**Project Title** 

**Fungicide Profiles in Casing** 

**Project Number** 

M 30a

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**Previous Reports** 

None

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#### **Practical Section for Growers**

## **Background**

The Project M 30 (Grogan & Gaze, 1998) examined the efficacy of three fungicides Sporgon 50WP, Bavistin DF and Hymush, to control two isolates of cobweb which had different fungicide resistance profiles. During the course of that project casing samples were taken throughout the duration of the crops for each of the different methods of fungicide application.

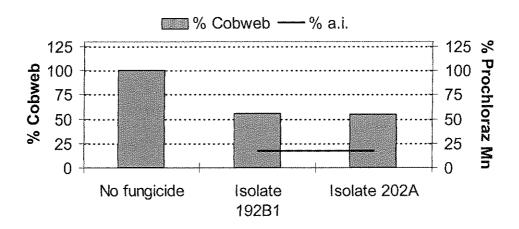
The objective of this project (M 30a) has been to determine the concentration of active ingredient in the casing over time and thus more fully understand the efficacy results described in project M 30.

#### Results

**Sporgon 50WP:** Most of the active ingredient was present in the upper half of the casing throughout the duration of the crop. However, the active ingredient was rapidly lost and by the end of the second flush had fallen to less than 20% of what had been applied. From previous experiments (M 14a, Grogan, Gaze & Scruby, 1996) the resistance data would indicate the expectation of moderately good control of both isolates by Sporgon 50WP. The project M30 indicated practical control to be only about 50%. The results of this project seem to indicate that this level of control is due to the combined effects of weak resistance and rapidly diminishing levels of Sporgon 50WP in the casing (Figure 1).

# Figure 1.

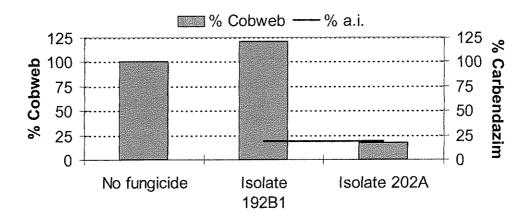
Area of cobweb present and amount of active ingredient remaining (% of total a.i. applied) at the end of 2nd flush following treatment with 2 x 120 g doses of Sporgon in 180 litres/100m<sup>2</sup>.



**Bavistin DF:** The levels of active ingredient dropped steadily throughout the crop, unlike Sporgon, where there was the opportunity to replenish levels with a second application. Previous results (M 30) showed that the partially resistant isolate 192B1 was not controlled by Bavistin SF whilst control of the sensitive isolate was largely achieved (Figure 2). The disappearance of active ingredient from casing was sufficiently rapid to make practical control of the partially resistant isolate difficult. This makes it appear, therefore, to be more resistant to the chemical than it actually is.

Figure 2.

Area of cobweb present and amount of active ingredient remaining (% of total a.i. applied) at the end of 2nd flush following treatment with  $1 \times 250$  g dose of Bavistin DF in  $200 \text{ litres}/100\text{m}^2$ .

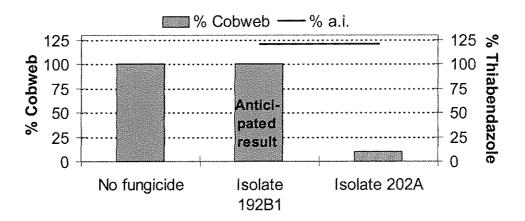


Where no fungicide is applied, the area of cobweb that develops is taken as 100%. The area of cobweb that develops for isolates 192B1 and 202A after the application of Bavistin DF is expressed as a percentage of the area of cobweb developed with no fungicide application.

Hymush: The number of application options for this chemical are sufficiently varied to make generalization a little difficult but high levels of active ingredient were maintained over a period of 30 days. Levels diminished only at the very end of the crop. Project M 14a showed that cobweb isolates were either highly resistant (192B1) or weakly resistant (202A) to this chemical. In cropping experiments, the weakly resistant isolate, 202A, was very effectively controlled by Hymush. Highly resistant isolates (like 192B1) would not be controlled however (Figure 3). It was therefore considered unnecessary to carry out an inoculation experiment to demonstrate that Hymush would be ineffective against this isolate.

## Figure 3.

Area of cobweb present and amount of active ingredient remaining (% of total a.i. applied) at the end of 2nd flush following treatment with 1 x 230 g dose of Hymush in 200 litres/100m<sup>2</sup>.



Thiabendazole levels are high. This could be due to experimental errors in application and extraction or due to unexpected activity in the casing.

