MUSHROOM PEST & DISEASE

MU16003 fact sheet #4

Cladobotryum spp. – Cobweb disease

GROWERS' NOTES

- Cobweb infection is characterised by a white fluffy mycelium which becomes dense and granular.
- While the initial source of Cobweb infection onto the farm is unclear, the main source of spores becomes the diseased growing rooms and increased preventative hygiene is always necessary.
- Often the first sign of Cobweb in a crop is spotting of mushroom caps caused by spores introduced to new crops via cross-contamination.
- Because Cobweb has rapid growth and produces large numbers of aerial spores, early detection and immediate action to contain the outbreak using spot treatment is essential.
- The recommended method for spot treatment is to carefully cover the infected area with damp paper to trap the spores and then apply salt on top of the paper, beginning at the edges and working into the middle of the paper.
- In a growing room containing disease, spot treatment should be continued right to the end of the crop.
- During an outbreak, ALL crops must be monitored for infection. Well-trained harvesters are critical to ensure constant surveillance. During an outbreak crops should also be checked on non-picking days as watering onto untreated disease is a common method for the disease to spread more widely.
- Fungicides with the active ingredient Prochloraz-manganese can be legally used against Cobweb at the time of casing. Apply according to the label rate or as a split application according to APVMA PER12645. The split application counteracts degradation of the fungicide on the casing. When watering on the fungicide solution it is important to deliver the correct amount of fungicide in 1 litre/m^2 of bed surface.
- *Cladobotryum asterophorum* is a Cobweb-causing pathogen unique to Australian *Agaricus* cultivation.

INTRODUCTION

Cobweb is the common name given to a fungal disease affecting *Agaricus bisporus* crops in mushroom growing regions worldwide.

It is caused by species of the genus *Cladobotryum* (formerly *Dactylium*), primarily *Cladobotryum mycophilum* and *Cladobotryum dendroides*. The pathogen grows rapidly over the casing surface and colonizes mushrooms at all stages of development with a white aerial mycelium, causing a destructive soft rot. *Cladobotryum* sporulates heavily and the spores are easily spread around the farm causing secondary infections. Spores landing on mushroom caps incite browning, causing loss of quality.

Until the early 1990s, cobweb outbreaks had little impact and were easily controlled by available fungicides and routine hygiene practices. But during the early 1990s, the incidence and severity of cobweb on British mushroom farms increased and in 1994/95 the disease reached epidemic proportions, regularly causing up to 40% crop loss.

In Australia, the disease was of little significance until it became epidemic in 1999-2000. No single reason was identified, but contributing factors were thought to be reduced sensitivity to fungicides, relaxed attitude to hygiene, incorrect disease treatment, poor fungicide application and a change in the main casing ingredient leading to a warmer, moister growing environment that wasn't adequately managed.

In Australia, the primary cobweb pathogen is C. *mycophilum* but more recently *Cladobotryum asterophorum* has been identified from an outbreak of a fungal disease expressing Cobweb-like symptoms.

Figure 1 Fluffy white *Cladobotryum* mycelium growing over the casing surface. Colonies often develop from a central point of origin such as a mushroom stump (arrow). *Photograph: Judy Allan*

Figure 2 Cobweb-like *Cladobotryum* mycelium (arrow) colonising a mushroom stipe. *Photograph: Warwick Gill*

SYMPTOMS

The most obvious symptom of cobweb infection is the appearance of a white, fluffy aerial mycelium which rapidly grows over the casing surface decreasing the productive area of the bed. The mycelium grows in a more or less circular colony, often around a distinct point of origin near the centre, which is usually an infected stump or piece of mushroom debris remaining from the previous harvest (Fig. 1).

Cladobotryum mycelium infects healthy mushrooms at all developmental stages, enveloping them in a delicate white mycelium which gives the disease its common name, 'Cobweb' (Fig 2). The mycelium colonizes all tissues of the mushroom including the stem, gill and cap (Fig. 3). Infected mushroom tissue becomes brown and areas of soft rot develop (Fig. 4) resulting in collapse of the mushroom. As the infection matures, the mycelium becomes denser and granular or powdery due to prolific sporulation (Fig. 5). Cobweb mycelium may produce a pink-red pigment, depending on the species of *Cladobotryum* present (Fig. 6).

