A Report carried out for the HDC

by

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Contract No. M14a

Part I Survey of fungicide resistance in the mushroom pathogens *Dactylium*, *Trichoderma* and *Aphanocladium*

Part II The persistence of carbendazim (Bavistin) in casing during cropping

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Project Leader	:	Helen M Grogan
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Part II

Degradation of carbendazim in casing

Summary

The objective of this project was two fold. The main objective was to see if there were any signs of fungicide resistance among mushroom pathogems. Eighty-five isolates were tested of which 57 were *Dactylium* spp., 24 were *Trichoderma* spp. and 4 were *Aphanocladium album*. A number of isolates were found which were completely resistant to Hymush (a.i. thiabendazole) particularly among *Dactylium* isolates. The majority of isolates tested (*Dactylium, Trichoderma*) were partially resistant to Bavistin (a.i. Carbendazim) being capable of reduced growth at concentrations of up to 50 ppm, only *A. album* isolates showed significant resistance to Bavistin. The majority of isolates were also partially resistant to Sprogon (a.i. Prochloraz manganese) being capable of significant but reduced growth at 20 - 50 ppm. These results are based on laboratory tests but there are indications that some *Dactylium* isolates are more resistant to Sporgon in the casing than others.

A second objective of this project was to determine what happened to carbendazim (a.i. in Bavistin) following its incorporation into, or drenching onto, casing. The results indicated that a good distribution of a.i. was obtained when the product was mixed into the casing at preparation but when drenched on that it remained in the top layer only. Additionally, the active ingredient decreased with time to the point where very little was detected at day 18 after casing when it was drenched on and at day 27 when mixed in. This information must be taken into account when considering the implications of the results of the resistance testing.

Action points for growers:

1. If fungicides appear to be ineffective in controlling disease outbreaks the isolate should be tested for resistance to commonly used fungicides. This can help to determine if resistance is a factor in loss of control and also identify the most effective fungicides.

2. Check to see that the correct fungicide dose is being applied and, if drenching that an even distribution of the drench is being applied.