# Action points for growers.

- In the light of the results from both projects M 30 and M 30a it would seem essential to establish the resistance profile of cobweb isolates on individual farms.
- If isolates are highly resistant to thiabendazole (Hymush) then the only effective fungicide currently available is Sporgon 50WP and it may only give 50% control.
- Due to rapid disappearance of Sporgon active ingredient following its application to casing, two additional factors should be considered:
- ⇒ Efficiency of application technique. Ensure you apply 100% of every dose to the area to be treated. Losses through run-off or under dosing mean even lower levels of active ingredient in the casing.
- ⇒ Hygiene measures. The contribution of good hygiene measures to gain control of early outbreaks of disease are of paramount importance.
- For those isolates shown to be thiabendazole sensitive two fungicides are theoretically available:
- ⇒ Bavistin DF can be expected to achieve good control but it should be aided by very careful application and stringent hygiene measures as control is likely to be reduced as levels of the fungicide in the casing fall.
- ⇒ Hymush will give much better control in later flushes due to higher levels of active ingredient in the casing. However this product is no longer available for purchase.
- A SOLA for another thiabendazole product would be helpful.

# **Practical benefits**

These results will enable a more efficient use of pesticides. They also indicate some important factors which need to be considered when choosing and using fungicides.

## 1. Introduction

Fungicides are used to reduce or eliminate the growth of fungal pathogens which may occur during the course of a mushroom crop. At present, there are four fungicides available for use on mushrooms, namely, Sporgon 50WP (prochloraz manganese), Bavistin DF (carbendazim), Hymush (thiabendazole) and Bravo 500 (chlorothalonil). There is little information available on the concentration of active ingredients in casing following the application of a fungicide treatments. With the evolution of fungicide resistant pathogens, it is important to be able to determine whether or not any loss of control experienced by growers is due (i) to the isolate in question being resistant, or (ii) to fungicide concentrations in the casing being insufficient. Fletcher et al (1980) demonstrated that benomyl, the active ingredient in Benlate, gradually disappeared from the casing during the cropping period, but that on one farm it disappeared within three days due to the presence of a high population of bacteria in the casing capable of degrading this fungicide. These results indicate that fungicide efficacy can be compromised by factors other than fungicide resistance and so there is a need to more fully understand the fate of fungicides in casings so that any potential problems, such as degradation can be addressed.

A previous HDC report, M 30, (Grogan & Gaze, 1998) examined the efficacy of three fungicides, Sporgon 50WP, Bavistin DF and Hymush, to control two isolates of cobweb, which had different fungicide resistance profiles. During the course of that experiment, casing samples were taken throughout the duration of the crop in order to determine the concentration of active ingredient present in the casing over time following different methods of fungicide application. This report presents these results and relates them to the efficacy results described in report M 30.

#### 2 Materials and Methods

#### 2.1 Crop details

Compost produced by the HRI Mushroom Unit was used for three cropping experiments. Compost no. 16/97 was used for the Hymush experiment and Compost 17/97 was used for the Sporgon 50WP and Bavistin DF experiments. Wooden trays, measuring 91cm x 61cm x 17cm (1 x b x h) were filled with 50 kg of spawned (Sylvan A12) compost and spawn run for 17 days at 25°C. Trays were cased to 4-5 cm with a black peat/sugar beet lime casing (Tunnel Tech English) and case-run at 25°C. One treatment in the Sporgon 50WP experiment used a sphagnum peat and sugar beet lime casing mix prepared by the HRI Mushroom Unit (300-litre bale Shamrock Peat moss; 75kg Bumpacrop lime; 50 litres water). Crops were aired on Day 7 after casing over a three day period, by which time the air temperature was reduced to 18°C. Two or three flushes were harvested from each crop, depending on the extent of disease development following the inoculation experiments being done at the same time (See report M 30).

## 2.2 Fungicides

The three fungicides Sporgon 50WP, Bavistin DF and Hymush were applied to individual crops during the course of a fungicide efficacy trial in 1997 (See report M 30). Details of the fungicides are sumarised in Table 1.

Table 1. Fungicides used in experiments

Fungicide	Active ingredient (a.i.)	% (a.i.)
Sporgon 50WP	Prochloraz manganese	46% w/w
Bavistin DF	Carbendazim	50%
Hymush	Thiabendazole	60%

## 2.3 Fungicide applications

Fungicides were applied at a number of different rates as outlined in Table 2. A separate cropping house was used for each fungicide as it was impossible to accommodate all fungicide treatments in one house. All rates used, except one, follow label recommendations. Bavistin DF applied in 100 litres/100m² is not on the label. This rate was included to see if it would concentrate the active ingredient in the upper layer of casing.