Although mycelium growing over the mushroom bed is the most obvious symptom, it may not be the first sign of Cobweb infection. The disease often first appears as cap spotting when spores from an infected, usually older crop, are introduced into an unaffected room and land on the mushroom caps. The spores cause a browning reaction in the mushroom tissue resulting in irregular spotting which maybe indistinguishable from spotting caused by other pathogens. Cobweb spots sometimes form pits at their centres (Fig. 7) and they may coalesce to form extensive areas of browning.

Given the right conditions, the spores on the cap will germinate, producing the characteristic white, fluffy aerial mycelium which then sporulates (Fig. 8) and continues the infection process.

Figure 3 Granular sporulating Cobweb mycelium colonising stem and gill tissues. *Photograph: Warwick Gill*

Figure 4 *Cladobotryum* growing on mushroom tissue causes a brown soft rot symptom. *Photograph: Warwick Gill*

Figure 5 Sporulating Cobweb mycelium appears dense and granular. *Photograph: Warwick Gill*

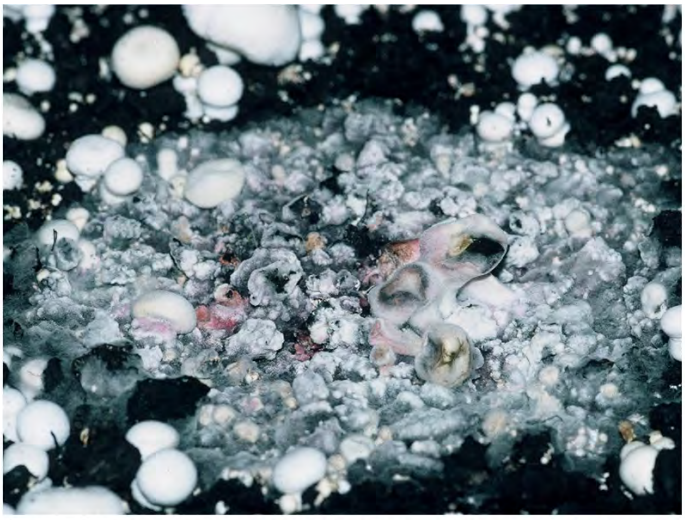

Figure 6 Mature Cobweb mycelium may produce a pink pigment, depending on the species present. *Photograph: Judy Allan*

Because mushrooms continue to respire and metabolise after being picked, *Cladobotryum* spores on the cap at harvest will cause symptoms to express post-harvest resulting in rejection of the product at the market door or loss of quality on the shelf.

In Australia, a third species of *Cladobotryum* has been reported as causing Cobweb disease on *Agaricus bisporus* mushrooms. *Cladobotryum asterophorum* expresses similar symptoms on mushroom beds to other recognised Cobweb pathogens but *C. asterophorum* mycelium appears to be grey/green rather than white (Figs 9, 10).

DISEASE DEVELOPMENT

Initial infection pathway

The initial route of cobweb infection onto the farm is often unclear. Mushroom pests and pathogens are eliminated from compost by an effective Phase II, so compost is not considered a source of infection.

Casing on the other hand is not treated before being applied to the crop, so infected raw ingredients may provide a possible pathway onto the farm. Spores released from natural *Cladobotryum* infections on wild mushrooms outside the farm may blow directly into grow rooms or they can contaminate poorly stored casing and casing ingredients and be introduced to the crop at casing. Irrespective of the route of initial contamination, the greatest cause of Cobweb infection of successive crops is the transfer of spores and/or mycelial fragments from older diseased crops on-farm.

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

Cobweb is more common in older flushes and may be severe, particularly if the infection is poorly managed in earlier flushes.

