Treatment 3, which had normal levels of *Agaricus* mycelium in the casing. However, sporulation intensity in the limited or no-*Agaricus* treatments was always very light (Figure 20). In addition, many of the colonies had disappeared by the end of the study, and were no longer sporulating. In contrast, when normal *Agaricus* levels were present, sporulation was initially delayed while colonies were growing, but once sporulation began, it became more intense as time progressed. Despite high levels of sporulation throughout the cabinet, strong air movements and continued watering of the pots, no colonies developed on the control pots. Spores were however liberated towards the end of the study as spotted mushrooms were harvested from control pots at the end of the first flush.

### 3.3.4 Discussion

Whilst other casing factors have been shown to have little effect on the development of disease symptoms through the casing layer the presence of *A. bisporus* mycelium has been shown to have a very significant effect. The results from this study suggest that the growth of cobweb colonies within the casing layer is greatly dependent upon the presence of *A. bisporus*. Cobweb growth over casing was rapid, and continued throughout the entire study period, only under the conditions associated with a normal *Agaricus* crop, however, *Cladobotryum* was unable to grow saprophytically when *A. bisporus* was absent. The small amount of pathogen growth on treatments with little or no *A. bisporus*, observed one or two days after inoculation, should not be confused with weakly saprophytic growth. This can be explained by the nutrient reserves held in the agar plugs used to inoculate the casing.

These results indicate that the relationship between *Cladobotryum* and *A. bisporus* is a parasitic one where *Cladobotryum* growth is dependent on *A. bisporus*, resulting in a reduction in mushroom yield.

### 3.3.5 Conclusions

- Casing is unable to support the growth of *Cladobotryum* in the absence of developing mushrooms.
3.4 Summary

The work described in this section on the biology of *Cladobotryum* in mushroom casing has revealed that *Cladobotryum* growth is not severely affected by either casing formulation or casing moisture content or matric potential. Despite a wide variety of experimental conditions, ranging from different peat sources and rates of sugar beet lime, to very wet or very dry growing conditions, Cobweb colonies still developed in all cases. Some minor effects on growth were detected such as 30% sugar beet lime rates and wetter growing conditions resulting in larger cobweb colonies. However, results were not clear cut and some ambiguity occurred. In a second experiment the establishment of cobweb colonies was quicker in wetter casings but subsequent colony growth was faster in drier casings.

*Cladobotryum* growth on casing was shown to be dependent on *Agaricus* with practically no growth or sporulation occurring in its absence. Thus, *Cladobotryum* spores landing on casing materials would not be capable of further growth. Such contamination of casing ingredients could however result in subsequent development of cobweb disease and it would be useful to know how long any such spores can remain viable and potentially infectious. This is an aspect of *Cladobotryum* biology that future work could focus on.

3.5 General Conclusions

- Statistical analysis of cobweb colony diameters at the end of the first flush suggest that the rate of sugar beet lime inclusion has a significant effect on the establishment of cobweb, with larger colonies occurring when high (30%) rates of sugar beet lime are used.
- Cobweb colonies were capable of significant growth over a wide range of matric potentials in casings that were very dry to periodically waterlogged.
- Drier casings slowed down the establishment of cobweb while wetter casings led to rapid establishment but subsequent growth rates were marginally higher in the drier casings (41 mm/day as compared with 34 mm/day).
- Management of casing matric potential in a bulk peat and sugar beet lime casing mix is unlikely to have any major effect on cobweb control.
- Casing is unable to support good growth and sporulation of *Cladobotryum* in the absence of developing mushrooms.
4 Conidia dispersal

Conidia are a common asexual means of propagation within the Ascomycetes. As exact copies of the parent they perform the role of a dissemination tool and are free to be dispersed by whatever means applicable as soon as mature (Soper, R. 1986). In many cases conidia are the predominant means by which an ascomycete disease spreads itself to infect new hosts or tissue. Cobweb disease of mushrooms, caused by Cladobotryum spp., (asexual forms of Hypomyces spp.), is no exception. Cladobotryum conidia are generally considered to be the major means of dispersal of this disease although other means of dispersal have been considered and remain possible means of disease transmission (Sinden, 1971; Atkins, 1974; Lane et al., 1991; Gaze, 1995(b); Gaze, 1995(c); Dar, 1997).

There is a general lack of knowledge regarding the epidemiology of cobweb disease, in particular knowledge regarding conidial dispersal and movement. Many studies have described the distinctive morphological characteristics of the conidia (Plate 6) in order to aid classification, but few have examined their biological significance or the manner in which they are liberated. Dar (1997) assessed water splash, water run off, scarid flies, and air currents for their ability to disperse Cladobotryum conidia. He concluded that all four were capable of dispersing conidia but to various distances: water splash and air currents were considered to disperse conidia locally, to 40 and 75cm maximum respectively, whilst water run off and flies were considered capable of spreading disease further.

4.1 Conidial release pattern

4.1.1 Introduction

The importance of conidia to many plant pathogens as a means of dispersal has ensured that several have been studied in depth. Not only have dispersal mechanisms been investigated thoroughly but also movement of conidia within contained environments similar to mushroom cropping houses such as glass houses (Frinking, 1991; McCartney, 1991; Kerssies, 1993a; Kerssies, 1993b; Jenkinson & Parry, 1994; Pederson et al., 1994; Rodriguez et al., 1996; Madden et al., 1996; Lacey, 1996; Ntahimpera et al., 1997; Williams et al., 1998). However, few studies exist relating to either the cobweb pathogen conidial liberation or even general conidial liberation within the somewhat unique environment of a mushroom cropping house. Gandy (1972) briefly investigated the dispersal of Verticillium malthousei conidia within a mushroom crop, and as previously described, Dar (1997) highlighted potential means of cobweb disease dissemination within a growth room.

