deformed primordia will grow together to form a single bubble as the individuals increase in size. As the bubble matures, the surface develops an off-white grey velvety texture indicating the pathogen is sporulating. The bubble may begin to turn brown as the mushroom reacts to the infection.

Under high pathogen load due to a high concentration of initial inoculum and poor disease management, Dry Bubble may exude a clear fluid (Fig. 1b). This characteristic is not to be confused

Figure 1 Dry Bubble symptoms expressed when young developing primordia are infected by *Lecanicillium fungicola* **a)** characteristic dry bubble **b)** bubble exuding fluid **c)** stipe blowout. *Images: Judy Allan and Warwick Gill*

Figure 2 Dry Bubble symptoms expressed when mature mushrooms are infected by *Lecanicillium fungicola* **a)** necrotic lesions (red arrow) and blue/gray patch (yellow arrow) caused by sporulating *Lecanicillium* **b)** necrotic lesions coalesce to form areas of extensive browning on the cap. Patches of sporulating *Lecanicillium* also coalesce (yellow arrow) **c)** warty outgrowths. *Images: Warwick Gill*

with Wet Bubble (*Mycogone perniciosa*). The production of fluid by mushrooms is a generic response to a physiological stress and is not indicative of any single pathogen.

A third common symptom of primordium infection is stipe blow-out (Fig. 1c). The affected mushrooms are often mildly deformed but retain a recognisable mushroom shape. Stipe deformation is often accompanied by splitting or peeling of the stipe.

Blow-out occurs when one side of the mushroom becomes infected as the stipe is beginning to elongate. As the 'clean' side elongates at the normal rate, the affected side is retarded by the infection resulting in the stipe literally pulling itself apart.

When infections of more developed mushrooms occur, bubbles do not develop. Because the tissues of more mature mushroom have already differentiated, the influence of *Lecanicillium* on tissue differentiation and development is much reduced. Consequently, the symptoms expressed are more superficial and are likely due to spores landing and germinating on the mushroom cap.

Initially, dark, chocolate brown lesions form which are moderately pitted. Patches of sporulating *Lecanicillium* can often be seen alongside the necrotic lesions (Fig. 2a). As the infection matures, the necrotic lesions coalesce to form extensive areas of browning (Fig. 2b), as do the areas of sporulating *Lecanicillium*.

A third common symptom observed on mature mushrooms is warty cap (Fig. 2c). The cause of the warty outgrowths is unknown, but it may be a defensive mechanism of the mushroom which isolates *Lecanicillium* spores by encapsulating them in mushroom tissue.

3. DISEASE DEVELOPMENT

Initial infection pathway

The route of initial introduction of *L. fungicola* onto the farm can be difficult to establish. Early sampling suggested that casing ingredients, particularly peat, were sources of *Lecanicillium.* It has since been shown that spore-laden dust accumulating on peat bales in storage is the more likely origin of infection.

External sources of *Lecanicillium* may include returnable containers, vehicles and personnel but regardless of the original route of entry onto farms, it is certain that the primary source of recurring infection originates on-farm from a build-up of viable *Lecanicillium* spores and hyphal cells/mycelial fragments.

Lecanicillium spores can survive at least one year in moist soils while spores and mycelium can survive up to eight months under dry conditions and accumulate in the grow room dust, in cracks and joins in grow room floors and in the soil around the premises. Once the spore-laden dust is disturbed and becomes airborne, often through strong winds, on-site earthworks, building renovations or simple maintenance tasks like replacing grow room ducting, the spores land on mushroom beds, germinate in the presence of growing *Agaricus* mycelium and infect the crop.

Mushrooms at all stages of development and in all flushes are vulnerable to Dry Bubble. The disease may express in early flushes if the concentration of inoculum is high enough and infection occurs at casing. This can happen when *Lecanicillium* spores and mycelium are carried over from the previous crop due to poor sanitation or if the pathogen is introduced during casing.

Once a crop is infected, it in turn becomes a source of Dry Bubble infection as the pathogen population peaks in the 3rd flush. With each bubble estimated to produce 30,000,000 spores/ hr, the older crops represent a significant source of inoculum.

Pathogen growth and development

Lecanicillium fungicola is unable to infect *A. bisporus* mycelium in compost and it is only when spores land on casing that Dry Bubble can express. However, germination is inhibited by metabolites produced by the casing microflora, a situation called 'soil fungistasis'. It is not until the casing is colonised by *A. bisporus* mycelium that the spores are able to germinate and the disease develops.

After germination, *Lecanicillium* hyphae grow alongside and attach to *A. bisporus* hyphae in the casing. Although not able to infect vegetative hyphae, *Lecanicillium* actively invades the hyphae of developing fruit bodies by the enzymatic breakdown of the host cell and the production of specific infection structures. These structures are able to pierce the weakened host cell walls which often collapse resulting in browning of the affected tissue.

As the infection matures, *Lecanicillium* mycelium on the surface of bubbles and affected mushrooms begins to sporulate. An erect conidiophore surrounded at intervals by whorls or verticils of spore producing cells (Fig. 3) rises above the mushroom surface, giving the bubble its characteristic off-white to blue-grey velvety texture. Small thin-walled spores are produced at the tips of the spore producing cells forming spherical, slimy heads held together in a sticky

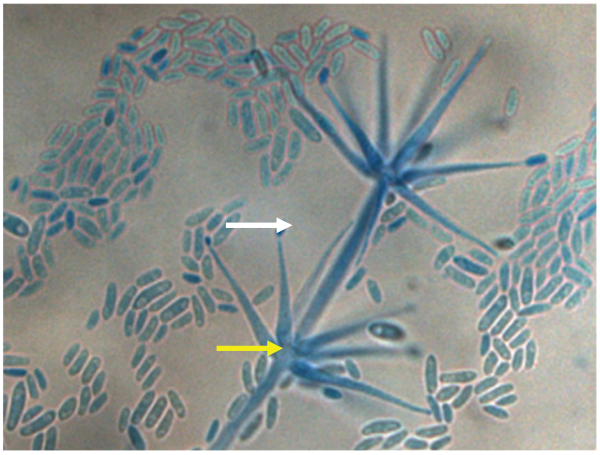

Figure 3 *Lecanicillium* conidiophore with verticils of conidiogenous cells (yellow arrow) arranged spaced along the axis. Small thin-walled conidiospores are produced at the apices of the conidiogenous cells (white arrow). *Image: Warwick Gill*