# 2.3.1 Incorporation into casing.

Bavistin DF was the only chemical for which an incorporation treatment was included. The calculated amount of Bavistin DF per batch of casing (equivalent to 250 grams/100m²) was added to 5 litres of water and incorporated as the casing was being mixed.

### 2.3.2 Casing drench.

Fungicides which were applied in the first watering after casing, or after the 1st or 2nd flush, were added to the fungicide tank at rates equivalent to those listed in Table 2. The fungicide solution was then drenched onto the cased mushroom trays using a hand held lance with a No. 2 Rose in conjunction with a 0.5 HP self-priming electric pump. The mushroom trays used had a surface area of 0.6 m<sup>2</sup> which required a fungicide doses as follows:

	1.1 litres/tray 0.55 litres/tray	(Sporgon 50WP at 120 g/180 litres/100 m <sup>2</sup> ) (Sporgon 50WP at 120 g/90 litres/100 m <sup>2</sup> )
	1.2 litres/tray 0.6 litres/tray	(Bavistin DF at 250 g/200 litres/100m <sup>2</sup> ) (Bavistin DF at 250 g/100 litres/100m <sup>2</sup> )
•	1.2 litres/tray 1.2 litres/tray 1.2 litres/tray	(Hymush at 230 g/200 litres/100m <sup>2</sup> ) (Hymush at 85 g/200 litres/100m <sup>2</sup> ) (Hymush at 115 g/200 litres/100m <sup>2</sup> )

**Table 2**. Fungicide treatments applied to three separate crops.

Fungicide Treatment		Rate of application	Time	Casing type
Sporgon 50W	/P (C	Crop 1)		
A		120 grams/100m <sup>2</sup> in 180 litres water	at casing	Black peat/sugar beet lime
	&	120 grams/100m <sup>2</sup> in 180 litres water	after 1st flush	
В	&	120 grams/100m <sup>2</sup> in 180 litres water 120 grams/100m <sup>2</sup> in 180 litres water	at 1st watering after 1st flush	Sphagnum/chalk
C		120 grams/100m <sup>2</sup> in 90 litres water	at 1st watering	Black peat/sugar beet lime
	&	120 grams/100m <sup>2</sup> in 90 litres water	after 1st flush	occi mne
Bavistin DF (	Crop	0 2)		
A		250 grams/100m <sup>2</sup> in 200 litres water	at 1st watering	Black peat/sugar beet lime
В		250 grams/100m <sup>2</sup> incorporated into mix	at casing	Black peat/sugar beet lime
C*		250 grams/100m <sup>2</sup> in 100 litres water	at 1st watering	Black peat/sugar beet lime
Hymush (Cro	p 3)			
A		230 grams/ $100$ m $^2$ in 200 litres water	at 1st watering	Black peat/sugar beet lime
В		230 grams/ $100$ m $^2$ in 200 litres water	at 1st watering	Black peat/sugar beet lime
	&	85 grams/ $100 \text{ m}^2$ in 200 litres water	after 1st flush	
	&	85 grams/100 m <sup>2</sup> in 200 litres water	after 2nd flush	
C		115 grams/100m <sup>2</sup> in 200 litres water	after 1st flush	Black peat/sugar beet lime
	&	115 grams/100m <sup>2</sup> in 200 litres water	after 2nd flush	·

<sup>\*</sup> This is not a label-recommended application

## 2.4 Determination of fungicide concentrations in casing

Samples of casing were removed at regular intervals during the cropping cycle, depending on the fungicide in question and the application times (Table 3). Five cores of casing (26 mm diameter, 50 mm approx. deep), were taken from each tray on each sampling day. The cores were split in half transversely to give 'top' and 'bottom' sub-samples which were frozen (-15°C) until analysed.