Because conidia are passively liberated, that is to say the fungus does not actively eject them, dispersal vectors such as air currents, water splash, invertebrates and vertebrates are required to liberate and/or disseminate them. One of the most common dispersal vectors is air movement. Airborne dissemination was chosen for study during this investigation over other dispersal vectors highlighted by Dar (1997) for several reasons.

Most importantly, experience of working with the disease suggested airborne dispersal was possibly an important means of conidial dissemination. Not only were conidia produced terminally on erect hyphae, but also conidia have been observed as being released into the atmosphere when a sporulating area of disease was disturbed. A significant release and dispersal of conidia by air currents would explain the occurrence of severe spotting symptoms.
in the absence of heavy fly infestation. Additionally, conidia from many other pathogens have been shown to be both liberated and dispersed by air currents. For example, Chastagner et al (1978) found wind speeds of 0.11 m/sec (comparable to those recommended for mushroom cultivation) were sufficient to disperse conidia of Botrytis cinerea.

It was the aim of this study therefore to monitor conidial liberation and airborne dispersal within a mushroom crop infected with cobweb disease. Continuous monitoring of the conidial load in the air should identify patterns of conidial dispersal that can be cross-referenced to records of cropping operations and movements within the house.

4.1.2 Materials and methods

4.1.2.1 Spore trapping

Cobweb conidial movements within a crop of mushrooms was studied with the aid of a Burkard Seven-Day Recording Volumetric Spore Trap. This style of spore trap draws air through a fixed aperture (2x14mm) and over a strip of adhesive cellophane tape attached to a cylinder that revolves once every seven days (2mm/hour). Particles contained in the air, i.e. conidia, dust, etc. are impacted onto the tape where they remain fixed in the adhesive, providing a chronological record of air contaminants.

The advantages of the Burkard spore trap over many other styles include:-
- it allows continuous sampling of the atmosphere over a seven day period
- conidial numbers/m³ of air can be calculated because airflow and aperture dimensions are known
- the occurrence of any one spore can be attributed to a specific period of time enabling its occurrence to be correlated to other activities
- trapped spores on the cellophane tape can be permanently slide-mounted and therefore examined at leisure.

During the course of the experiments described in this section, the airflow through the aperture of the Burkard spore trap was set at 10 litres/min. The spore trap was placed on the floor of the mushroom house, facing the back wall, immediately after the crop was aired and prior to inoculation of the crop with cobweb. The spore trap was maintained throughout the cropping period, which involved changing the battery, monitoring and adjusting airflow, winding the clockwork mechanism, and changing the tape every seventh day.

The cellophane strip was removed from the cylinder after seven days and cut into daily sections measuring 48 mm in length. Each daily section was mounted between glass slides and cover slips in a mounting gel containing 100 ml distilled water, 50 ml glycerol, 35 g 'Gelvatol', and 2 g phenol. Sections were then examined at 400x magnification. Several passes, each 0.5 mm wide, were made along the length of every slide and the distance of all Cladothromyces conidia from the start of the tape was recorded. The distance (mm) from the start of the tape was then converted into time with 2 mm equating exactly to 1 hour. For a given period of time (e.g. 15 minutes, 24 hours) the total number of conidia trapped was calculated as follows:
\[ O^n \times \left( \frac{W^t}{W^s} \right) = T^n \]

where \( O^n \) = number of conidia observed for a given time period; \( W^t \) = total width of tape (mm); \( W^s \) = width of tape sampled (mm); \( T^n \) = total number of conidia trapped for a given time period.

This figure can then be further transformed to show the number of conidia/m\(^3\) air as follows:

\[ T^n \times \left( \frac{1}{V} \right) = N \]

where \( T^n \) = total number of conidia trapped for a given time period; \( V \) = volume of air sampled during that time period (m\(^3\)); \( N \) = number of conidia/m\(^3\) of sampled air.

The number of conidia/m\(^3\) of air was calculated for each 15 minute interval, or averaged for every 24 hour period.

### 4.1.2.2 Crop management

A mushroom crop was established and managed as outlined in sections 3.1.2.1, 3.1.2.2, 3.1.2.3, and 3.1.2.4. Half the pots were inoculated with the thiabendazole-resistant *Cladosporium* isolate 192B1. Disease measurements were taken as outlined in section 3.1.2.5. In addition, a record was kept of entry and exit times, and the time and duration of every cropping operation.

### 4.1.3 Results

The mean number of conidia trapped/m\(^3\) air varied on a daily basis (Figure 21). The first conidia were trapped on the 15th day after casing, the same day that disease was first observed and only four days after inoculation with the disease. During the early stages of cropping the mean numbers of conidia trapped were very low at <10 conidia/m\(^3\), however, this increased dramatically after the 1st flush. On the first day after the 1st flush (the 23rd day after casing), and coinciding with a salting operation to reduce the disease area on the casing, over 1000 conidia/m\(^3\) of air were trapped. The numbers of conidia trapped/m\(^3\) of air remained high until the onset of the second flush when again the numbers fell to <10 conidia/m\(^3\), despite a significant increase in the area of disease present on the casing. Interestingly, no conidia were recorded at all on the 31st day after casing - the final day of the 2nd flush.

The effect the number of airborne conidia had on the incidence of spotting can be observed in figure 22. When studied in conjunction with figure 21, it shows how the number of conidia trapped/m\(^3\) of air related to the relative number of spotted mushrooms harvested. Spotted mushrooms were not observed in any numbers until the final two days of the flush (21 and 22 days after casing). For example, on the final day of the 1st flush, approximately 50% of the mushrooms harvested from both inoculated and control pots were spotted. Thus, it appeared the low levels of airborne conidia witnessed prior to the 1st flush were sufficient to cause sizeable losses during the later stages of the 1st flush. This suggested a lag period of about
Figure 21. Number of *Cladobotryum* conidia trapped using a Burkard spore trap, and area of cobweb disease developing on casing following inoculation of a mushroom crop.
Figure 22. Number of clean and spotted mushrooms harvested over two flushes from an intentionally cobweb infected crop.