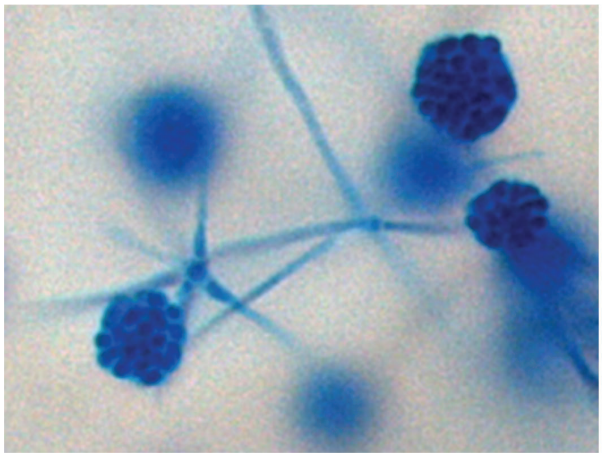

Figure 4 *Lecanicillium* conidiophore with clusters of conidiospores held in globose heads of sticky mucilage. *Image: Warwick Gill*

mucilage (Fig. 4). Adjacent heads may touch and coalesce forming large slimy, sticky masses of spores. The conidiophores hold the masses of sticky spores aloft presenting them to a passing human or fly vector to which the spores adhere. The spores are carried to another location where, if the conditions are favourable, Dry Bubble infection starts anew.

Spread

The reproductive biology of *L. fungicola* is particularly suited to thrive and persist in the mushroom growing environment. The sticky spores are primarily vectored by Sciarid flies and to a lesser but still significant extent by Phorid flies and by mites.

Flies walk onto symptomatic caps (Fig. 5), touch a sticky spore mass and then fly off to another location transferring the spores. With their six legs and therefore six points of inoculation, flies are particularly efficient disease vectors. Because Sciarids specifically lay their eggs in 10 to 15 separate clusters close to developing *Agaricus*

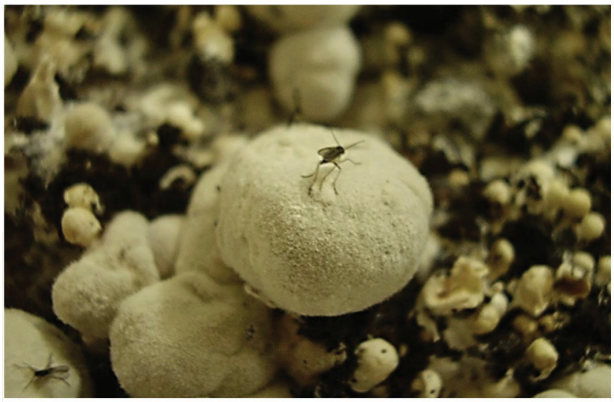

Figure 5 Sciarid fly walking over the surface of a mature bubble. *Image: Judy Allan*

Figure 6 'Incidental touches'. Symptomatic mushrooms (asterisks) within a cluster of clean mushrooms which were missed during crop inspection. *Image: Warwick Gill*

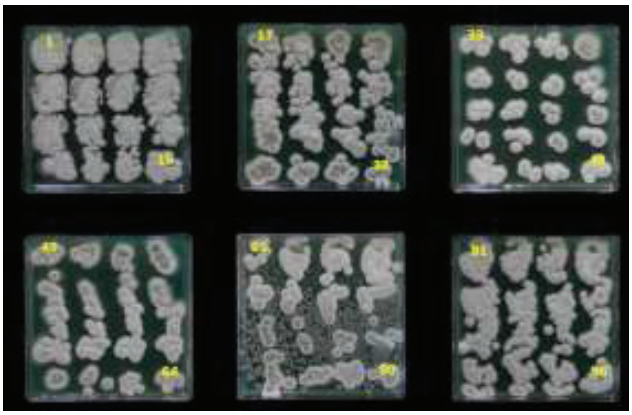

Figure 7 Transmission of sticky *Lecanicillium* spores. From a single touch of a gloved finger onto a symptomatic mushroom, spores were still being transferred after 96 touches onto agar. *Image: Warwick Gill*

mycelium, the *Lecanicillium* spores are transferred directly to the casing where soil fungistasis has been broken due to the presence of *Agaricus* hyphae, allowing the spores to germinate immediately and infect the mushroom hyphae.

Mites are also direct vectors as they too walk over the caps of symptomatic mushrooms, pick up the sticky spores and transfer them to another fertile location on the casing surface. One mite species is known to feed on *Lecanicillium* spores and mycelium and viable spores have been recovered from their faecal pellets.

Humans also actively vector *Lecanicillium* spores by touching affected mushrooms. An example of this is 'incidental touches' where small bubbles, concealed within a cluster of healthy mushrooms, are overlooked during crop checks (Fig. 6).

Harvesters pick the cluster of mushrooms, unknowingly touching the bubbles. *Lecanicillium* spores are then spread from harvesters' gloves to other surfaces that they subsequently touch. The spores are very easy to pass on and a glove inoculum can be very persistent. Even after 90 touches onto an agar surface, spores were still being shed after a single contact with a bubble (Fig. 7).

Viable *Lecanicillium* spores occurring on the bed are not confined just to bubbles and other expressed symptomologies. Due to fungistasis, viable spores can survive on the casing surface but remain ungerminated. Harvesters touching the casing will pick up a significant number of spores on their gloves. Furthermore, up to 25% of asymptomatic mushrooms at harvest are contaminated with *Lecanicillium* spores. It is likely that a significant number of these spores have been transferred onto 'clean' mushrooms from harvesters' contaminated gloves.

Asymptomatic mushrooms carrying *Lecanicillium* spores will unfortunately lose quality on the retail shelf post-harvest. More importantly, spores from asymptomatic mushrooms and probably the occasional incidental bubble contaminate reusable boxes and returnable crates so they can potentially carry spores back into the grow room.

Standard cultivation practices are also responsible for the spread of *Lecanicillium*. Watering onto an area of untreated disease not detected during a crop inspection is the major cause of spread within an affected room. Likewise, harvesting and poorly performed spot treatment are significant contributors to the spore load on the floor.