3. If symptoms occur in the second or third flush only, use a fungicide approved for inter-flush application.

Summary Table of Results

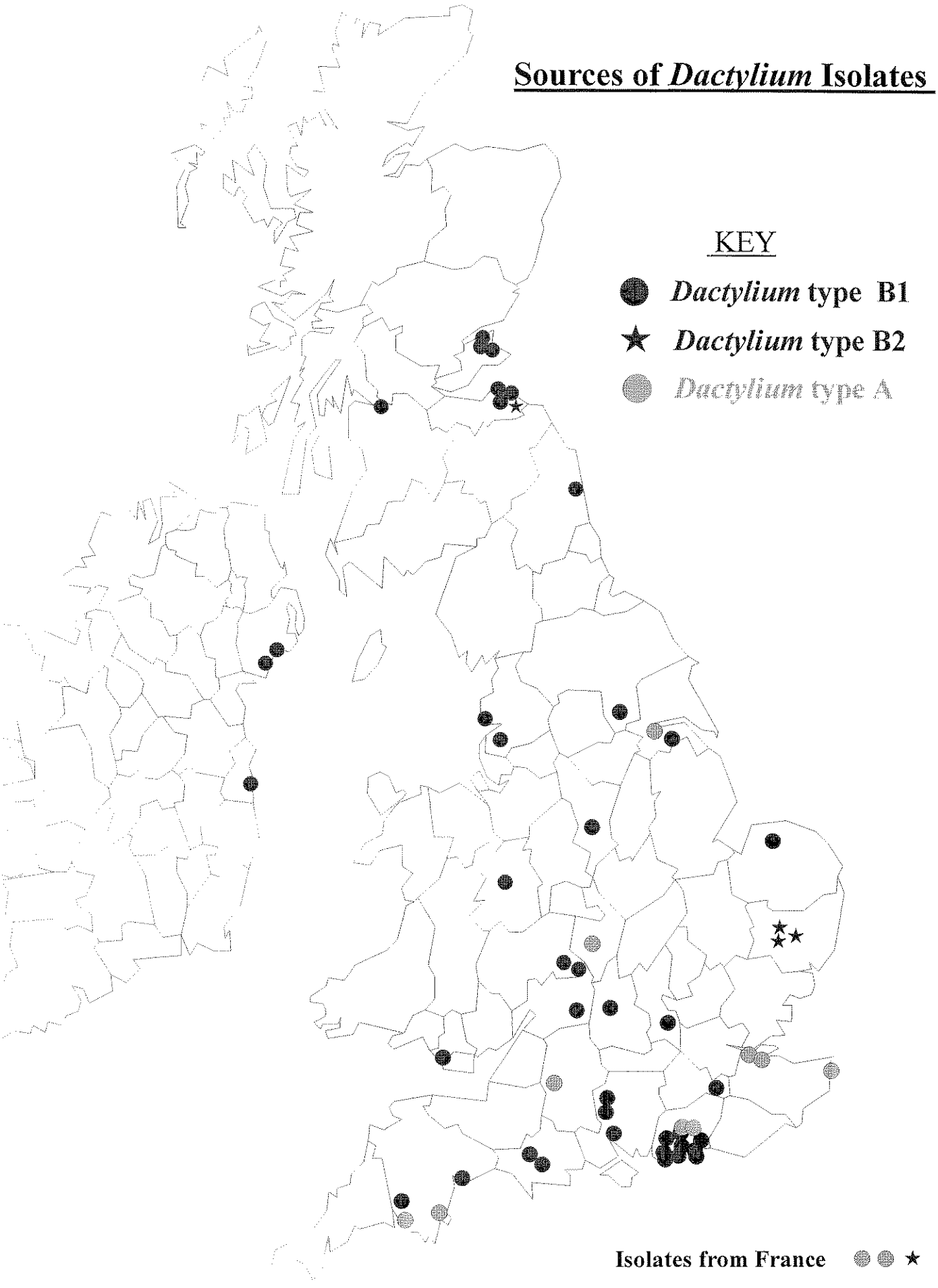
Summary of fungicide resistance profiles for various mushroom pathogens

Organism (number of isolates)	Hymush (a.i. thiabendazole)	Bavistin (a.i. Carbendazim)	Sporgon (a.i. Prochloraz manganese)
Dactylium dendroides			
Group A (11)	Partially Resistant*	Sensitive	Partially Resistant (more variable?)
Group B1 (41)	Resistant	Partially Resistant	Partially Resistant
Group B2 (5)	Partially Resistant	Sensitive	Partially Resistant (more sensitive?)
Trichoderma			
T. harzianum (14)	Sensitive ** (some Partially Resistant)	Sensitive ** (some Partially Resistant)	Partially Resistant (some more sensitive?)
T. viride (4)	Partially Resistant	Sensitive	Partially Resistant
T. koningii (1)	Partially Resistant	Sensitive	Partially Resistant
T. longibrachiatum/ pseudokoningii (3)	Sensitive	Sensitive	Partially Resistant
T. hamatum (1)	Sensitive	Sensitive	Sensitive
Gliocladium virens (1)	Partially Resistant	Sensitive	Sensitive
Aphanocladium album (4)	Resistant	Resistant	Partially Resistant

* Partially resistant: growth inhibited at concentrations of 50 ppm active ingredient

** Majority of isolates tested were sensitive.

Sources of *Dactylium* Isolates



Part I - Fungicide resistance among mushroom pathogens

1. Introduction

An increase in mushroom disease levels and growing concern in relation to fungicide resistance prompted the commissioning by the HDC of this study on the incidence of fungicide resistance among the mushroom pathogens *Dactylium, Trichoderma* and *Aphanocladium*. The near epidemic levels of *Dactylium* during 1995 confirmed the wisdom of this decision. There are few fungicides approved for use on mushrooms and since the development of resistance to beonomyl by *Verticillium* (Bollen & Zaayen, 1975; Gaze & Fletcher 1975) the threat of fungicide resistance among other pathogens is a very real one. There is a need to be aware of any changes in fungicide resistance profiles so that one can prepare a strategy to cope with the situation where there is a total loss of fungicidal control. There are already a number of cases where pathogens (of other crops) have developed resistance to carbendazim, thiabendazole and prochloraz following intensive use over an extended period (Eckert 1990; Hollmon *et al.*, 1990). Fletcher & Jaffe (1993) screened a number of mushroom pathogens for fungicide resistance and indicated that with respect to *Dactylium*, isolates from only one out of 16 farms were resistant to thiabendazole while the situation was unclear for prochloraz manganese. With respect to *Trichoderma* sp. some isolates were resistant to benomyl, thiabendazole or prochloraz manganese but only two isolates were resistant to all three. The purpose of this current survey is to reassess the fungicide resistance situation by looking at pathogen isolates from around the country.