Figure 7 Brown spots and pitting caused by *Cladobotryum* spores landing on the mushroom cap. *Photograph: Warwick Gill*

Figure 8 Sporulating Cobweb mycelium produced from *Cladobotryum* spores which have germinated on the mushroom cap. *Photograph: Warwick Gill*

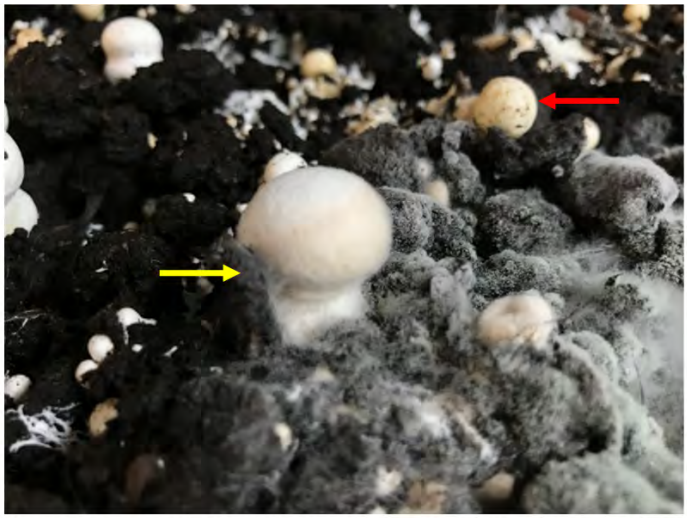

Figure 9 *Cladobotryum asterophorum* grey/green mycelium colonising casing and mushrooms. Note cap spotting (red arrow) and 'cobweb' mycelium (yellow arrow). *Photograph: Supplied by grower*

Figure 10 A brown rot on mushrooms caused by *Cladobotryum asterophorum* infection (arrow). *Photograph: Supplied by grower*

Growth and development

Cobweb infection begins when spores land on the mushroom bed or mushroom cap and rapidly germinate in the mushroom growing climate. Optimal conditions for *Cladobotryum* spore growth and development are 25°C with 90% RH. Under these conditions, spores begin to germinate after three hours and 100% of the spores germinate within nine hours.

At germination, a single hypha emerges from each cell of the spore (Fig. 11) and begins dividing to form the mycelium. *Cladobotryum* mycelium grows very rapidly at 1 or 2 cm per day and quickly develops into visible white fluffy colonies, the so-called Cobweb mycelium (Fig. 4). As the mycelium matures and food sources such as mushrooms are encountered, the mycelium begins to sporulate.

Vertical structures called conidiophores (Fig. 12) are formed which raise the reproductive conidiogenous cells above the mushroom bed and into potential air currents and within contact of passing vectors such as flies and humans. The conidiophore branches intermittently at nodes into two or three groups each of three conidiogenous cells. Large, dry and solitary multicellular conidiospores are then formed at the tips of the conidiogenous cells where they remain attached until they are dislodged through physical impact or by watering.

Cladobotryum infection spreads mainly by huge numbers of airborne spores. The spores are firmly attached to the mycelium but are dislodged by physical disturbance caused by standard cultivation practices such as watering, harvesting and poorly performed spot treatment. Once dislodged, the spores become airborne and air currents transport them around the farm. Spores that find their way to the floor by water splash or from dropped casing and mushroom debris become part of the floor dust.

When the dust dries, spores are spread by air currents or by pressure washing the floors. Although Cobweb spores are not sticky like Dry Bubble spores (*Lecanicillium fungicola*), flies and humans are still significant factors in the spread of Cobweb and control of both vectors is a critical aspect of Cobweb disease management.

Cobweb can also be spread by hyphal fragments, which are formed when infected casing is disrupted during harvesting. The Cobweb mycelium is broken up into individual hyphal cells which can then be distributed in the casing on pickers' gloves. Hyphal fragments also end up on the floor in dropped casing and mushroom debris.

Like spores, hyphal fragments become part of the dust microflora which can then be spread by air currents and pressure washing. When they land on a mushroom bed, hyphal fragments begin growing like spores and develop into the characteristic Cobweb mycelium to continue the infection cycle.