Watersplash will disperse spores directly to clean areas of the bed for a new infection to develop. Watersplash may also disperse spores onto the waterer who will transfer the spores on their clothing to other areas of the grow room or farm. Watersplash can also disperse spores to a nearby wall or equipment so they can be picked up and vectored to another location to start a new infection.

Once on the floor, spores combine with the organic material and water and develop a significant infection reservoir, particularly if they are compacted into cracks and joins in the floor. The spores are carried on wheeled equipment out of the room and deposited along the concrete apron outside the room or along the utility corridor.

Staff also pick up the spores on their footwear and deposit them throughout the farm. Significantly, they can be carried to the shared lunch room where they will cross-contaminate production staff and be tracked back to the casing line and other 'clean' areas of the farm. Spores adhering to harvesters' footwear can be introduced to the mushroom bed by water being cast off their shoes as they climb up on picking trolleys or picking lorries.

Once the organic material on the grow room floor has dried, a different method of dispersal comes into consideration. Long-lived and resilient *Lecanicillium* spores become a component of farm dust once the organic material dries out. While the air velocity in a grow room is insufficient to dislodge *Lecanicillium* spores, it is capable of dispersing airborne dust created by activities such as dry sweeping.

Spore-laden dust accumulates inside the facility in roof spaces, ducting and on most flat surfaces while outside, the dust settles on production equipment and stored casing materials, on gravel roadways and in soil. When these reservoirs are disturbed during, for example, farm renovations or earthworks on a nearby property, wind will disperse the viable spores contained in the dust through the farm, initiating new infections.

4. DISEASE MANAGEMENT

Like any significant mushroom disease, the most efficient and cost-effective control measure for Dry Bubble is to prevent infection by ensuring rigorous hygiene throughout the farm.

Effective control of Dry Bubble rests on a solid foundation of meticulous hygiene, effective exclusion, rigorous fly control and careful monitoring backed up with a speedy and appropriate response to any infection. Early detection and immediate action are essential to manage Dry Bubble and to prevent it getting out of control.

Dry Bubble is an insidious persistent disease which is prolonged by a continual cycle of on-farm infections, a direct result of the large number of sticky spores it produces.

Because the spores can remain viable for a long time and are efficiently dispersed by multiple vectors, only a whole-farm approach in which all personnel diligently participate, can hope to control the disease (See Table 1).

Arthropod vectors

Flies are significant vectors of *Lecanicillium* spores and control of Dry Bubble is not possible while their population remains unchecked. Fly control can be achieved through a combined approach of exclusion/containment and chemical control. While mites perform a lesser role in Dry Bubble vectoring, the potential for mite swarms and the ability of some species to consume *Lecanicillium* spores and deposit viable spores in their faeces means they mustn't be overlooked.

Casing and filling operations

Infection of the mushroom crop at casing is most likely due to casing ingredients becoming contaminated by *Lecanicillium* spores carried in airborne dust which is transferred to the casing during mixing. Ideally, casing inputs ought to be stored under cover wherever possible and peat bags and bales must be sanitised before being opened.

Casing equipment must be sanitised before use to remove accumulated dust and immediately after use to prevent the accumulation of compost which can harbour dust and spores and carry them over into the next compost batch. To prevent dust contamination during operations, the mixing area must be sanitised before work begins and at completion and if possible, casing operations ought to be carried out under positive pressure.

Contamination of compost by *Lecanicillium* is unlikely to lead to Dry Bubble infection of the crop. But filling equipment must still be sanitised immediately prior to start of work and cleaned again at completion to avoid the accumulation of spore-laden dust within compost residues which may transfer to the casing surface by wind. Whether filling trays or filling shelves, the concrete apron surrounding the operation must be sanitised immediately before and after work.

For both filling and casing, other grow rooms must not be emptied and the doors of diseased grow rooms must be kept closed during both operations to avoid possible cross-contamination from spore-laden airborne dust, particularly if the farm performs 'chemical cookout' rather than steam cookout.

It is extremely important that staff wear freshly laundered clothing before beginning work and that production staff do not share facilities such as break rooms and toilets with staff from harvest and post-harvest sections of the farm to avoid cross-contamination with *Lecanicillium* spores.

Grow room

In an infected grow room, Dry Bubble control is achieved by a combination of:

- **containing** the pathogen to the room and the affected the bed by spot treatment;
- **excluding** arthropod vectors and carefully managing human vectors and their activities; and
- **eliminating** the pathogen by effective crop termination.

Ensure crops are closely inspected and made 'safe' before human activity begins in a grow room on an affected farm. The checker, usually a supervisor or grower very familiar with Dry Bubble symptomology, identifies areas of disease on the beds and marks them for immediate spot treatment which:

- prevents watering on to the disease and splashing spores onto walls, the floor and personnel;
- prevents contact with arthropod and human vectors; and
- suppresses the growth of the pathogen.

Appropriate door signage is necessary for diseased rooms. Ideally, a sign needs to clearly warn staff that Dry Bubble is expressing in the room accompanied by photographs of symptoms as a reminder to harvesters of what to look for when working in the room.

Consider a second sign including a plan of the room which can be updated after each daily crop inspection with the locations of diseased areas. This provides growers and supervisors with a disease situation report at a glance. Also consider updating with regular monitoring data.

It is important that the disease is detected early and that appropriate action is taken immediately to prevent the disease getting away and becoming unmanageable.

Farms often rely on harvesting staff to detect disease early as they are in the grow rooms for extended periods on a daily basis. It is critical then that harvest staff are:

- well-trained in early symptom recognition;
- aware of the marking and reporting procedures and chain of communication;
- aware of the effect that non-reporting of Dry Bubble symptoms may have on their job security; and
- assured that abnormal observations reported by them will be taken seriously.

Well-trained and prepared harvesters are a major weapon in the battle against Dry Bubble as many eyes and time spent with the crop ensure constant surveillance.

Harvesting must begin in young, disease-free rooms and finish in the oldest or most heavily affected rooms in which harvester numbers are kept to a minimum. Consider forming a dedicated team restricted to symptomatic and/or older rooms; these harvesters will need their own facilities to avoid cross-contaminating other harvesting and production staff.