Figure 23. Number of cobweb pathogen conidia (strain 192b1) trapped per m$^3$ of air every 15 minutes on the 28th day after casing.

Figure 24. Number of cobweb pathogen conidia (strain 192b1) trapped per m$^3$ of air every 15 minutes on the 24th day after casing.
Figure 25. Number of cobweb pathogen conidia (strain 192b1) trapped per m³ of air every 15 minutes on the 23rd day after casing.

Figure 26. Number of cobweb pathogen conidia (strain 192b1) trapped per m³ of air every 15 minutes on the 26th day after casing.

Figure 27. Number of cobweb pathogen conidia (strain 192b1) trapped per m³ of air every 15 minutes on the 29th day after casing.
four days existed from the time the conidia were liberated to the development of spotting symptoms.

All mushrooms harvested during the 2nd flush were spotted. This followed six days of heavy conidial loading of the air (Figure 21 & 22). In addition, spotting symptoms were more intense. Whereas spotted mushrooms from the 1st flush had only one or two spots per cap, 2nd flush mushrooms had far more (10+), indicating the higher spore load in the air during the interflush period.

When the spore-trap data was presented on a 15-minute basis rather than a 24-hour basis it was evident that the conidial loading of the air was not constant. For the best part of each day the air was relatively free of conidia, however, occasionally large numbers of conidia were liberated into the air (Figures 23, 24, 25, 26). The number of conidia trapped rose sharply, peaking after around 1 hour before falling with equal rapidity. The whole period of conidial build up and settling out took no more than three hours and often less than two (Figure 24). Conidia numbers during one of these events increased to over 30,000/m³ which equated to around 2.5 million within the cropping house (80m³ total) during that 15 minute period (Figure 25). Although such high numbers of conidia were unusual, 5,000 - 10,000 conidia/m³ was not (Figure 23).

The peaks of airborne conidia occurred when certain cropping operations were carried out. Whilst the relatively low background conidia loading of the air didn’t seem to follow any discernible pattern, the large peaks of loading always coincided with a period of salting, watering, or both. This is most obvious on day 26; one period of salting and watering and two periods of watering alone were each closely followed by a period of conidial loading of the air (Figure 26). The first application of salt, followed by watering, liberated the majority of conidia observed that day, with the subsequent two applications of water liberating fewer conidia.

Measuring and picking did not liberate as many spores as salting and watering. Relatively few conidia were liberated during the 29th day after casing when measuring and picking took place but salting and watering did not (Figure 27).

4.1.4 Discussion

In contrast to the findings of Gandy (1972) when studying Verticillium malthousei the seven-day volumetric spore trap has proved to be a useful tool for the monitoring of Cladosporium conidia within a mushroom cropping house. Due to the relatively contained nature of the mushroom cropping house a limited range of spores was trapped (Plate 7). Only A. bisporus spores, Penicillium spp., and cobweb pathogen conidia were trapped in any great numbers. Thus, identification was simplified. Identification was also helped by the distinct nature of cobweb pathogen conidia. Cobweb pathogen conidia are relatively large and septate, whereas A. bisporus and Penicillium spp. spores are smaller and non-septate. Identification of cobweb pathogen conidia, despite their distinct appearance, was however not always simple. During cropping periods A. bisporus spores were liberated, and trapped in such numbers they made identification of any other spores difficult, regardless of size or distinct appearance (Plate 8). One further point to be made with this technique’s suitability for this purpose is that conidial viability cannot be assessed. It can therefore not be categorically stated that each conidium observed had the ability to produce a new colony or spotting symptoms.
The importance of early disease-identification and containment in reducing the severity of disease symptoms was demonstrated clearly. Small, young disease colonies were shown to liberate enough conidia to cause quite significant spotting symptoms in the first flush. Also quite evident is the fact that if larger areas of sporulating disease colonies are watered over, it can cause the liberation of literally millions of conidia, leading to massive spotting. However, somewhat more worrying, is the discovery that salting, a process employed to contain this disease has a propensity to exacerbate the problem. The conidia liberating capabilities of this operation were highlighted by peaks of trapped conidia following salting, but also by a lack of airborne conidia if no salting was carried out. For example, during the 2nd flush when salting and watering ceased, a large reduction in the number of conidia liberated into the atmosphere was observed, despite the fact that the area of cobweb on the casing was increasing considerably. Consequently, the development of a non-disruptive but effective means of treating disease colonies on the casing is required.

4.1.5 Conclusions

- *Cladobotryum* conidia are liberated into the air in great abundance when disease colonies are disturbed by watering or salting without any protection
- Picking operations and other activities in the cropping chamber do not result in significant numbers of conidia being released.
- The vast majority of conidia settle out within a few hours of liberation
- Even low numbers of conidia/m³ of air can cause significant spotting symptoms
4.2 Spatial dispersal of cobweb disease pathogen conidia

4.2.1 Introduction

Several factors will affect the distribution of conidia within a mushroom cropping house including strength and direction of air movements, density of conidia, and aerodynamics of the conidia (Ingold, 1965). Of these factors it is the strength and direction of air currents which may vary most significantly during a crop and thus having the greatest effect on the distribution of conidia. For example, cobweb conidia of any given strain will remain relatively consistent with regards to size and shape whereas air currents can be far more variable. The strength and direction of air currents can not only change with an alteration of the fan speed but also with an alteration of house layout such as the movement of shelves or other equipment, the opening of doors, etc.

The objective of this study is to elucidate spatial patterns of cobweb conidial dispersal within a mushroom house. Several alternative means of trapping conidia will be examined and the effect of shelving on spore dispersal will also be investigated.