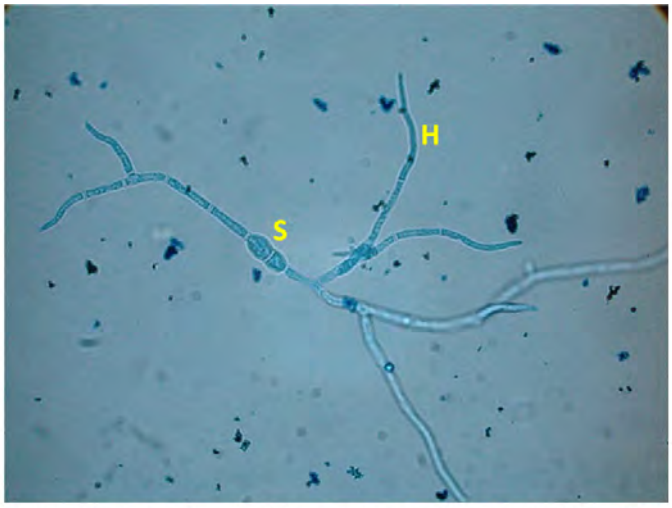

Figure 11 A 2-celled *Cladobotryum* spore (S) germinating. Each cell of the spore produces a hypha (H) composed of hyphal cells laid end-to-end. *Photograph: Warwick Gill*

Figure 12 An erect *Cladobotryum* conidiophore (C) bearing conidiogenous cells (CC) which give rise to multicellular spores (S). Production of conidiophores and spores gives the mature Cobweb mycelium its granular/powdery texture. *Photograph: Warwick Gill*

DISEASE MANAGEMENT

Effective control of cobweb is built 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. Because of its rapid growth and heavy sporulation, early detection and immediate action are essential to manage Cobweb and to prevent it getting out of control.

With respect to Cobweb disease, mushroom farms fall into one of two broad categories; those that have recent experience of, or are regularly affected by Cobweb and those that have little or no history of infection. Farms that have experience of Cobweb are in a better position to manage the disease as the farm personnel are familiar with the problem and can detect and manage infections early.

On the other hand, farms with little or no experience are vulnerable because if the early symptoms of Cobweb are not recognised by staff, infection will establish before action can be taken.

In order to be farm-ready, staff, particularly the harvesters, must:

- be trained in early symptom recognition and the corrective actions to be taken
- be aware of the marking and reporting procedures and chain of communication
- be aware of the potential effect that non-reporting of Cobweb symptoms may have on their jobs
- be assured that abnormal observations reported by them will be taken seriously

Well-trained and prepared harvesters are a major tool against Cobweb as many eyes and time spent with the crop ensure constant surveillance.

Casing

The primary source of crop infection, particularly of early infections, is thought to be from spores introduced into casing materials by contaminated personnel, unsanitary machinery, wind-blown dust and flies. Infection of the casing raw ingredients can occur in storage or the casing may be contaminated during the casing process, often by spore-laden dust collected on the peat bags in storage. Ideally, the casing ought to be prepared indoors under positive pressure to prevent entry of airborne spores and, if not used immediately, stored under cover. Both the casing areas and the machinery must be cleaned and sanitized immediately before operations begin and immediately after, using a registered sanitizer according to the manufacturer's label instructions. Likewise, the peat bags must be sanitized before they are brought into the sanitized casing area as any accumulated dust may contain *Cladobotryum* spores.

Casing personnel must wear fresh clothes and be isolated from other farm staff before casing begins and they should be issued dedicated boots and overalls. Disposable gloves may not be robust enough for casing operations so washable work gloves which can be regularly disinfected should be made available. All operations on the farm which could release *Cladobotryum* spores from infected zones must be suspended during casing. Activities which create dusts and aerosols, such as pressure washing floors after emptying a grow room must also stop.