**Table 3.** Timing of casing samples for fungicide residue analysis

Fungicide Treatment	Day 0/1	Day 14	Day 21	Day 22	Day 28/29	Day 32	Day 35
AND THE TREE		Androgen Andronomo Normano Processor de constitucio de la constitució de la constitució de la constitució de l					
Sporgon 50WP			.,				
A: 120 g/180 litres Black peat	#		#	#	#		
B: 120 g/180 litres Milled Peat	#		#	#	#		
C: 120 g/90 litres Black peat	#		#	#	#		
<b>Bavistin DF</b>							
A: 250 g/100 m <sup>2</sup> Incorporated	#	#	#		#		
B: 250 g/200 litres	#	#	#		#		
A: 250 g/100 litres	#	#	#		#		
Hymush							
A: 230 g/200 litres	#		#			#	#
B: 230 g/200 litres	#		#	#	#	#	#
+ 85g/200 litres after 1st Flush							
+ 85g/200 litres after 2nd Flush							
C: 115g/200 litres aft 1st Flush				#	#	#	#
+ 115g/200 litres aft 2nd Flush		RAGINAN KONTON KANDON KAND		EPAPONO FINISIONA HARANANA MANAGATI ON CANA			

After defrosting the samples were weighed and mixed. Dry weights were determined by drying samples of casing to constant mass in a microwave oven. Residues were extracted from casing (20 g) with methanol (60 ml, hplc grade) by tumbling end-over-end for 1 hour. The extracts were filtered through filter paper (Whatman No. 5) before further analysis. Prochloraz manganese and carbendazim were analysed by high performance liquid chromatography using a Spectra Physics SP8810 pump, Cecil CE1200 uv detector and a 250 x 4.6 mm Spherisorb C8 column. The mobile phase was methanol:acetonitrile:water (60:20:20). The flow rate was set at 1.5 ml/min for prochloraz manganese and 1.25 ml/min for carbendazim giving retention times of 4.1 and 3.8 minutes, respectively. Detection was by uv absorbance at 220 nm.

Thiabendazole was analysed by gas chromatography using a Hewlett Packard 5890 gas chromatograph fitted with a split/splitless injector, 12 m x 0.53 mm SGE BPX5 column and a np detector. Injection was made in the splitless mode at 260°C. The detector was set at 250°C and the column temperature was raised from 85 °C to 235 °C at 40 °C/min giving a retention time of 4.7 min with helium carrier gas at 8 ml/min.

Analytical efficiencies were assessed by fortifying untreated casing samples with standard fungicide solutions in methanol at 5 - 100 mg a.i./kg dry casing. Recoveries were always in the range of 90-110% and results were not corrected for analytical loss.

# 2.5 Statistical design

Six replicate trays were prepared for each of the three treatments for each fungicide. Within each house, plots were positioned according to a randomized block design, consisting of 6 blocks with one replicate of each treatment per block. Data were analysed by Analysis of Variance (ANOVA) and the least significant difference between means was calculated according to the formula:

(lsd) = (sed) x 
$$t_{(p=0.05, d.f.)}$$

(lsd = least significant difference; sed = standard error of deviation; d.f. = residual degrees of freedom).

## 3. Results and Discussion

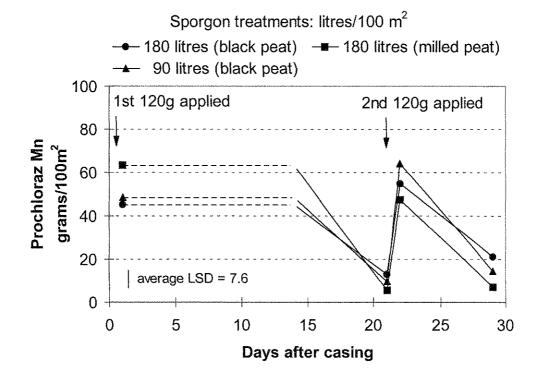
# 3.1 Sporgon 50WP (prochloraz manganese in casing)

Approximately 55g grams of active ingredient/100m<sup>2</sup> should be recovered from casing following a single 120 g product/100m<sup>2</sup> application of Sporgon 50WP to the bed area. The target volumes of fungicide per tray (100%) were achieved for both applications using 180 l/100m<sup>2</sup>. The target volumes for the 90 l/100m<sup>2</sup> treatment was under-delivered (78%) for the first application and over-delivered (118%) for the second application to give an average total delivery of 98%.

In this experiment, between 45 and 63g a.i./100m² were recovered after the first Sporgon 50WP drench application, with significantly more active ingredient being recovered from the milled peat casing type as opposed to the bulk black peat casing type (Figure 1). However, by the end of the first flush, day 21 after casing, levels in all casing types had fallen considerably to between 6 and 13g a.i./100m². Following the application of the second 120g application of Sporgon 50WP on Day 22, the amount of active ingredient present in the casing increased again to between 47 and 54g a.i./100m². However, levels dropped again by the end of the second flush to less than 20g a.i./100m².

Figure 1.