4.2.2 Materials and methods

4.2.2.1 Trapping techniques

Four trapping techniques were used to monitor conidial numbers in a mushroom cropping house where Cladosporium conidia were released from a single cobweb colony. The house contained a three-shelf unit and was laid out as shown in Figure 28.

a) Burkard Seven-Day Recording Volumetric Spore Trap

The setting up, maintenance and analysis of the spore-trapping tape from the Burkard spore trap followed the protocol outlined in section 4.1.2.1. The trap was activated the day before conidial liberation and allowed to run for the duration of the experiments (3 days).

b) Rotorods

Rotorod traps were developed by Perkins in 1957 and are based on the principle that small airborne particles are deposited with high efficiency on narrow cylinders (Gregory, 1951). The trap relies on two narrow vertical metal arms rotating quickly around an axis (Figure 29). The leading edge of each arm is covered with transparent tape coated with a petroleum jelly based adhesive (similar to that used for the Burkard spore trap) that traps small airborne particles and which can be later removed for microscopic examination.

Revolving at a constant 3100 rpm, the elastic properties of the brass arms ensures the vertical uprights rotate at 90° to the horizontal arms. After three hours of sampling the rotorods were stopped and the adhesive tape mounted onto a glass slide. Sterile forceps and scalpel were used to carefully remove the adhesive tape covering the leading edge of each arm, which because still tacky could be mounted directly onto a glass slide. Each length of tape was then covered with mounting gel (section 4.1.2.1) and cover slip. Following drying of the mounting gel the slides were microscopically examined. The number of conidia trapped per unit area of tape was calculated and recorded for each trap position.
Figure 28. Distribution of conidial traps on each of three shelves and position of cobweb disease and Burkard spore trap within the experimental mushroom house.

Figure 29. Rotorod apparatus used to trap cobweb conidia from the air at several trap positions within a mushroom house.
c) Mushrooms
Pots containing first-flush mushrooms at the small closed-cup stage of development, were used to provide information on the pattern of spotting symptoms which would develop, following the liberation of conidia within a cropping chamber. Immediately prior to the liberation of conidia, six pots of growing mushrooms were positioned on each of the three shelves as indicated in Figure 28. The diameters of all developing mushrooms at each position on each shelf were recorded and the mushroom surface-area for that position was calculated. The number of spots that subsequently developed was expressed per unit area of mushroom at the time that conidia were liberated.

d) Trap plates
Malt Extract Agar (MEA) (3% Oxoid) was prepared and sterilised according to the manufacturers recommendations. Upon cooling to 50°C, an aliquot of 1000 ppm liquid thiabendazole was added to the MEA to give a final concentration of amended MEA of 100 ppm thiabendazole. The amended MEA was then dispensed into Petri dishes and allowed to solidify overnight.

Before liberation of the cobweb conidia took place, 15 thiabendazole-amended plates were placed on the shelves as indicated in Figure 28 and marked with the position and time of exposure. Immediately prior to liberation of the cobweb conidia the plates were uncovered in a specific order and the time noted. Exactly 15 minutes later the exposed plates were covered (following the same order as for uncovering) and replaced with fresh thiabendazole-amended plates. These were also exposed immediately in the same order as the first plates and had also been marked with the position and time. Fresh plates were exposed every 15 minutes for three hours, giving 12 trap plates for each position. All exposed plates were incubated at 25°C. Any emerging fungal colonies were recorded on a daily basis and identified.

4.2.2.2 Conidial release
The liberation of cobweb conidia was brought about by introducing a pot of mushroom compost with developing first flush mushrooms, on which had been established a colony of cobweb measuring 150 mm diameter. The conidia were released by watering the cobweb colony using a tri-nozzle hose attachment that dispensed 40ml of water over a period of 4 seconds from a height of 30cm.

4.2.2.3 Shelving effects
Two experiments were carried out in order to ascertain the effects of shelving on the movement of air, and hence conidia, around the house. In the first experiment, conidia were released in an empty house with open shelves, allowing the free movement of air through the aluminium mesh shelves. In the second experiment, each shelf was covered with plastic sheeting simulating the presence of a mushroom crop. The various traps were positioned for each experiment as described in 4.2.2.1 and the two experiments were carried out on consecutive days.
4.2.3 Results

4.2.3.1 Individual trapping technique results

a) Burkard spore trap
Conidial peaks similar to those described in the previous section were clearly distinguishable at around the time of the controlled conidial liberation for both the open and closed shelf systems i.e. 08:10hrs on both days (Figure 30). Whilst the open-shelf peak coincided exactly with the liberation of conidia the closed-shelf peak appeared to occur slightly after the conidia were liberated (about 15-30 minutes). Additionally a smaller peak occurred just before the main peak for the closed-shelf experiment. This smaller peak occurred about 30-60mins before the controlled release of conidia and it coincides with the time when the pot containing the cobweb colony was brought into the house.

b) Rotorods
Conidial numbers trapped on each shelf from the open-shelf system followed the same broad pattern. The highest numbers of trapped conidia occurred close to the source with the bottom shelf trapping the highest number of all. As the distance from the source increased however, there was virtually no difference between shelves in the number of conidia trapped (Figure 31). This contrasted with the closed-shelf system, where there was very little difference in the number of conidia trapped with increasing distance from the source of liberation (Figure 32).

c) Mushroom trap crop
Mushroom spotting was heavy throughout the house following liberation of conidia in both open-shelf and closed-shelf systems (Figures 33 – 38). The number of spots per cm² of mushroom tissue in any single trap position was never less than 1.5 and frequently greater than 4.

d) Trap plates
The vast majority of conidia (≥90%) were trapped in first 15 minutes after liberation in both the open-shelf and closed-shelf systems (Figure 39). Each subsequent 15-minute period demonstrated an approximately ten-fold reduction in the number of conidia trapped. An hour and a half after liberation almost all conidia had settled out of the air in both systems. Numbers of conidia trapped in the open-shelf system were consistently lower in comparison to the closed-shelf system. This may be a reflection of the colony from which conidia were liberated rather than an effect of the two shelf systems examined.