2. Materials and Methods

2.1 Fungicides

The three fungicides Sporgon, Bavistin and Hymush were used throughout this study. The concentration of active ingredient in each product is given in Table 1.

Table 1. Active ingredient present in fungicides

Fungicide	**Active ingredient**	
Sporgon	Prochloraz manganese	50% w/w
Bavistin DF	Carbendazim	50% w/w
Hymush	Thiabendazole	60% w/w

A stock solution of each fungicide was prepared to give a concentration of active ingredient of 1,000 parts per million (ppm). Fresh stock solutions were prepared for each new batch of medium. A greater range of stock solutions were prepared for use in the ED50 experiments. These included stock concentrations of 10, 100, 500, 1,000 and 10,000 ppm.

2.2 Growth medium

Standard Malt Extract Agar (Oxoid) was used throughout the experiments. The autoclaved medium was allowed to cool to 50°C prior to the addition of the fungicides. Volumes of fungicide stock solutions were added to give a range of concentrations from 1 to 50 ppm for initial experiments. A selected number of isolates were subsequently grown on media containing fungicides at concentrations ranging from 0.01 to 500 ppm active ingredient.

2.3 Isolates

A total of 57 *Dactylium* isolates, 22 *Trichoderma* isolates and 4 *Aphanocladium* isolates were obtained from various farms and research institutes in the UK, France and Ireland (Table 2).

Table 2. Number and origin of isolates examined

Organism	**Country of Origin**			
	GB	NI	France	Ireland
Dactylium	51	2	3	1
Trichoderma	20	-	2	-
Aphanocladium	4	-	-	-

Of the 51 *Dactylium* isolates from Great Britain, 39 were obtained as single isolates from 39 farms. The remaining 12 isolates were obtained as multi isolates from 5 farms (Table 3).

Table 3. Farms providing more than one isolate

Farm	**Isolate Ref. No**	**No. of Isolates**
A	193	3
B	202	2
C	215	2
D	217	3
E	229	2

All isolates were subcultured onto malt agar for use in experiments. Slopes of all cultures were maintained at 4°C during the experimental period and cultures of all isolates were placed in the HRI culture collection in liquid nitrogen.

2.4 Experimental procedures

2.4.1. Fungicide resistance profiles

Fungicide resistance profiles were determined for each isolate with respect to the three active ingredients prochloraz manganese, carbendazim and thiabendazole. Isolates were grown on media containing 0, 1, 2, 10, 20 or 50 ppm active ingredient and growth along two radii was measured daily over a period of up to 21 days. Growth at each concentration of active ingredient was plotted against time in order to determine the period of linear growth. The growth during this period was used to calculate the linear growth rate. The linear growth rate at each concentration of active ingredient was expressed as a percentage of the control growth rate. The resulting data were used to give a broad estimation of the ED50 value.

2.4.2 ED50 determination

Two sub-groups of *Dactylium* isolates were identified on the basis of their growth characteristics. The resistance profiles obtained from 2.4.1 above suggested that some isolates within the sub-groups had different resistance profiles. Since isolates were examined over a period of about six months further tests were done to compare a number of isolates together and to increase the range of fungicide concentrations tested based on the initial results. Isolates were grown on media containing different concentrations of active ingredient over the range 0.01 to 500 ppm. In view of the relatively fast growth rate of many isolates on control media, Petri-dishes were inoculated to one side of the plate and growth was measured along one radius.