Recycled boxes and reusable crates are a significant Dry Bubble reinfection pathway not just within a farm but when product is transferred between farms. Confine recycled boxes to diseased or 3rd flush crops – ideally do not store them anywhere on-farm or use them when the farm is under high disease pressure. Reusable crates must be sanitised before being introduced to early flushes.

Disposable gloves must be worn at all times and changed regularly – at least between crops and when leaving the grow room for a break. Because viable ungerminated *Lecanicillium* spores can exist on the casing surface and on asymptomatic mushrooms, gloves ought to be changed more often in infected rooms.

Ideally, leave soiled gloves in a dedicated bag or bucket in the grow room for careful disposal instead of carrying infected gloves out to communal rubbish bins in the utility corridor and shared spaces.

The farm's glove policy must be clearly written and issued to all harvesters. Consider training in safe glove removal techniques to prevent spread of casing and mushroom debris from soiled gloves.

Mushroom beds must be kept clean by removing stumps and debris which may contain *Lecanicillium* to prevent the pathogen accumulating. At the end of harvest, avoid creating airborne dust by dry sweeping. Instead, use high volumes of low pressure water and a squeegee to clean the floors. Check that the drains are clear of mushroom debris, casing and compost at the end of clean-up.

Harvesting equipment, including picking trolleys, squeegees, knives, buckets and hoses must be washed and sanitised before starting work, between rooms and at the finish of work. At the end of the day, picking trolleys must be thoroughly sanitised, paying particular attention to the wheels and wheel brackets where spores from the floor accumulate. Towels, clothing and other textiles which come into contact with the diseased crop must be collected and washed daily in hot soapy water at a temperature exceeding 60°C.

Because the spores and mycelial fragments of *Lecanicillium* ultimately end up on the floor when disease areas are disturbed (Fig. 8), foot dips are essential tools in managing spread by personnel and wheeled equipment. Foot dips must be safe, fit for purpose and placed in the doorway of infected rooms to contain the pathogen and in the doorway of clean rooms to exclude the pathogen.

During harvest, it is important that the foot dip is always used on exit and entry and that it is suitable for wheeled equipment to pass through. A 'mat' – a carpet offcut or strip of artificial turf soaked with sanitiser which spans the width of the door – makes a very effective foot dip (Fig. 9). Staff cannot avoid stepping on it and picking trolleys, forklifts and other wheeled equipment can pass over it without impediment.

Figure 9 Artificial turf foot dip placed in a grow room doorway. *Image: Screen shot from Farm Hygiene Video*

Carpet can be simply hosed off and replenished with fresh sanitiser from a bucket. Like all disinfectants, check that runoff does not contravene local EPA regulations. However, the mat must be long enough for the largest wheels to make at least one complete revolution in contact with it. In times of high disease pressure, doorway disinfecting mats ought to be strategically placed around the facility in places like in front of the

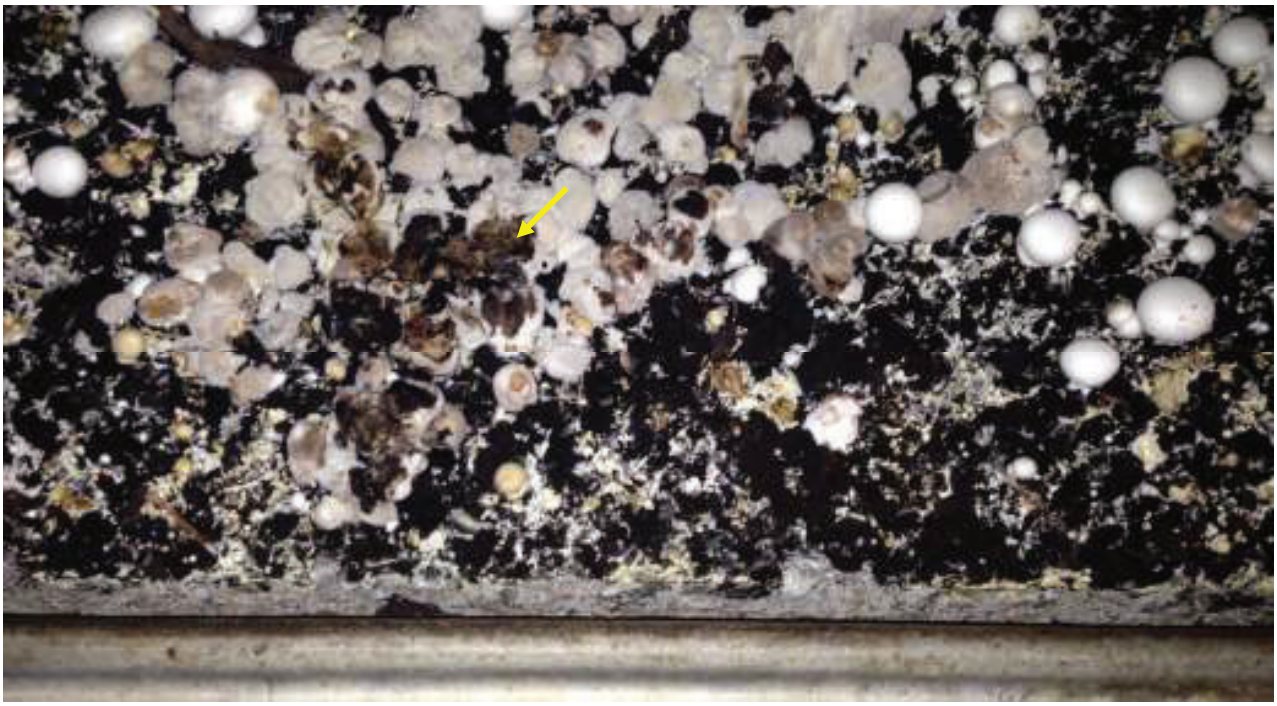

Figure 8 Widespread area of Dry Bubble infection caused by watering on a patch of disease. To make this situation worse, someone has climbed the shelving to inspect upper levels and stood on the Dry Bubble (arrow). *Image: Judy Allan*