Conidia were trapped at all positions on all shelves in both the open-shelf and closed-shelf systems (Figures 40 – 45). In the open-shelf system, there was a clear, though sometimes small, reduction in the number of conidia trapped with increasing distance from the source (Figures 40, 41 & 42), similar to the rotorod data in Figure 31. The number of conidia trapped with increasing distance from the source in the closed-shelf system was more homogenous (Figures 43, 44 & 45), again similar to the rotorod data.
Figure 30. Number of cobweb pathogen conidia (isolate 192b1) trapped in 15 minute periods by the Burkard Seven-Day Recording Volumetric Spore Trap following two controlled liberations at 08:10hrs on two consecutive days.
Figure 31. Number of cobweb pathogen conidia trapped using rotorods at different distances and heights from the point of conidial liberation in an open shelf system.

Figure 32. Number of cobweb pathogen conidia trapped using rotorods at different distances and heights from the point of conidial liberation in a closed shelf system.
Figures 33 (bottom), 34 (middle), & 35 (top). Distribution and intensity of spotting symptoms developing on mushrooms placed on three open shelves within a mushroom house following a controlled liberation from a single disease colony located at the front of the house.
Closed shelves – Top shelf

Closed shelves – Middle shelf

Closed shelves – Bottom shelf

Figures 36 (bottom), 37 (middle), & 38 (top). Distribution and intensity of spotting symptoms developing on mushrooms placed on three closed shelves within a mushroom house following a controlled liberation from a single disease colony located at the front of the house.
Figure 39. Total number of *Cladosporium* conidia trapped in 1 hour on trap-plates distributed throughout a mushroom house following a controlled release of conidia in either an open-shelf or closed shelved system.

Conidia were trapped at all positions on all shelves in both the open-shelf and closed-shelf systems (Figures 40 – 45). In the open-shelf system, there was a clear, though sometimes small, reduction in the number of conidia trapped with increasing distance from the source (Figures 40, 41 & 42), similar to the rotorod data in Figure 31. The number of conidia trapped with increasing distance from the source in the closed-shelf system was more homogenous (Figures 43, 44 & 45), again similar to the rotorod data.

**4.2.4 Discussion**

The most significant result to emerge from these data is that very high numbers of *Cladosporium* conidia were trapped throughout a cropping house following disturbance of a single patch of cobweb. All four trapping techniques demonstrated this. The mushroom spotting results clearly demonstrated the devastating effect of a single spore liberation event on mushroom quality with over four spots per cm² of mushroom cap area developing. These results therefore do not agree with Dar (1997) who suggested that air currents were only capable of dispersing cobweb conidia relatively short distances (75cm). A standard commercial airflow pattern within the growing room used in these experiments was clearly sufficient to distribute cobweb conidia throughout the room, up to at least 4 m away from the source.

A few dispersal patterns were apparent although the high number of conidia trapped throughout the house may have made the observation of distinct patterns more difficult. However, there was a distinct impression that the partitioning of the house by the inclusion of closed shelving resulted in a more even distribution of conidia throughout the house. When the shelves were open, and conidia allowed to fall freely to the ground, more conidia settled out close to the source as might be expected. However, a great many conidia were still carried throughout the house, up to 4 m away from the source to where the burkard spore trap was positioned.

The burkard and trap plates were deemed the most suitable techniques for further investigations of this type. The Burkard trap, despite small problems associated with its
Figures 40 (bottom), 41 (middle), & 42 (top). Number and distribution of conidia growing on trap plates positioned on three open shelves within a mushroom house following a controlled liberation from a single disease colony positioned at the front of the house.
Figures 43 (bottom), 44 (middle), & 45 (top). Number and distribution of conidia growing on trap plates positioned on three closed shelves within a mushroom house following a controlled liberation from a single disease colony positioned at the front of the house.
imprecise clockwork mechanism, allowed continuous monitoring of the atmosphere. Analysis of the spore-trap tape revealed an unexpected release of conidia when the cobweb colony was brought into the cropping house prior to the start of the closed-shelf experiment. This minor liberation of cobweb conidia, which was associated with moving the disease colony, would have been undetected by any of the other techniques. The ability to divide the recording tape into 15-minute periods also gives this technique a sensitivity that only trap plates can easily better. However, dividing the tape into 15-minute sections is also misleading as in reality each 15-minute section of tape is exposed for one hour as it passes the aperture through which conidia are drawn. Thus, the apparent gradual build up of conidia over a 45-minute period prior to the main peaks in Figure 30, although apparent on the Burkard tape, does not actually occur. This was highlighted by the results from the trap plates, which demonstrated that the majority of conidia settled out during the first 15 minutes following their release.

Previous results (section 4.1) regarding conidial release demonstrated the ease with which conidia are released by watering and salting activities. The results presented above now compound this problem by demonstrating that once released, conidia are distributed widely and in large numbers by the air-flow normally employed in commercial mushroom cultivation.

Thus, two target areas for further investigation have been highlighted. The first is to reduce the liberation of conidia and the second to limit the dissemination of conidia should liberation occur.

4.2.5 Conclusions

- *Cladobotryum* conidia that are released from a single source are quickly distributed throughout the cropping house.
- *Cladobotryum* conidia become quickly distributed throughout the house whether or not the system is open (i.e. single layer of bags) or contains barriers to movement (i.e. full shelves or stacked trays).
- Three methods of measuring conidial dispersal within a cropping house all gave comparative results.
4.3 Preventing conidial liberation and spread

4.3.1 Introduction

The results presented in the previous two sections indicate that *Cladobotryum* conidia are liberated most frequently and in greatest numbers when disease colonies are either accidentally watered over or intentionally salted. Whilst only more thorough disease-detection could reduce the impact of unintentionally watering over disease patches, perhaps the method of salting disease patches, used to kill off disease, could be modified to reduce the extent of conidial release. More gentle application of the salt and/or containment of the conidia once liberated are considered to be the possible techniques to reduce the magnitude of conidial release. Not treating disease patches is not really an option, although if they are left alone and undisturbed they will liberate only few conidia. An unchecked disease colony would rapidly grow and sporulate heavily making the accidental disturbance and concomitant conidial release a real possibility.