In addition to comparing isolates under the same experimental conditions the data from the above experiment was used to calculate the ED50 value for each isolate/fungicide combination. The ED50 value is the concentration of fungicide required to reduce the growth of the organism by 50%. In this study the ED50 value was calculated using growth rate data. The growth rates of the isolates were calculated for each concentration of fungicide tested and expressed as a percentage of the control growth rate.

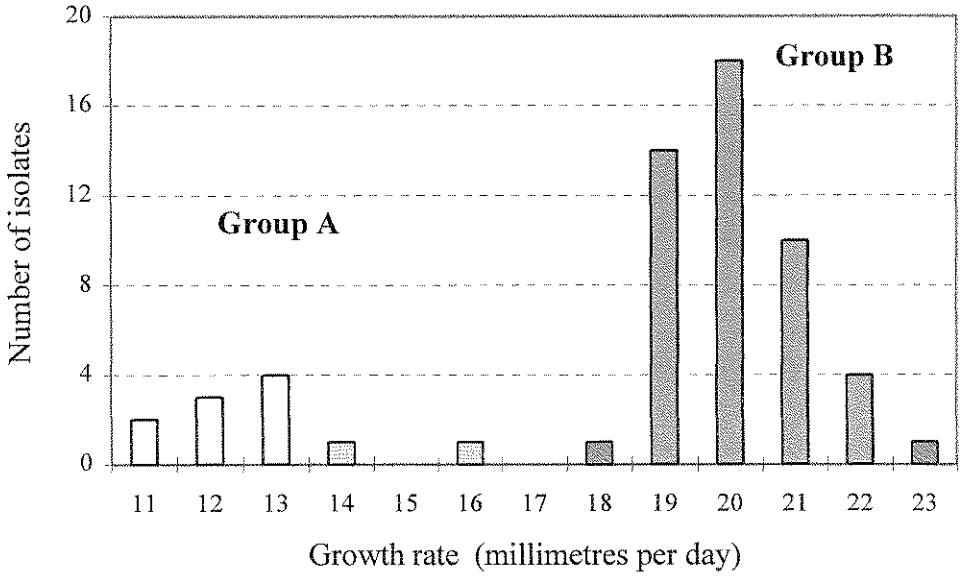
3. Fungicide resistance among *Dactylium* isolates

3.1 Growth of Dactylium isolates

The growth rates of the 57 *Dactylium* isolates examined ranged from 11 to 23.1 millimetres per day (Figure 1). Growth rates fell into two main groups. A small group of 11 isolates had growth rates of between 11 and 16 mm/day and were designated as Group A. A larger group of 46 isolates had growth rates of between 18 and 23 mm/day and were designated as Group B. Many (but not all) of the isolates with the slower growth rate (Group A) produced a distinctive odour, grew more restrictedly on nutrient media, and produced mainly one and two celled spores. These isolates have been tentatively identified as *Cladobotryum (Dactylium) mycophilum* but further studies are required to determine more fully the taxonomic relationship between the two groups of isolates.

Figure 1.

Control growth rate of all *Dactylium* isolates at 25° C



3.2 *Growth response of Dactylium isolates to fungicides*

3.2.1 Thiabendazole

Of the 46 faster growing Group B isolates tested 41 were resistant to thiabendazole with in excess of 75% growth being recorded at 50 ppm (Figure 2). This large sub group was designated as Group B1. The five remaining Group B isolates were more sensitive to thiabendazole with significant growth inhibition occurring at 2-10 ppm. These were designated as Group B2. The 11, slower growing, Group A isolates were also sensitive to thiabendazole, responding in a way similar to Group B2 isolates.

Multi-isolates taken from a number of farms gave thiabendazole growth response curves of only one type.

3.2.2 Carbendazim

Two growth response curves were identified for Dactylium isolates grown in the presence of carbendazim (Figure 3). The 41 Group B1 isolates were capable of 80-100% growth at concentrations of 1-2 ppm but severe growth inhibition occurred at concentrations of 10 ppm or higher. The 5 Group B2, and the 11 Group A isolates were much more sensitive to carbendazim with severe growth inhibition occurring at 1 ppm.