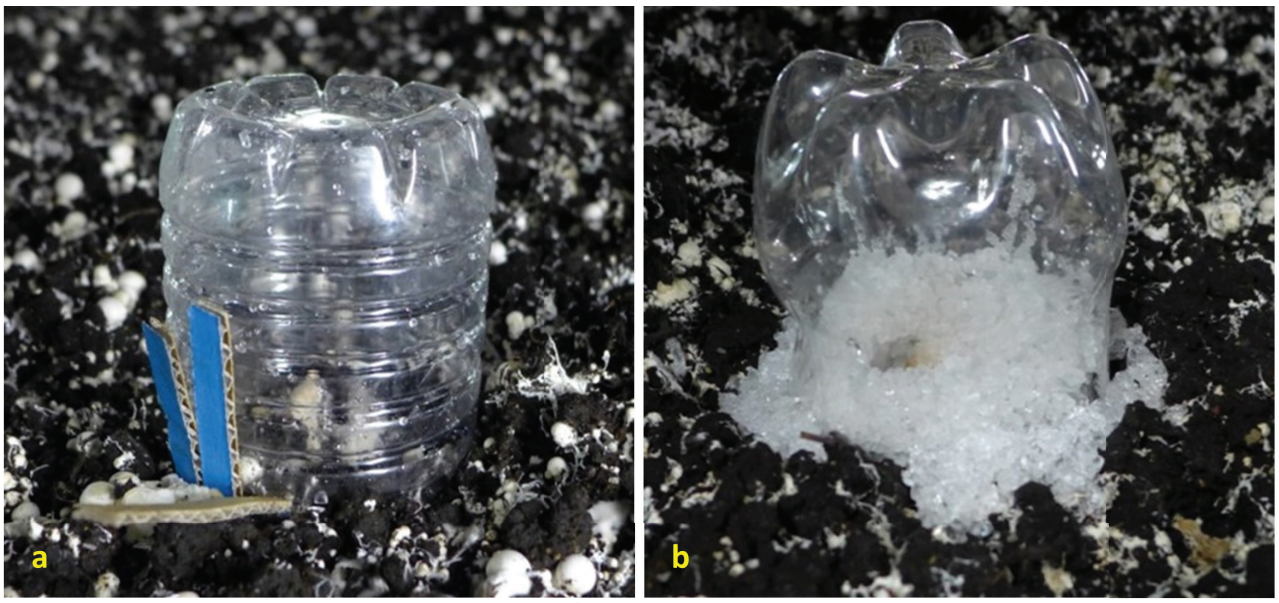

Figure 10 Pot technique for spot treatment of Dry Bubble **a)** inverted pot without salt. Note the blue marker used to identify disease patches for treatment **b)** inverted pot containing salt. The farm has used the bottoms of soft drink bottles for pots. *Images: Judy Allan & Warwick Gill*

set back or spawn run room and the main forklift entry point to the facility from the production area. This is particularly important on tray and block farms.

Environmental control

In the past, the time from *Lecanicillium* spore germination to bubble development was around 14 days with some variation in summer due to increased temperature and humidity. But with the widespread adoption of deep-dug peat as a casing ingredient, and hybrid strains which grow at a slightly higher temperature, the grow room environment has become generally wetter and warmer, conditions which *Lecanicillium* prefers, resulting in the average time from spore germination to cap spotting being 3-4 days and to bubble development around 7-10 days.

As a result, more spores are going to be produced over a shorter time frame. Air handling units and sensors may need to be calibrated to ensure good environmental control. Doors of diseased rooms must be kept closed at all times and the seals need to fit tightly. All vents and other wall penetrations need to be fitted with quarantine mesh to exclude flies and to prevent resident flies leaving the room.

Spot treatment

For control of Dry Bubble once it is expressing on the bed, it is necessary to both isolate the disease from the grow room environment and to contain the infection. This is achieved by spot treatment which is a time-consuming and laborious process but effective if done correctly and diligently. Spot treatment must be carried out by trained 'disease teams' as poorly performed spot treatment can worsen the infection.

Unfortunately, hygiene and disease treatment are often regarded as 'punishments' and the staff undertaking these critical tasks do not apply due diligence. It is essential for the continued operation of the farm and therefore job security that these critical tasks be performed correctly and effectively.

There are two viable treatment options depending on where the area of infection has been identified; the pot technique and removal and salting. For early infections in first and early second flush where a small number of individual bubbles have been recognised, the pot technique is very useful.

A clear plastic cup, which may contain salt, is inverted over the affected mushroom and firmly pushed through the casing layer as far as the compost (Figs 10a,b). Depending on how soft the casing is, the pot may need to be twisted to get it below the bed surface.

This is a relatively rapid method and is very effective at isolating the bubble from water splash and from contact with flies and humans, preventing dispersal of spores. The cup also contains the infection as *Lecanicillium* cannot grow beyond the confines of the cup and growth of the pathogen is suppressed by the salt, if used.

The cup can be left in the bed for the life of the crop, or it may be removed at the end of the flush, the bubble removed and the area salted. In early infections, the pot method also assists in monitoring the crop as the pots are easy to see and they are (usually) associated with a single bubble.

Bubble removal followed by salting is the most common spot treatment method and it is especially appropriate when mushrooms are growing close together and/or the casing is too dry to push a plastic cup into. It is also the best method to use when infection is widespread after a patch of disease has been watered.

Once the affected area is identified, a disease team member places a gloved hand inside a small plastic bag and picks a bubble with the bagged hand and pulls it from the casing holding the bubble firmly (Fig. 11a). Being careful not to drop infected casing on a clean area of bed, the bag is then pulled over the hand holding the bubble, twisted and tied off. The bagged bubble can then be disposed of very carefully or placed into a bucket and left in the grow room for cookout.

Once the bubble is removed, a generous amount of salt is poured over the area extending about 5cms beyond where the bubble was removed from (Fig. 11b). While suppressing *Lecanicillium* growth, presumably because of the salinity in the casing, the salt also prevents vectors from touching the infected area and protects from water splash. Treated areas must be monitored regularly to ensure there has been no regrowth of the pathogen around the site.

It is critical that the bagging and removal process is performed as prescribed. To prevent cross-contamination, the removal process must follow the 'one bubble-one bag-one touch' rule. That is, one bubble must be removed with a single bag in one movement or touch and then tied and discarded. Cross-contamination occurs when one bag is used to pick up multiple bubbles, resulting in contaminated casing being dropped onto areas of clean bed and the floor and effectively dispersing the pathogen rather than containing it. The operator's gloves also become contaminated with *Lecanicillium* spores and the disease cycle continues.