As demonstrated in the preceding two sections, *Cladobotryum* conidia are distributed throughout cropping houses in high numbers by the air currents routinely employed in commercial mushroom production. Velocities in the order of 10-20 cm/second are used to mix the air within the mushroom house and thereby prevent stratification of the atmosphere and associated temperature and relative humidity variation (van den Boomen, 1988; Noble & Gaze, 1993). These air currents are essential to homogenous environmental conditions within the mushroom house and are produced by high volume fans. The air is normally introduced into the cropping house through perforated ducting running along the length of the ceiling. The objective of this section is to discover if switching the fans off for a short period of time during, and after a controlled liberation, has any effect on the patterns of conidial dispersal already described.

4.3.2 Materials and methods

4.3.2.1 Experiment 1 – Effect of fan speed on conidial dispersal

The computer controlled air-circulation fans within mushroom cropping houses at HRI operate at about 25% capacity to give an air speed of 10-20 cm/second, which is fairly standard within the mushroom industry. Conidial liberation experiments were conducted with the fans set at normal speed (Fan on) or with the fans switched off (Fan off). The Burkard spore trap and trap plate methods were selected to trap conidia in these experiments. Each experiment was replicated three times.

A pot of mushrooms with a growing *Cladobotryum* colony, measuring on average 158 x 154 mm, was located on the middle shelf of a 3-shelf unit in a mushroom cropping house. Each shelf was covered with plastic to simulate the conditions when the shelves are full of mushroom compost, which would provide physical barriers to the movement of conidia. A Burkard spore trap was placed at the back of the house on the floor, and monitored and recorded as described in section 4.1.2.1. Trap plates were placed in 4 trapping positions on each shelf, 50 to 100 cm from the source of conidia, to compare conidial distribution between shelves (Figure 46). In addition 4 trap plates were positioned at increasing distance from the source on the middle shelf only to determine relative movement of conidia from the source (Figure 46). All trap plates were replaced at 15 minute intervals for a period of 1 hour after conidial release. Trap plates were prepared and recorded as described in section 4.2.2.1(d).
Figure 46. Position of trap plates on three shelves in a mushroom cropping house in order to study the dissemination of conidia within the house when the air circulation fan is on and off.

Figure 47. Position of trap plates on the middle shelf of a three shelf unit examining the dissemination of conidia following four different salting techniques.
The controlled liberation of conidia was achieved by watering over a cobweb colony with a tri-nozzled lance. A volume of 40ml of water was applied in four seconds from a distance of 30cm. Average cobweb colony diameters were 158 x 154 mm, but some variation occurred. All colonies used were sporulating heavily.

4.3.2.2 Experiment 2 – Effect of different salting techniques on conidial dispersal

Four different salting techniques were tested to determine their ability to liberate cobweb pathogen conidia:

I. **Salting** – Regular table salt applied directly to the disease, either using a trowel or by hand.

II. **Tissue salting** – Damp tissue (at least 3 cm larger in all directions than the colony area) placed over the disease colony before salting. The tissue is then pinned down around the edges with salt before the central area of tissue is gently covered.

III. **Air filtration** – Salting is conducted as in technique I but in addition the air above the salted area is constantly drawn through a fine filtration unit (Draper dust extractor – reference No. 2097D050N; Plate 10).

IV. **Tissue salting & dust extractor** – Tissue and salt are applied as in technique II in conjunction with air filtration as in technique III.

All four salting techniques were tested three times with the fan off and techniques II, III & IV were tested three times with the fan on. It was not considered necessary to re-test the simple salting technique (I) with the fan on as previous experiments have shown that liberation and distribution is both severe and widespread with this salting technique (see section 4.2.3).

As in the previous experiment, a pot of mushrooms with a growing Cladobotryum colony, measuring on average 158 x 154 mm, was located on the middle shelf of a 3-shelf unit in a mushroom cropping house. Each shelf was covered with plastic to simulate the conditions when the shelves are full of mushroom compost, which would provide physical barriers to the movement of conidia. A Burkard spore trap was placed at the back of the house on the floor, and monitored and recorded as described in section 4.1.2.1. Twelve trap plates were placed on the middle shelf only, at various distances from the source, to give an impression of conidial distribution around the source following the various salting techniques (Figure 47). Trap plates were exposed for 20 minutes in order to trap the majority of conidia following conidial release (see 4.3.2.1(d) & Figure 39). Trap plates were prepared and recorded as described in section 4.2.2.1(d).

4.3.3 Results

4.3.3.1 Experiment 1. Effect of the air circulation fan on conidial dispersal

The concentration of conidia in the air sampled by the Burkard spore trap at the back of the cropping house was much higher when the fan was on compared with when the fan was off (Figure 48). The vast majority of the conidia were trapped within the first two hours following conidial liberation, with very few conidia being trapped after this time.

More conidia were trapped at various positions on the shelves by trap plates when the fan was off than when it was on (Figure 49). The vast majority of these conidia were confined to the middle shelf where the source of conidia was located (Figure 50). In addition, 90% of them were deposited within 50 cm from the source of the conidia compared with > 30% when the
Figure 48. Number of conidia trapped/m$^3$ of air during a four-hour period following controlled liberation of conidia in a mushroom cropping house with the air circulation fan switched on or off. Spores trapped using the Burkard spore trap. Data are means of three replicate experiments.

Figure 49. Number of conidia trapped at 12 positions (4 on each shelf) in a 1 hour period following a controlled liberation of conidia with the air circulation fan switched on or off. Data are means of three replicate experiments.

Figure 50. Number of conidia trapped in 1 hour at four positions for each shelf following a controlled liberation of conidia with the air circulation fan switched on or off. Data are means of three replicate experiments.
fan was on (Figure 51). This more concentrated deposition around the source when the fan is off, would have reduced the concentration of conidia in the air that was to be eventually sampled by the Burkard spore trap located at the back of the room. Thus, when the fans are off, conidia are deposited in high numbers close to the source, which results in a lower concentration of conidia in the air throughout the rest of the house.