Multi-isolates taken from a number of farms gave carbendazim growth response curves of only one type.

3.2.3 Prochloraz manganese

The growth responses of all isolates to prochloraz manganese varied considerably with no clear patterns emerging in constrast to the responses observed for thiabendazole and carbendazim. There was significant variation in the growth of cultures at low concentrations both within and between experiments but at concentrations of 10 ppm or higher there was some degree of uniformity (Figures 4 and 5). On this basis Group B1 isolates appeared to

Figure 2.

Response of *Dactylium* isolates to Thiabendazole

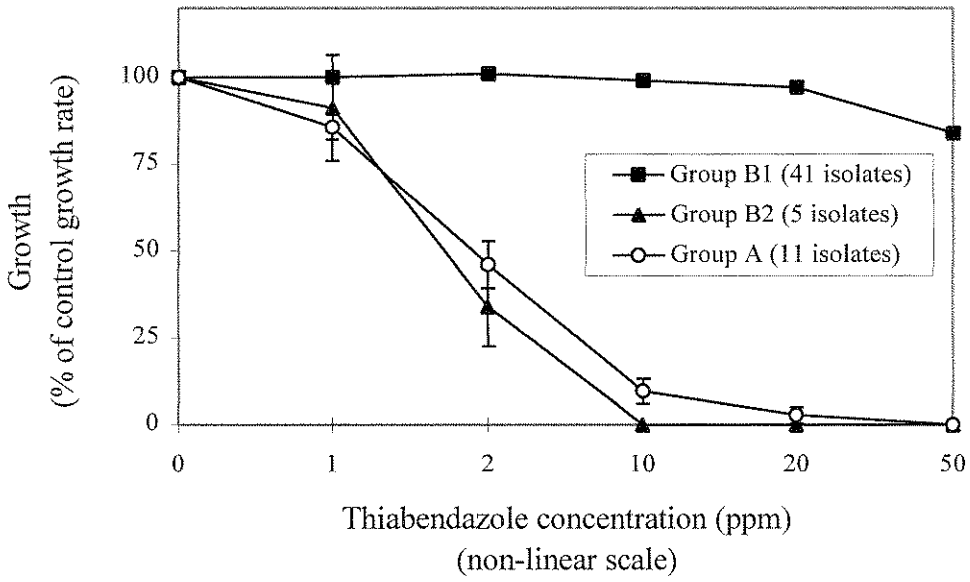


Figure 3.

Response of *Dactylium* isolates to Carbendazim (Bavistin)

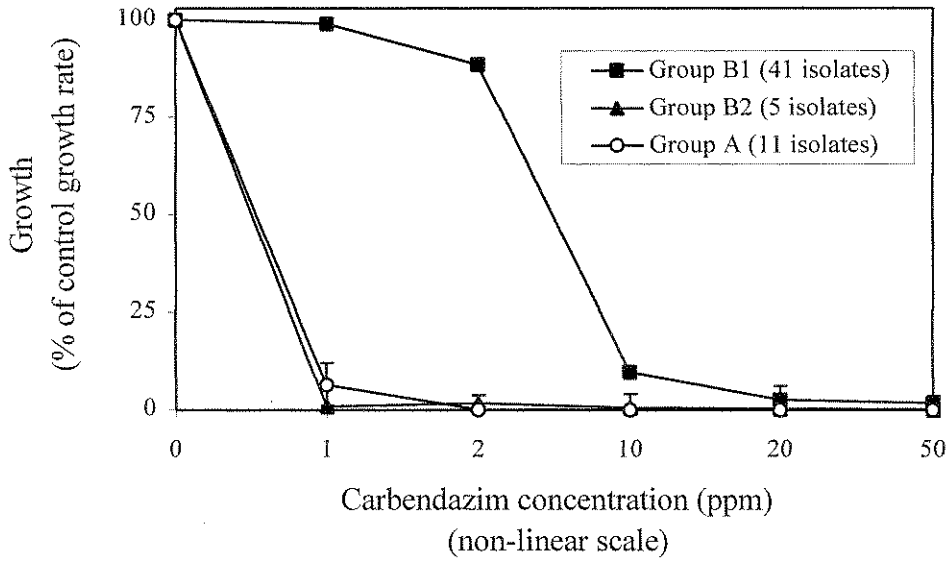


Figure 4.