As Dry Bubble never sleeps, 'disease teams' need to be rostered on for non-picking days, weekends and public holidays, particularly when a farm is experiencing an outbreak or navigating out of an outbreak.

The crop must also be treated right up until the day the crop is terminated. When a room is shut down, the environmental conditions encourage *Lecanicillium* growth and sporulation and increasing fly populations. This results in many millions more spores becoming available and a greater number of flies emerging to carry them to new rooms.

Crop termination

If the Dry Bubble is left uncontrolled, the level of *Lecanicillium* increases so that by the end of the crop the pathogen population will reach a maximum. Crop termination is the most effective method of Dry Bubble control as it breaks the on-farm reinfection cycle by reducing the pathogen population and allowing the next crop to start clean. If Dry Bubble is expressing heavily in 2nd and 3rd flushes, early crop termination ought to be considered to reduce chances of the disease spreading. It is estimated that early termination may reduce the pathogen population on the farm by up to 90%.

By far, the most effective termination procedure is cookout performed *in situ* where the undisturbed

Figure 11 Removal and salting technique for spot treatment of Dry Bubble **a)** one bag inverted over a gloved hand is used to grasp and dispose of one bubble **b)** salt is applied to the bed where the bubble was removed from. *Images: Judy Allan*

crop is treated with steam in the grow room and it must kill pathogens and pests within compost and netting on shelf farms and compost and trays on tray farms. An effective cookout prevents contamination of subsequent and adjacent crops which occurs when spent substrate contaminated with viable pathogens and pests is removed from a grow room.

Prior to the introduction of steam, all grow room wall penetrations, drains and vents must be closed off and the door seals checked for integrity to prevent flies escaping. Steam is then gently introduced so the inrush of steam does not force flies and/or spores out of the room through small holes that have been overlooked.

To achieve an effective kill of *L. fungicola* in compost, casing and the grow room environment, it is recommended that the compost temperature be held at 65-70°C for 9-12 hours. This will account for the pathogen, which has a minimum thermal death time of 2 hours at 60°C (or 4 hours at 55°C) and mites and adults and larvae of Phorids and Sciarids which all have a thermal death time of 5 hours at 55°C. The steam must be given longer to penetrate all the nooks and crannies of the wooden trays and shelf construction and netting which may harbour viable *Lecanicillium* and its arthropod vectors.

Following cookout and cool down, the spent substrate must be emptied from the room and removed from the farm premises immediately. Stockpiled spent compost is a breeding ground for flies which will vector *Lecanicillium* spores from the farm environment to infect new crops and continue the disease cycle.

Trays must be stored where they cannot become re-contaminated. As *Lecanicillium* can grow on tray timbers, a second steaming of the empty trays ought to be considered if the farm is under high disease pressure. Similarly, nets must be washed, disinfected and dried before being stored in a way so as not to become re-contaminated. Nets will need to be sanitised again prior to fill if Dry Bubble is expressing heavily on the farm.

For farms that either cookout in a dedicated cookout room or rely solely on disinfection to terminate a crop, the crop must be terminated *in situ* with an appropriate disinfectant before being moved. The grow room air must first be shut off then the surface of the crop sprayed with an appropriate disinfectant. The grow room should then be closed down for an hour or so to let the disinfectant act and for airborne spores and dust to settle before being emptied.

If the room cannot be emptied within 12 hours of disinfection, the crop must be re-sprayed on the day that it is to be emptied. Mushrooms, pathogens and pests will continue to develop within the compost and casing because the disinfectant acts only on the surface. The application equipment must be capable of applying a good coverage of disinfectant so that *Lecanicillium* spores and mycelium and fly adults and larvae are eliminated and are not released into the farm environment while the terminated crop is being moved.

Good disinfectant coverage is indicated if the remaining mushrooms turn uniformly brown after spraying. Uneven browning indicates that the disinfectant has not been applied evenly and must be re-applied to ensure the crop is safe to move. Farms that rely on 'chemical cookout' must ensure that the terminated crop is removed from the farm immediately to prevent pests and pathogens emerging from the substrate into the farm environment.

Once the room has been emptied, both the forklift(s) and the route taken to the cookout room or discarded compost truck must be thoroughly sanitised to eradicate viable *Lecanicillium* within spilt compost, casing and mushroom debris.

Grow room sanitation following crop termination

Once spent compost has been removed, the grow room must be thoroughly sanitised by a seven-step process to ensure the next crop starts free of *Lecanicillium*. This process includes:

- gross cleaning of mushroom debris, compost and casing by shovel and water;
- pre-rinse, working from the ceiling to the floor;
- detergent wash to remove stubborn organic material from surfaces;
- post-rinse to remove detergent residues which will affect disinfectant efficacy;
- disinfectant which can be applied manually or by fogging or spraying;
- terminal rinsing if directed by the product label or if food contact surfaces have been treated; and
- drying, which allows disinfected surfaces to maintain their hygienic state for longer.

Particular attention must be paid to the floor and floor cracks and joins where *Lecanicillium* is known to accumulate and be buffered from effects of both temperature and disinfectants by compacted organic material. Keep the grow room doors closed and personnel out of the clean room until fill. Ideally, fog the grow room immediately prior to fill to ensure the crop starts clean.

Power washing or pressure cleaning is a popular method for removing stubborn organic material and biofilm from grow room walls and floors. The high-pressure jet of water produced by power washers aerosolises organic material, including infective *Lecanicillium* spores and mycelium, particularly if floor cracks and joins have been cleaned out with the power washer.

A power washer must be used in a closed room to prevent aerosols being spread around the farm and it must *only* be used prior to final sanitation. The room can be disinfected about an hour after power washing, once aerosols have settled.

Chemical control

The application of fungicides to control Dry Bubble infection is one of the tools available to the grower, but it must be done only in response to an outbreak.

Routine incorporation of fungicide into the substrate to prevent future infection is a significant cause of chemical resistance, particularly in a closed system like mushroom cultivation, and must be avoided. This is particularly important considering the small number of chemical controls available to the mushroom industry and the inability to rotate chemicals based on different active ingredients.

The only fungicidal products registered for application to mushroom crops in Australia are those containing Prochloraz (as the manganese chloride complex) at 462g/kg. Prochloraz can either be incorporated into the casing or applied as a surface spray after the first flush has been harvested.