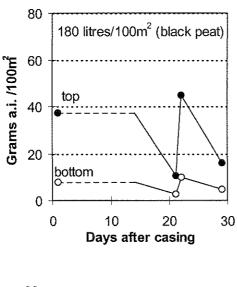
Total prochloraz manganese (a.i.) recovered from casing following 2 x 120 gram Sporgon 50WP applications in either 180 or 90 litres/100m<sup>2</sup>. Arrows indicate application times.

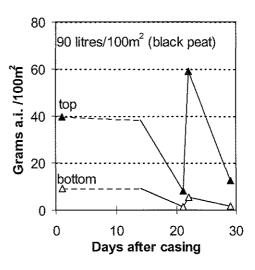


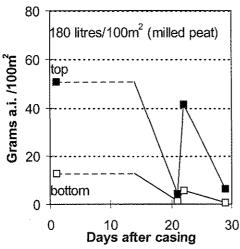
Most of the active ingredient was present in the upper half of the casing layer throughout the duration of the crop for all casing types and Sporgon 50WP treatments indicating that the active ingredient was not moving down the profile as the casing was being watered (Figure 2).

There were no consistent effect of one treatment over another in terms of active ingredient recovered throughout the experimental period due to variation in the amount of active ingredient recovered. Active ingredient was rapidly lost from all casing types and Sporgon 50WP treatments dropped by the end of the 2nd flush to a level of less than 20% of what had been applied.

Figure 2. Prochloraz manganese (a.i.) recovered from top and bottom layers of casing over time following different Sporgon 50WP treatments.





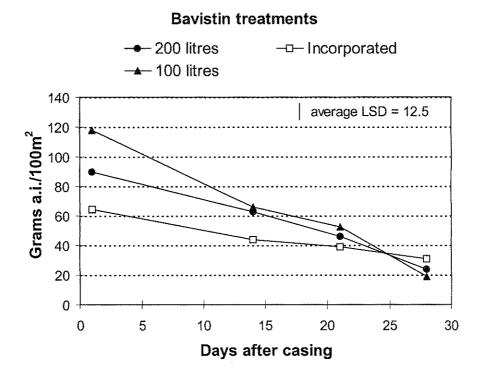


#### 3.2 Bayistin DF (carbendazim) in casing

Approximately 125 grams of carbendazim/100m<sup>2</sup> should be recovered from casing following a single 250g Bavistin DF application/100m<sup>2</sup>. The target volume for treatment A (250g/200 litres) was over-delivered to a level of 111% while the target volume for treatment C (250g/100 litres) was achieved (100%). Treatment B was by incorporation at mixing and the required amount/batch of casing was added (100%).

In this experiment there was considerable variation in the amount of carbendazim recovered following each treatment. Casing from the low volume treatment had 118g a.i./100m², just short of the expected value of 125ga.i./100 m² (Figure 3). Casing from the standard 200 litre volume treatment and the incorporation treatment had 90, and 64g a.i./100m², respectively, well below the expected value. The reasons for this are unclear, particularly the very low level recorded from the incorporation treatment which was about half of what was expected. The amount of carbendazim recovered from all treatments had dropped considerably by Day 14, and continued to drop further during the first and second flush.

**Figure 3.** Total carbendazim (a.i.) recovered from casing following the application of 250 grams Bavistin DF /100m<sup>2</sup> in various ways.

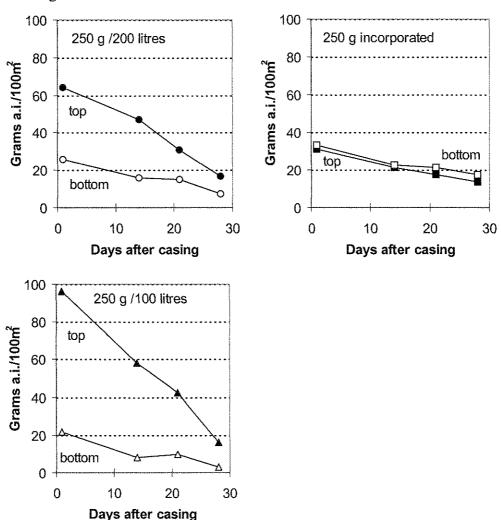


When the fungicide was incorporated during casing preparation there was an even distribution of the active ingredient throughout the profile as would be expected (Figure 4). When it was drenched on in the standard volume of 200 litres/100 m², 71% of the active ingredient was present in the top half of the casing layer, but when only 100 litres/100m² was used, a higher proportion (82%) of the active ingredient was always recovered from the top layer. This may be explained by the fact that the larger volume of water would move to greater depths, while the smaller volume would be more likely to be preferentially absorbed by the top layer.