Response of two *Dactylium* isolates from one farm to Prochloraz manganese (Sporgon)

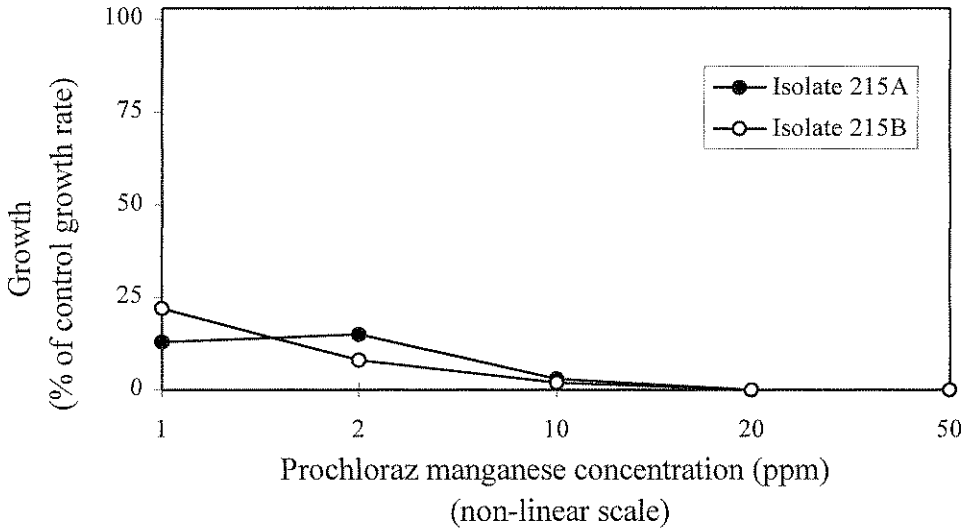
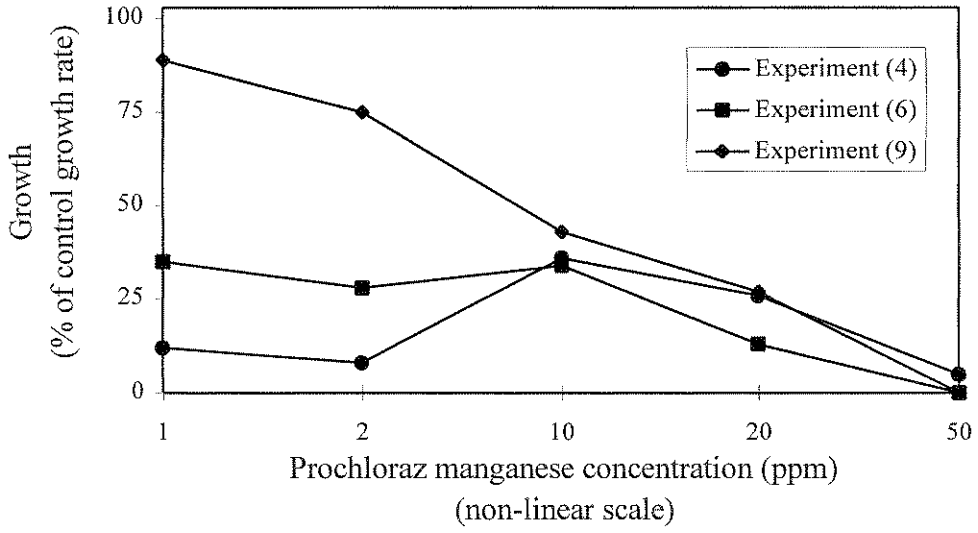


Figure 5.