Cobweb can be treated with the registered fungicide Prochloraz-manganese at casing either at the manufacturers' label rate or as a split application according to the APVMA-issued permit PER12645 delivering the correct amount of fungicide in 1L/m^2 water. The split application counteracts degradation of the fungicide within the casing. To ensure maximum effectiveness, the fungicide must be applied at the correct rate and full coverage must be assured by minimising overspray. The application of fungicide alone will not eradicate Cobweb disease.

Cropping

In the grow rooms, there are a number of steps that can be taken to prevent the spread of Cobweb. Because Cobweb grows so rapidly, crops on an affected farm must be monitored continuously to detect and treat infection before the mycelium sporulates, minimising the number of spores. Constant monitoring also ensures that areas of disease are located and treated before watering and harvesting, reducing the number of spores released into the air. Because *Cladobotryum* mycelium grows rapidly and sporulates prolifically, arrangements must be made to ensure the crop continues to be inspected on non-picking and non-watering days so that emerging Cobweb colonies can be identified early and treated immediately.

Spore dispersal on an infected farm can be reduced by exclusion – check that all door seals are intact and that all wall penetrations and cracks are sealed. Foot dips must be fit-for-purpose and be regularly maintained. Both incoming and exhausted air must be filtered to prevent spores leaving an infected room and entering a clean room. Restrict entry to infected rooms by posting warning signs on the doors and keep picking staff to a minimum in rooms where Cobweb is present. Consider turning the air off during harvest in an infected room to prevent dislodged spores from becoming airborne. However, it is very important that the air is not left off for long periods of time as the increased temperature and humidity will favour Cobweb growth and sporulation.

Because *Cladobotryum* mycelium may grow and sporulate on mushrooms after harvest, ensure that reusable boxes and crates used in an infected room are not subsequently used in a clean room before they are sanitized. Older crops must be harvested before younger, cleaner crops to prevent transfer of *Cladobotryum* on trolley wheels, harvesting equipment, clothing and tools.

While it should always be standard practice to remove stumps and picking debris from the bed surface after harvest, it is particularly important to do so during a Cobweb infection as infections often start on stumps and mushroom debris. If the infection is severe, consider terminating the crop early to reduce the inoculum levels. The growing environment may also be manipulated to slow the growth and sporulation rate of *Cladobotryum* by maintaining room temperature and RH at less than 18°C and less than 92% respectively.

Figure 13 The recommended method for spot treatment of Cobweb is to cover the infection with a damp paper towel to trap the spores and then apply salt over the paper. *Photograph: Judy Allan*

Figure 14 Mushrooms growing beneath paper applied during spot treatment. *Photograph: Judy Allan*

Figure 15 Mature Cobweb infection on mushrooms in a room 'shut down' before cookout. *Photograph: Judy Allan*

Once located, the recommended action for Cobweb infection detected on the mushroom bed is spot treatment. This is a laborious and time-consuming process but when done correctly and repeated as required, is effective in reducing spore dispersal and spread of the disease. A dedicated, well-trained spot treatment team should be established who will take the time and effort to apply treatment correctly. The project team have created an instructional video describing spot treatment of Cobweb and Dry Bubble, available online at http://bit.ly/2TeP7xh.

For effective spot treatment of Cobweb, very gently place a wet paper towel over an area extending about 5cm beyond the affected area so that spores are not dislodged. Gently cover the edges of the paper towel with salt to trap spores beneath the towel. Then cover the rest of the towel, working in from the edges to the centre (Fig. 13).

If the paper towel has been placed over mushrooms, then it will be necessary to go back the next day and carefully remove the growing mushroom and re-treat the area as these will lift the paper (Fig. 14) and allow spores to escape. To remove affected mushrooms, place a gloved hand inside a plastic bag and gently grasp the mushroom. Lift the mushroom from the bed and pull the plastic bag over both your hand and the mushroom and tie the neck of the bag tightly. Dispose of the sealed bag carefully or leave it in a bucket in the room for cookout or other method of disposal.