While the amount of active ingredient recovered from all three treatments dropped considerably over time, there is some evidence to suggest that, when the fungicide had been applied in 100 litres/100m<sup>2</sup> compared with 200 litres/100m<sup>2</sup>, the disappearance from the upper layer of casing occurred more quickly (Figure 4). The rate of loss of active ingredient also appeared to be lower when the fungicide had been incorporated. The rate of loss from the bottom layer of the casing was similar for all three fungicide applications. This also indicates that the active ingredient was being removed by some process rather than being washed down the profile, in which case the levels in the bottom layer would rise as the levels in the top layer fell.

Figure 4.

Carbendazim (a.i.) recovered from top and bottom layers of casing over time following different Bavistin DF treatments.



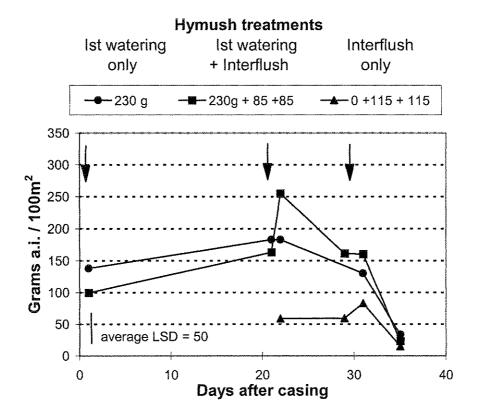
#### 3.3 Hymush (thiabendazole) in casing

Approximately 138 grams of thiabendazole/100m<sup>2</sup> should be recovered from casing following a single 230 g Hymush application. An additional 51 or 69 grams of thiabendazole /100m<sup>2</sup> should be recovered following an interflush Hymush application of either 85 or 115 grams, respectively. All treatments received between 100 and 104% of the target volume/tray.

In this experiment, between 100 and 138 grams of active ingredient was recovered from casing following a first application of 230 g Hymush/100m<sup>2</sup> on Day 1 after casing (Figure 5). This is in the region of what was expected although one set of trays appeared to have less thiabendazole present than another set of trays although both had received the same first application of 230 g Hymush/100m<sup>2</sup>, from the same tank of fungicide. This is likely to reflect variation in the distribution and application of the fungicide despite the fact that the target volume was fairly accurately applied. Levels of thiabendazole in the casing were still high by the end of the first flush at Day 21 and they either remained high, in the absence of further applications, or rose following second and third applications, until Day 32. The last sample was taken on Day 35 towards the end of the third flush by which time the casing was beginning to dry out. There was a significant reduction in the amount of active ingredient recovered from all treatments at this time.

Figure 5.

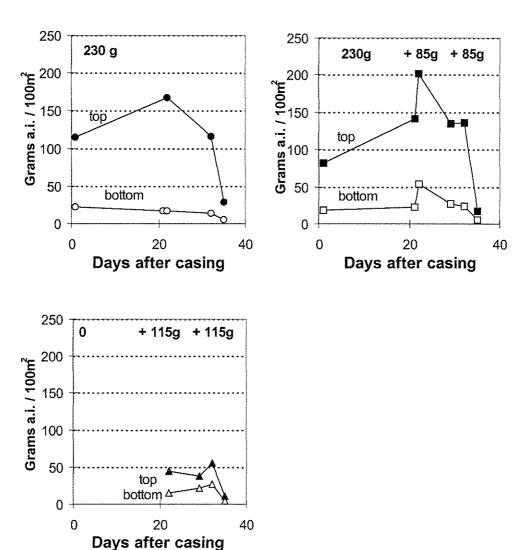
Total thiabendazole (a.i.) recovered from casing following different Hymush applications to 100m². Arrows indicate application times.



Most of the active ingredient (80-90%) remained in the top half of the casing layer following drench applications, except when only interflush applications were made. This treatment resulted in only 50-80% of the active ingredient remaining in the top layer (Figure 6). This may be due to the casing structure being less uniform following the harvesting of mushrooms.

Applying Hymush immediately after casing resulted in high levels of active ingredient being recovered throughout the cropping period up until the end of the third flush. The two 115 g interflush treatments (= 230 g total) did not result in as high a level of active ingredient as a single 230 g drench after casing. This is likely due to changes in the structure of the casing with time, especially the effects of drying out during cropping, which would also lead to a significant amount of runoff, and therefore loss, of the fungicide.