Response of *Dactylium* 192B1 to Prochloraz manganese (Sporgon)



be roughly grouped into those which were largely sensitive to prochloraz manganese at concentrations of 10 ppm or above (Figure 4) or those which showed some resistance to prochloraz manganese at concentrations of 10-20 ppm (Figure 5). Within these "groupings" however there was wide variation. A number of isolates from both "groupings" were re-examined together in order to ascertain if the "groupings" were valid or not. Particular attention was paid to reducing experimental variation to a minimum. The results from this second experiment indicated that the groupings were false and that the responses of 7 selected Group B1 isolates to prochloraz manganese were similar when compared under exactly the same experimental conditions (Figure 6). Isolates which appeared to be sensitive to prochloraz when initially screened showed a similar growth response to isolates which initially appeared to have some resistance. The repeat experiment indicated that Group B1 isolates were capable of significant growth in the presence of prochloraz manganese at concentrations of up to about 1 ppm but at higher concentrations growth was significantly inhibited. Growth at 10 and 20 ppm was still quite significant ranging from about 5-30% of control and growth at higher concentrations was recorded for some isolates.

The growth responses of the (thiabendazole-sensitive) Group B2 isolates showed some variability in the initial experiments but this variability was maintained when isolates were compared in a second experiment (Figure 7). Group B2 isolates appeared to be more sensitive to prochloraz manganese than Group BI isolates, with significant inhibition of growth at 1 ppm.

The growth responses of the slower growing Group A isolates to prochloraz manganese were variable in the initial experiments and this variability was maintained when selected isolates were compared in a second experiment (Figure 7).

Figure 6.

Response of selected *Dactylium* Group B1 isolates to Prochloraz manganese (Sporgon)

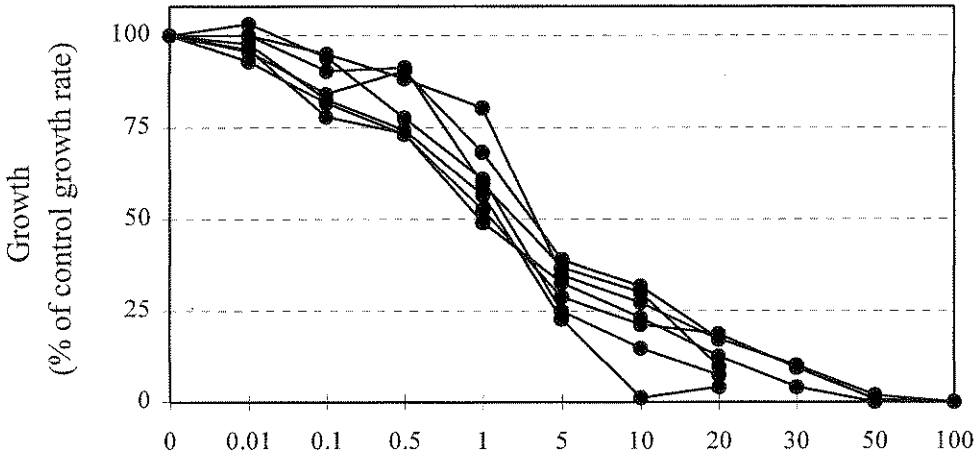
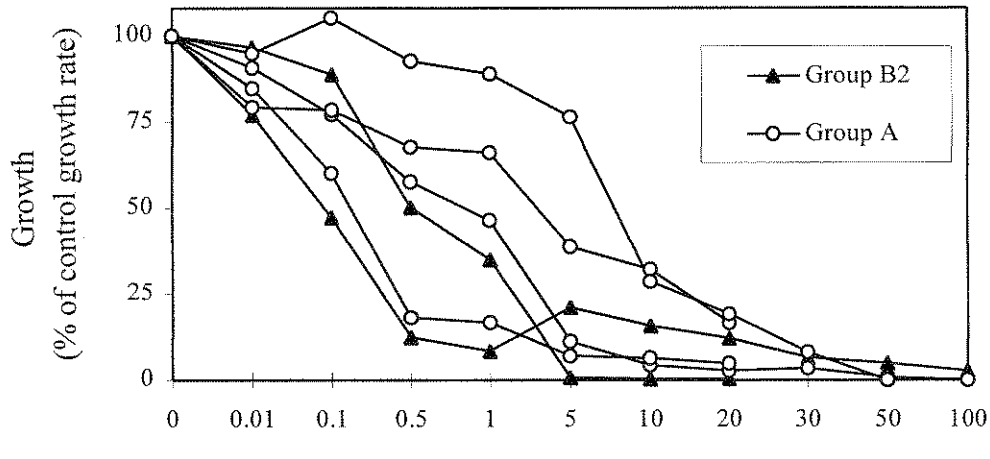


Figure 7.