Whilst switching the fan off affected the dissemination of conidia it did not affect the rate at which conidia settled out of the air. As previous experiments had shown, settling out of conidia was rapid, with the majority (>90%) deposited on trap plates within 15 minutes of the controlled liberation (Figure 52).

![Graph of conidia trapped on trap plates at increasing distances from the source expressed as a percentage of the total number of conidia trapped (on the middle shelf only). Data are means of three replicate experiments.](image1)

**Figure 51.** Conidia trapped on trap plates at increasing distances from the source expressed as a percentage of the total number of conidia trapped (on the middle shelf only). Data are means of three replicate experiments.

![Graph of number of conidia trapped on trap plates at 15 minute intervals at 12 trap positions (4 on each shelf) following a controlled liberation of conidia with the air circulation fan switched on or off. Data are means of three replicate experiments.](image2)

**Figure 52.** Number of conidia trapped on trap plates at 15 minute intervals at 12 trap positions (4 on each shelf) following a controlled liberation of conidia with the air circulation fan switched on or off. Data are means of three replicate experiments.
4.3.3.2 Experiment 2. Effects of different salting techniques on conidial dispersal

Straightforward salting did not prevent conidia being liberated into the air throughout the cropping house whether the fan was off (Figure 53) or on (see Figure 23). However, very few conidia were trapped when salting of cobweb colonies was done in conjunction with tissue or air filtration (Figures 54, 55 & 56). With these techniques, results were similar when the fan was switched on or off.

Salting of a cobweb colony with the fan off resulted in the majority of conidia being deposited close to the source (Figure 57 and Plate 9), similar to the pattern described earlier for a controlled liberation of conidia (see Figure 51). However, very few conidia were trapped on trap plates when salting of cobweb colonies was done in conjunction with tissue or air filtration (Figures 58, 59 & 60). Results were also very good for these treatments when the fan was switched on (Figures 61, 62 & 63).

4.3.4 Discussion

It has been demonstrated in this section that if a cobweb colony is disturbed when the air circulation fan is on, the concentration of *Cladosporium* conidia in the air is fairly uniform throughout the house. If the fan is switched off, the majority of conidia will precipitate out close to the source but a small number will still get carried to other shelves as well as some distance (at least 1.5 m) from the source. Switching the fans off will therefore not prevent the dissemination of conidia within a house, and new colonies and spotting symptoms would be expected to occur with decreasing frequency at a distance from the original source. Similarly, straightforward salting of cobweb colonies will also result in significant numbers of conidia being dispersed throughout the house, which will also be concentrated around the source if the fan is switched off during this operation. However, a dramatic and almost total reduction in conidia distribution was achieved when salting was done in conjunction with either the use of tissues or air filtration irrespective of whether or not the fan was on or off.

Whilst the benefits from reducing the number of conidia liberated are obvious, the benefits to the grower of minimising dissemination by turning the fan off are more subtle. Firstly, localised dispersal ensures secondary colonies are more likely to develop closer to the original source, which in turn can makes their detection more efficient, and likely. By increasing the probability that secondary colonies will develop close to the original, efforts to detect them can be concentrated in these areas. Secondly, the ubiquitous dispersal of conidia when the fan is on results in the ubiquitous spotting of mushrooms; a phenomenon demonstrated clearly in section 4.2. If dispersal of liberated conidia can be minimised by switching the fan off during salting and/or watering, it is possible that the spotting damage inflicted upon that crop may also be minimised.

As well as showing that switching the fan off can reduce dispersal generally around the house these data also suggest that this action may give greater protection to upper shelves with no disease. Airborne conidia liberated on the middle shelf will, through the process of gravity, be deposited on the bottom shelf. However, without updrafts conidia will not be deposited on the upper shelf. The fact that some conidia were trapped on the top shelf implies that updrafts carrying conidia had occurred, even though the fan was off.
Fan OFF – Burkard spore trap

Figures 53 - 56. Number of cobweb conidia trapped using a Burkard spore trap following four different salting techniques when the air circulation fan was switched off. Data are means of three replicates.
Fan OFF – Trap plates

Figure 57

Technique I - Salting

Figure 59

Technique III - Air filtration

Figure 57 – 60. Number of cobweb conidia trapped on trap plates positioned on the middle shelf of a three shelf unit following four different salting techniques in conjunction with the air circulation being switched off. Cobweb colony was positioned in the centre of the shelf (see Figure 47). Data are means of three replicates.

Figure 58

Technique II - Tissue salting

Figure 60

Technique IV - Tissue salting & air filtration
Fan ON – Trap plates

Technique I – Salting

(Distribution similar to Figure 44)

Figure 61.

Technique II – Tissue salting

Figure 62

Technique III – Air filtration

Figure 63

Technique IV – Tissue salting & air filtration

Figures 61 - 63. Number of cobweb conidia trapped on trap plates positioned on the middle shelf of a three shelf unit, following three different salting techniques in conjunction with the air circulation fan being switched on. Cobweb colony was positioned in the centre of the shelf (see Figure 47). Data are means of three replicates.
The most suitable technique for application within the mushroom should be decided by practicality. Using this criterion it is felt that tissue salting is the most appropriate. This technique is effective, cheap, simple to use, and relatively rapid. Air filtration, although effective, simple to use, and even more rapid than tissue salting, would require the purchase and maintenance of a dust extractor, costing in excess of £400.