Figure 6. Thiabendazole (a.i.) recovered from the top and bottom layers of casing over time following different Hymush treatments/100m².



#### 3.4 Fungicides in casing and control of cobweb.

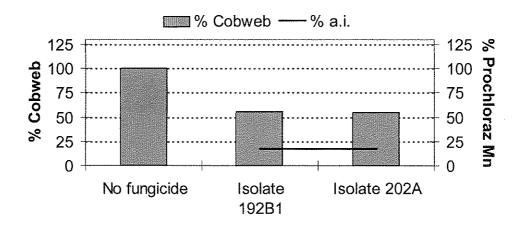
The fungicide data described in the previous pages was gathered during the course of an experiment which was examining the efficacy of the three fungicides Sporgon 50WP, Bavistin DF and Hymush to control two cobweb isolates, 192B1 and 202A, which had different fungicide resistance profiles (see HDC Report M 30). Isolate 192B1 contains the benzimidazole resistance mutation while Isolate 202A does not (Grogan & Gaze 1999, McKay *et al* 1998).

# 3.4.1 Sporgon and cobweb control

In *in vitro* tests, both cobweb isolates 192B1 and 202A were able to tolerate Sporgon 50WP at concentrations of 20 ppm of active ingredient (prochloraz manganese) and they were both considered to be weakly resistant to this chemical.

Figure 7 shows that by the end of the 2nd flush, cobweb symptoms of both isolates were reduced by almost 50% when Sporgon 50WP had been used. The fungicide data reported in the previous pages however indicates that the level of the active ingredient at this time was only 19% (approx.) of the total amount which had been applied indicating that the level of control being achieved is likely to be reflecting both the tolerance-level of the isolates to prochloraz manganese as well as the low levels of the active ingredient in the casing at a time when the pathogen is present. Anything which would enhance the retention of the active ingredient in the casing is likely to improve the level of control obtained.

Figure 7. Area of cobweb present and amount of active ingredient remaining (% of total a.i. applied) at the end of 2nd flush following treatment with  $2 \times 120$  g doses of Sporgon 50WP in 180 litres/100m<sup>2</sup>.

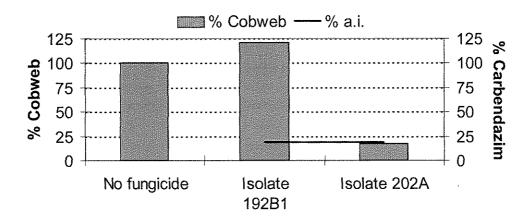


#### 3.4.2 Bayistin DF and cobweb control

Isolate 192 B1 grew well *in vitro* at concentrations of up to 5 ppm carbendazim (active ingredient in Bavistin DF). It possesses the benzimidazole resistance mutation but it is not totally resistant and growth is severely inhibited at carbendazim concentrations of 10 ppm and above. Isolate 202A does not possess the resistance mutation and is much more sensitive to this chemical with no growth occurring *in vitro* at 2 ppm or above.

Figure 8 shows that the cobweb symptoms caused by isolate 192B1 were not controlled by Bavistin DF at the end of the 2nd flush whereas there was an 80% reduction (approx.) in the amount of cobweb observed for isolate 202A, along with no spotting symptoms being recorded. The level of reduction was even higher than this as much of the cobweb on 202A treatments was due to cross contamination from heavily infected 192B1 plots however some 202A cobweb was confirmed from 202A plots at the end of the second flush. However, this is not surprising as the amount of carbendazim in the casing at this time had dropped to less than 20% so that even the very sensitive Isolate 202A was capable of growth. Bavistin DF is still a good chemical against isolates which do not have the resistance gene. Anything which would enhance the retention of the active ingredient in the casing is likely to improve the level of control obtained.

Figure 8. Area of cobweb present and amount of active ingredient remaining (% of total a.i. applied) at the end of 2nd flush following treatment with  $1 \times 250$  g dose of Bavistin DF in 200 litres/100m<sup>2</sup>.



Where no fungicide is applied, the area of cobweb that develops is taken as 100%. The area of cobweb that develops for isolates 192B1 and 202A after the application of Bavistin DF is expressed as a percentage of the area of cobweb developed with no fungicide application.