If the fungicide has been incorporated into the casing, it must not be applied later as a casing spray. For incorporation into casing, the fungicide is thoroughly mixed with the water used to wet the peat and is distributed evenly throughout prior to casing. Depending on the ratio of black peat to blond peat used in the casing, it may be difficult to mix the fungicide adequately – heavier, wetter casing mixes with higher rates of black peat are more difficult to mix. Also, by incorporating the fungicide into the casing, much of the fungicide is wasted as it is distributed throughout the entire casing and not is concentrated in the upper half where it is most needed to control *Lecanicillium*.

To apply as a casing surface spray, the fungicide is pre-mixed with a small volume of water then added to the required volume of water in the spray tank with agitator running to ensure thorough mixing and even application of the active ingredient.

The spray is then applied immediately after first flush harvest, again *only* if the fungicide has not previously been incorporated into the casing. There is no withholding period required if the product is applied according to label directions.

Prochloraz is known to degrade in the mushroom substrate so the effective dose is reduced as the crop matures. To mitigate the impact of degradation when applying to the bed surface, ensure that sprays are evenly applied and that there is no overspray ensuring the maximum amount of active ingredient is delivered to the crop.

Check the delivery pattern and the volume delivered at regular intervals. Check also for nozzle blockages which may occur if Prochloraz is not agitated sufficiently during delivery. Prochloraz must not be allowed to settle in the spray tank as the active ingredient will fall out of solution and the correct dosage will not be delivered to the mushroom bed.

Products containing 500g/L Carbendazim may also be incorporated into the casing or applied to the mushroom bed according to the conditions stipulated in permit PER14949. Carbendazim must be applied only once per crop. There is a 14-day withholding period following application of Carbendazim.

It is the growers' responsibility to consult and familiarise themselves with the product labels and permit conditions available on the APMVA's online PubCRIS portal **before** applying these control agents.

5. MONITORING

Monitoring is an important weapon in Dry Bubble disease management and control. Unfortunately, it is often neglected until a farm is in crisis, but it ought to be regarded as an investment in farm health and ongoing crop security. In addition to keeping track of disease during an outbreak, monitoring will also help assess the effectiveness of farm hygiene as it battles out of a disease outbreak. But most of all, it is an essential early warning tool to head off an outbreak before disease impacts quality and yield.

During a Dry Bubble outbreak, the crop is primarily monitored for evidence of spotting and bubble formation so that the symptoms can be treated and the crop made 'safe' before watering and harvesting begin.

If the farm is under severe disease pressure, monitoring ought to be carried out twice daily. In addition to mandatory signage on the grow room door warning of disease in the room, monitoring results can also be displayed, such as the number of disease patches and/or the locations of the affected areas drawn on a room map. This acts as a general alert to staff working in the room and gives growers and supervisors an idea of the disease status of the grow room at a glance.

Monitoring staff must be well-trained in disease symptom recognition and the chain of reporting so that supervisors and growers can act immediately to isolate and contain the disease to prevent its spread. Monitoring staff must also be rostered so that monitoring continues on non-watering and non-pick days as well as weekends and holidays.

As the farm works out of the disease, the focus of monitoring shifts to evaluating the effectiveness of farm hygiene. A reduction in the number of diseased areas indicates that hygiene protocols are having an impact. Displaying a declining infection on the grow room door is a great boost to embattled staff morale, while on the other hand, an increasing rate is a sign for staff to be more vigilant and that more effort is required for controlling the disease.

Because flies are so closely associated with the spread of Dry Bubble, monitoring fly numbers is critical as an indicator to an impending Dry Bubble infection. The Project Team are aware of different ways that farms do this, such as counting the number of 'zaps' per minute of an electronic fly zapper or counting the corpses in the fly zapper collection tray. But perhaps the best method is the standard yellow sticky trap.

Not only can the flies be readily counted, but if the numbers do get out of hand, Sciarids and Phorids can easily be distinguished and the appropriate species-specific control treatment undertaken.

If your farm does not have an established fly monitoring programme, NOW is the best time to start one. It can be as simple as two or three yellow traps hanging in a service corridor which are inspected weekly.

When times are good and disease pressure is low, you will be able to determine the number of flies that your farm can tolerate without impacting on the crop. Over time, this number will rise and fall, giving a background profile. At some point, the number of flies will rise more than the normal fluctuations and you may find Dry Bubble expressing. This will indicate the threshold number of flies for your farm.

By constant monitoring, you will know that when fly numbers approach the threshold level, it is time to review your farm's hygiene and be on the lookout for Dry Bubble.

If you farm is currently experiencing a Dry Bubble outbreak, fly monitoring will very quickly show if your fly control regime is having an effect on the primary disease vector. As the farm works through the disease, declining fly numbers will confirm that fly control is working and the chances of Dry Bubble spreading are reduced. Fly monitoring will take time and resources, but it is a very worthwhile investment.

To control Dry Bubble, an extremely rigorous and holistic hygiene programme must be implemented across the whole farm.