4.3.5 Conclusions

- When cobweb is prevalent, switching air circulation fans off during watering and straightforward salting operations should concentrate the incidence of subsequent disease or spotting symptoms to the areas closest to the initial location of the disease.
- Straightforward salting operations will result in conidia being disturbed and distributed around the cropping house.
- Salting in conjunction with the use of a tissue to initially cover areas of disease will dramatically reduce the dispersal of *Cladobotryum* conidia around the house.
4.4 Summary

The work described in this section on the dispersal of *Cladobotryum* conidia within a mushroom house clearly demonstrates that standard watering operations applied to a cobweb disease colony, or a straightforward salting of that colony, will result in a major release of *Cladobotryum* conidia. These conidia will then proceed to be dispersed throughout the house where they can cause spotting symptoms or new disease colonies to occur. The pattern of dispersal will vary depending on whether the air circulation fan is switched on or off, with conidia being more uniformly distributed throughout the house if the fan is on, or more locally distributed around the source, when the fan is off. Although conidial distribution can be more localised if the fan is switched off, small numbers of conidia will still succeed in moving to shelves above and below the source, as well as moving some distance from the original source. This widespread airborne dispersal of conidia following their disturbance emphasises the importance of early disease identification and isolation, if the spread of the disease is to be controlled.

Straightforward salting was shown to be virtually ineffective in preventing the spread of the disease. The technique MUST be used in conjunction with either (a) a tissue, which covers and contains the disease colony prior to salting, or (b) a hand held dust extractor fitted with a fine air filtration unit. If either of these techniques is used, then extremely few conidia are released into the air resulting in virtually no dispersal of disease propagules throughout the house.

4.5 General Conclusions

- *Cladobotryum* conidia are liberated into the air in great abundance when disease colonies are disturbed by watering or salting without any protection
- Picking operations and other activities in the cropping chamber do not result in significant numbers of conidia being released.
- The vast majority of conidia settle out within a few hours of liberation
- Even low numbers of conidia/m³ of air can cause significant spotting symptoms
- *Cladobotryum* conidia become quickly distributed throughout the house whether or not the system is open (i.e. single layer of bags) or contains barriers to movement (i.e. full shelves or stacked trays).
- When cobweb is prevalent, switching air circulation fans off during watering and straightforward salting operations should concentrate the incidence of subsequent disease or spotting symptoms to the areas closest to the initial location of the disease.
- Straightforward salting operations WILL result in conidia being disturbed and distributed around the cropping house.
- Salting in conjunction with the use of a tissue to initially cover areas of disease will dramatically reduce the dispersal of *Cladobotryum* conidia around the house.
5 Conclusions

Taxonomy:
- *Cladobotryum* isolates show significant variation in terms of their growth rate and conidial morphology.
- There is no relationship between the morphological data and the genetic RAPD data, suggesting that genetically similar isolates show a range of morphologies.
- The thiabendazole-resistant isolates associated with the cobweb epidemic in Britain in the early 1990's was genetically similar to *C. mycophilum*, but was morphologically different from current descriptions of this species.
- *Cladobotryum dendroides* appears to be less pathogenic than the thiabendazole-resistant isolates, the dominant isolate encountered during the cobweb epidemic in the early 1990's.

Biology:
- Statistical analysis of cobweb colony diameters at the end of the first flush suggest that the rate of sugar beet lime inclusion has a significant effect on the establishment of cobweb, with larger colonies occurring when high (30%) rates of sugar beet lime are used.
- Cobweb colonies were capable of significant growth over a wide range of matric potentials in casings that were very dry to periodically waterlogged.
- Drier casings slowed down the establishment of cobweb while wetter casings led to rapid establishment but subsequent growth rates were marginally higher in the drier casings (41 mm/day as compared with 34 mm/day).
- Management of casing matric potential in a bulk peat and sugar beet lime casing mix is unlikely to have any major effect on cobweb control.
- Casing is unable to support good growth and sporulation of *Cladobotryum* in the absence of developing mushrooms.

Epidemiology:
- When cobweb is prevalent, switching air circulation fans off during watering and straightforward salting operations should concentrate the incidence of subsequent disease or spotting symptoms to the areas closest to the initial location of the disease.
- Straightforward salting operations will result in conidia being disturbed and distributed around the cropping house.
- Salting in conjunction with the use of a tissue to initially cover areas of disease will dramatically reduce the dispersal of *Cladobotryum* conidia around the house.
6 References

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Appendix

Plate 1
Plate 2
Plate 3
Plate 4
Plate 5
Plate 6
Plate 7
Plate 8
Plate 9
Plate 10
Plate 1. Mushroom cap spotting caused by cobweb pathogen conidia.
Plate 2. Perithecia produced following the mating of isolate CC8 (C. dendroides Mt. b) with CC23 (C. dendroides Mt. a) under the conditions outlined in section 2.3.2. (Bar = 300μm).
Plate 3. Ascospore produced following the mating of isolate CC8 (*C. dendroides* Mt. b) with CC23 (*C. dendroides* Mt. a) under the conditions outlined in section 2.3.2. (Bar = 20μm).
Plate 4. Pathogenicity of four *Cladothyrium* spp. isolates recorded nine days after inoculation of an *Agaricus bisporus* crop (a = isolate 192B1, b = isolate CC10, c = isolate CC18, d = isolate CC4). Note extensive cobweb growth and sporulation in ‘a’, extensive growth but poor sporulation in ‘b’, poor growth and no sporulation in ‘c’, and no apparent growth or sporulation in ‘d’.
Formulation 3 = Fully spawn run compost with casings in casing layer.
Formulation 1 = No A. brassicae. Formulation 2 = Casings in casing layer only.

Plate 5. Covered colonization of three formulations of compost and casing containing various amounts of A. brassicae.
Plate 6. Cobweb pathogen conidia and conidiophores (isolate 192B1). (Bar = 20μm).
Plate 7. Limited range of particles trapped within a mushroom cropping house infected with a cobweb pathogen (isolate 192B1) when using a seven-day recording volumetric spore trap (Burkard). (Bar = 50μm).
Plate 8. High numbers of *A. bisporus* spores obscuring other particles making identification of those particles more difficult. (Bar = 50μm).
Plate 9. The physical disturbance of cobweb pathogen (isolate 192B1) conidia using salt granules such as those commonly used to cover cobweb colonies in a commercial situation.
Plate 10. Air filtration technique employed to reduce the number of cobweb pathogen conidia released into a mushroom house atmosphere during the salting process.