All treated areas must be checked regularly to ensure there has been no regrowth. Crop inspection and spot treatment must be carried out right up to the day of cookout because shutting the room down creates conditions of high humidity that favour cobweb development and the abandoned room will only breed more disease and increase the spore load on the farm (Fig. 15).

Crop termination

Effective crop termination is essential to prevent *Cladobotryum* carryover into the next crop and to prevent contamination of successive and adjacent crops. The most effective crop termination procedure is cookout *in situ*, where the crop is treated undisturbed in the grow room. Before cookout, the room must be made airtight by sealing all cracks and joins around doors, vents and other wall penetrations and capping the floor drains to prevent spores being forced from the room by positive pressure. Introduce steam slowly to reduce the pressure build up and minimize the strain on door seals. Maintain 65-70°C for a minimum of 8 hours to exceed he pathogen's thermal death point.

After emptying the room, remove spent compost from the farm immediately to prevent surviving spores re-infecting new crops and to prevent spores landing on the compost and germinating. Clean and sanitize the floor thoroughly as concrete floors seldom reach sufficient kill temperature. Clean and sanitize the nets and shelves and the concrete apron outside the room. If the spore load on the farm is high, consider laying the clean nets on the shelves and re-steaming to remove any lingering pathogens.

For farms that are unable to cookout, infected crops and floors must be sprayed with an approved disinfectant and left to stand to allow the disinfectant to act. After emptying, remove spent compost form the farm immediately. Floors, walls, shelves and netting must then be cleaned and sanitized thoroughly.

Table 1 Checklist of key action points for *Cladobotryum* spp. prevention and control

Location	✓	×	?	Action point
Filtration & air pressure	☑	☒	☐	5-micron air filters fitted to input and exhaust ducts in grow rooms
	☑	☒	☐	Quarantine mesh fitted to vents
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 airborne spores
	☑	☒	☐	Prochloraz-manganese applied according to the label or permit – split application effective
	☑	☒	☐	All equipment used for casing cleaned and sanitized before and after use
	☑	☒	☐	All casing employees wearing fresh clothes before casing operations begin
Grow room	☑	☒	☐	Affected mushrooms and casing are spot treated by covering with wet paper towel and salt
	☑	☒	☐	Staff rostered to spot treat disease on non-picking days
	☑	☒	☐	Crops are not harvested before they are inspected and treated
	☑	☒	☐	Untreated areas of disease are not handled or watered
	☑	☒	☐	Affected crops are treated right up until day of cookout or crop termination
	☑	☒	☐	Door seals checked, all joins and cracks in walls and around penetrations sealed
	☑	☒	☐	Temperature and RH held at <18°C and <92% respectively to arrest disease spread
	☑	☒	☐	Environmental controls effective and good evaporative conditions prevail
	☑	☒	☐	Air turned off while harvesting staff working
	☑	☒	☐	Staff managed so that clean rooms are harvested first while infected rooms are kept closed
	☑	☒	☐	Keep numbers of picking staff to a minimum in affected rooms to reduce chances of human vectoring
	☑	☒	☐	Ensure all stumps and mushroom debris removed from bed surface after harvest
	☑	☒	☐	Grow room doors kept closed during harvesting to prevent spore dispersal throughout farm
	☑	☒	☐	Signs attached to doors of affected rooms warning of disease
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 steam dispersing spores
	☑	☒	☐	Cookout room sealed tightly to prevent spore 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
Monitoring	☑	☒	☐	Crops monitored daily for evidence of cap spotting, the first indication of airborne spores
	☑	☒	☐	Crops monitored for evidence of spotting twice daily during an outbreak
	☑	☒	☐	Crops monitored on non-watering days and non-picking days
	☑	☒	☐	Action taken immediately cobweb recognised
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 placed strategically and are maintained regularly
	☑	☒	☐	General farm sanitation is of a high standard

Compiled from: Coles & Barber 2002; Curtis 2008; Grogan & Gaze 2008; Fletcher & Gaze 2008; Pyck & Grogan 2015

Priority Checklist Actions

1. .....

2. .....

3. .....

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