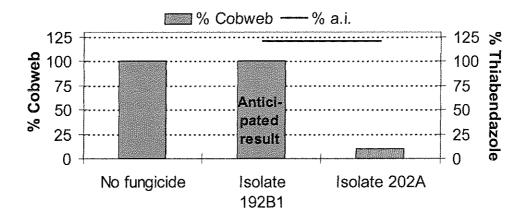
# 3.4.3 Hymush and cobweb control

Isolate 192B1 was totally resistant to thiabendazole (a.i. in Hymush) in *in vitro* tests, growing at concentrations of up to 500 ppm, and it was considered unnecessary to carry out an inoculation experiment to demonstrate that the chemical would be ineffective against this isolate. Isolate 202A however grew at concentrations of up to 10 ppm but not at 20 ppm or above, thus it was possible that Hymush would give some control of this isolate.

Figure 9 shows that Hymush reduced cobweb symptoms at the end of the second flush to 10% of what had developed in the absence of the fungicide. This level of control was also achieved in the third flush, thereby confirming that Hymush is an effective chemical against cobweb isolates which do not possess the resistance mutation.

Figure 9.

Area of cobweb present and amount of active ingredient remaining (% of total a.i. applied) at the end of 2nd flush following treatment with 1 x 230 g dose of Hymush in 200 litres/100m<sup>2</sup>.



Thiabendazole levels are high. This could be due to experimental errors in application and extraction or due to unexpected activity in the casing.

### 4. Conclusions

- 1. Levels of prochloraz manganese, (a.i. in Sporgon), dropped to low levels during the course of a mushroom crop to levels in the region of 20% of what had been applied. The reasons behind the reduction are not known but it could be a reflection of microbial decomposition or irreversible binding of the chemical to the peat as it dries out towards the end of each flush. This reduction in the amount of active ingredient in the casing is likely to reduce the effectiveness of the chemical in controlling any pathogens, in particular those which are able to grow *invitro* at low concentrations of the fungicide. Cobweb symptoms were reduced by 50% by the use of Sporgon during the course of this experiment. Greater retention of active prochloraz manganese in the casing is likely to give enhanced control and this is an area where future work could be directed.
- 2. Levels of carbendazim (a.i. in Bavistin) also decreased in the casing with time, to levels in the region of 25% of what had been applied by the end of the second flush. A cobweb isolate which was weakly resistant to carbendazim (isolate 192B1) was not controlled by this fungicide, but the low level of fungicide in the casing during the cropping period is likely to have rendered the fungicide treatment less effective. This is backed up by the fact that a carbendazim sensitive cobweb isolate (202A) was effectively controlled in the first flush but had begun to establish in the crop by the end of the second flush, by which time the level of carbendazim in the casing was much reduced. Microbial degradation has been implicated in the reduction in levels of another benzimidazole fungicide in casing, benomyl (a.i. in Benlate), and it is important to establish if this is also the reason behind the reduction in carbendazim levels, or if some other factor is responsible.
- 3. Levels of thiabendazole (a.i. in Hymush) remained high throughout the cropping period, unlike the other two chemicals tested. A cobweb isolate which was weakly resistant to thiabendazole (isolate 202A) was effectively controlled by this chemical well into the third flush and demonstrates that effective control of weakly resistant isolates can be obtained if fungicide levels in casing can be maintained. It would be very useful to know what factor affects the persistence of thiabendazole in casing as this is a very desirable trait for a fungicide to have. This is an area where future work could be directed.

# 5. References

- Fletcher, J.T., Connolloy, G., Mountfield, E.I., & Jacobs, L. (1980). The disappearance of benomyl from mushroom casing. *Annals of Applied Biology* 95, 73-82.
- Grogan H., Gaze R.H. & Scruby, A. (1996). I. Survey of fungicide resistance in the mushroom pathogens *Dactylium*, *Trichoderma* and *Aphanocladium*. *HDC Report M 14a*, 33pp. Horticultural Development Council, Bradbourne House, East Malling, Kent, ME19 6DZ.
- **Grogan H. & Gaze R.H. (1998).** Relationship between laboratory resistance and practical control of *Dactylium dendroides*. *HDC Report M 30*, 27pp. Horticultural Development Council, Bradbourne House, East Malling, Kent, ME19 6DZ.
- Grogan H. & Gaze R.H. (1999). Fungicide resistance among *Cladobotryum* spp. causal agent of cobweb disease of the edible mushroom *Agaricus bisporus*. *Mycological Research* (paper submitted).
- McKay, G.J., Egan, D., Morris, E. & Brown, A.E. (1998). Identification of benzimidazole resistance in *Cladobotryum dendroides* using a PCR-based method *Mycological Research*, **102**, 671-676.