Table 1 Checklist of key action points for Dry Bubble prevention and control

Location	✓	×	?	Action point
Filtration & air pressure	☑	☒	☐	5-micron air filters fitted to input and exhaust ducts in grow rooms and incoming air of Phase II rooms
	☑	☒	☐	Spawning operations carried out under positive pressure to prevent contamination by airborne dust
	☑	☒	☐	Quarantine mesh fitted to vents to prevent fly infestation
Filling & casing	☑	☒	☐	Casing material stored under cover
	☑	☒	☐	All dust removed from the outside of peat bags before opening
	☑	☒	☐	Casing mixing area sanitized before and after use
	☑	☒	☐	Casing performed under positive pressure to prevent contamination by spores in airborne dust
	☑	☒	☐	Prochloraz-manganese incorporated into peat, if required, according to product label directions
	☑	☒	☐	Concrete in front of grow room sanitised immediately before and after fill
	☑	☒	☐	All equipment used for casing and filling cleaned and sanitized before and after use
	☑	☒	☐	All casing and filling staff wear fresh clothes before operations begin
	☑	☒	☐	During operations, filling and casing staff do not share facilities with harvest and post-harvest staff
	☑	☒	☐	Diseased grow rooms kept closed, no grow rooms emptied during casing and filling
	☑	☒	☐	Flies and mites eradicated
Grow room	☑	☒	☐	Crops inspected and treated before harvesting and watering
	☑	☒	☐	Untreated areas of disease neither handled nor watered
	☑	☒	☐	Affected mushrooms and casing are carefully spot-treated
	☑	☒	☐	All stumps and mushroom debris removed from bed surface after harvest
	☑	☒	☐	Diseased crops are treated right up until day of termination
	☑	☒	☐	Picking staff trained in symptom recognition and disease reporting procedure
	☑	☒	☐	Staff managed so that clean rooms are harvested first while infected rooms are kept closed
	☑	☒	☐	Picking staff numbers kept to a minimum in affected rooms to reduce chances of human vectoring
	☑	☒	☐	Harvesters' gloves changed regularly; trained in correct removal; glove policy understood by all staff
	☑	☒	☐	Hand towels and other textiles and clothing collected daily for hot soapy wash (water >60°C)
	☑	☒	☐	Picking trollies, lorries and post-harvest trollies washed and disinfected daily at tend of shift
	☑	☒	☐	Door handles and broom and squeegee handles washed and sanitised daily
	☑	☒	☐	Staff rostered to spot-treat disease on non-picking days
	☑	☒	☐	Environmental controls are calibrated, effective and good evaporative conditions prevail
	☑	☒	☐	Door seals checked, all joins and cracks in floor and around wall penetrations are sealed
	☑	☒	☐	Signs attached to doors of affected rooms warning of disease
	☑	☒	☐	Pressure washing of floor done only *before* grow room is sanitized
	☑	☒	☐	Dust kept to a minimum reducing chances of airborne contamination – no dry sweeping after harvest
	☑	☒	☐	Flies and mites eradicated
	☑	☒	☐	Concrete apron and/or corridor floor outside affected grow room disinfected daily
Crop termination	☑	☒	☐	Cookout crop at 65–70°C for 9–12 hours
	☑	☒	☐	Concrete floor, particularly cracks and joins, is sanitized after cookout
	☑	☒	☐	Steam introduced gradually to prevent sudden in-rush of air dispersing spores and flies
	☑	☒	☐	Cookout room sealed tightly incl drains, to prevent spore and fly release during steam introduction
	☑	☒	☐	If cookout not possible, ensure crop is well covered with an approved disinfectant before emptying
	☑	☒	☐	Early crop termination considered especially if heavy infection in 2nd and early 3rd flushes
	☑	☒	☐	Spent mushroom compost removed immediately after cookout, not stockpiled on site
Monitoring	☑	☒	☐	Crops monitored daily for evidence of spotting and bubble formation
	☑	☒	☐	Crops monitored for evidence of spotting and bubble formation at least *twice* daily during outbreak
	☑	☒	☐	Crops monitored on non-watering days and non-picking days
	☑	☒	☐	Action taken immediately dry bubble is recognised
	☑	☒	☐	Fly and mite numbers monitored – control action implemented if numbers exceed threshold
General	☑	☒	☐	All returnable containers are sanitized before taking into grow room
	☑	☒	☐	Restrict cropping to a maximum of three flushes
	☑	☒	☐	Effective fly control is established and maintained
	☑	☒	☐	Foot dips are safe, fit for purpose, placed strategically and are maintained regularly
	☑	☒	☐	General farm sanitation is of a high standard

Compiled from: Overstijns 1998; Fletcher & Gaze 2008; Pyck & Grogan 2015

6. KEY REFERENCES

Berendsen RL, Baars JJP, Kalkhove SIC, Lugones LG, Wösten HAB, Bakker PAHM (2010) *Lecanicillium fungicola*: causal agent of dry bubble disease in white-button mushroom. *Molecular Plant Pathology* **11**:585-595

Berendsen RL, Kalkhove SIC, Lugones LG, Wösten HAB, Bakker PAHM (2012) Germination of *Lecanicillium fungicola* in the mycosphere of *Agaricus bisporus*. *Environmental Microbiology Reports* **4**:227-233

Beyer DM (1994) Get ready for summer "Vert". AMGA Journal, Spring:23-26

Beyer DM, Wuest PJ, Kremser JJ (2011) Verticillium Dry Bubble. Penn State extension article. Available online at https://extension.psu.edu/verticillium-dry-bubble

Curtis J (2008) 2008/2009 Mushroom Production Guide. Ministry of Agriculture and Lands, British Columbia, Canada

Fletcher JT, Gaze RH (2008) *Mushroom Pest and Disease Control: a Color Handbook*. Elsevier

Harvey Sorensen C (1982) Methods for control of *Verticillium* and *Mycogone*. *Mushroom News* November-December: 29-31

Nunes JS, de Brito MR, Zied DC, das Graças Leite EA, Dias ES, Alves E (2017) Evaluation of the infection process by *Lecanicillium fungicola* in *Agaricus bisporus* by scanning electron microscopy. *Revista Iberoamericana de Micología* **34**:36-42

Overstijns A (1998) The conventional Phase II in trays or shelves. *Mushroom journal* **584**:15-21

Piasecka J (2010) Molecular and Microbiological Methods for the Detection and Measurement of Dry Bubble Disease caused by *Lecanicillium* (*Verticillium*) *fungicola* on Mushroom Farms. PhD Thesis, National University of Ireland, Maynooth, County Kildare, Ireland. pp300

Pyck N, Grogan H (2015) Fungal diseases of mushrooms and their control. Factsheet 04/15, Agriculture and Horticulture Development Board

Shamshad A, Clift AD, Mansfield S (2009) Host-parasite interaction between cultivated mushroom, *Agaricus bisporus* hybrid strain Sylvan A15, and the mycoparasite *Verticillium fungicola*, a causal agent of dry bubble disease. *Australasian Plant Pathology* **38**:74-78

Wong WC, Preece TF (1987) Sources of *Verticillium fungicola* on a commercial mushroom farm in England. *Plant Pathology* **36**:577-582

Zare R, Gams W (2008) A revision of the *Lecanicillium fungicola* species complex and its affinity with the genus *Lecanicillium*. *Mycological Research* **112**:811-824

MU16003 - Pest and Disease Management and Research Services

TASMANIAN INSTITUTE OF AGRICULTURE

Hort Innovation Strategic levy investment | MUSHROOM FUND

This project has been funded by Hort Innovation using the mushroom research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au