Response of selected *Dactylium* Group B2 and Group A isolates to Prochloraz manganese (Sporgon)



3.3 ED50 Values

Table 4 lists the concentrations of the three active ingredients tested, thiabendazole, carbendazim and prochloraz manganese, at which the growth rate of selected *Dactylium* isolates was reduced by 50% compared to the controls. Group B1 isolates all had similar ED50 values with respect to the three active ingredients tested. All B1 isolates had an ED50 of >500 ppm for thiabendazole, 4-8ppm for carbendazim and 1-5ppm for prochloraz manganese. Group B2 and Group A isolates had much lower ED50 values with respect to thiabendazole and Carbendazim but ED50 values for prochloraz manganese were more variable. The two B2 isolates tested were more sensitive to prochloraz manganese than the B1 isolates while none of the Group A isolates had the same ED50 value. Some Group A isolates were more sensitive to prochloraz manganese while others were less sensitive.

Table 4

ED50 Values for Selected *Dactylium* isolates

Group	Isolate	Thiabendazole	Carbendazim	Prochloraz
B1	169	> 500	4 - 8	1 - 5
	192B1	> 500	4 - 8	1 - 5
	213	> 500	4 - 8	1 - 5
	214	> 500	4 - 8	1 - 5
	215	> 500	4 - 8	1 - 5
	239	> 500	4 - 8	1 - 5
	245	> 500	4 - 8	1 - 5
B2	193 A	≈ 2 - 5	0.1 - 0.5	0.5 - 1
	241 C	1 - 2	0.1 - 0.5	< 0.1
A	195A	5 - 10	0.1 - 0.5	1 - 5
	220B	2 - 5	0.1 - 0.5	5 - 10
	220D	2 - 5	0.1 - 0.5	0.1 - 0.5
	222	1 - 2	0.1 - 0.5	0.5 - 1

3.4 Discussion

Dactylium dendroides Group B1 isolates

Thiabendazole (Hymush). The results presented above indicate that of the 51 *Dactylium* isolates collected from around Britain, 38 of them (75%) had similar growth rates and fungicide resistance profiles. This large group of isolates, designated Group B1, were resistant to thiabendazole, the active ingredient in Hymush. Concentrations of 500 ppm had only a slight effect on the growth rate. Considering that the theoretical concentration of thiabendazole in casing following application of one dose is in the region of 30 - 40 ppm this product is unlikely to have any effect on Group B1 isolates.

Carbendazim (Bavistin). Carbendazim, the active ingredient in Bavistin had a greater effect on Group B1 *Dactylium* isolates than Hymush, with a concentration of 4-8 ppm causing a 50% reduction in growth rate. Growth was more severely inhibited at concentrations of between 10 and 50 ppm. The theoretical concentration of carbendazim in casing following the application of a single dose is in the region of 30 - 40 ppm. While such concentrations severely reduced the growth rates of B1 isolates to less than 5% of the controls, some growth and sporulation did occur with some isolates. These results lead to the conclusion that Group B1 *Dactylium* isolates are partially resistant to carbendazim - at least under laboratory conditions. What relationship this has to farm conditions remains to be tested and this may form part of a future HDC research proposal. The results presented in Part 2 of this report indicate however that the concentration of carbendazim in the casing decreases during cropping to a very low level by the end of the first flush. This would imply that there may not be any chemical present to control later outbreaks of the disease. The report also finds that very little carbendazim, if any, penetrates to the bottom of the casing if it is applied as a drench and, again, we do not know if this is significant in the control, or rather lack of control of *Dactylium* outbreaks. Clearly much more work is needed to clarify the situation and to perhaps critically review the recommended method and timing of Bavistin application.

Prochloraz manganese (Sporgon). Group B1 *Dactylium* isolates were also partially resistant to prochloraz manganese, the active ingredient in Sporgon. The concentration required to reduce the growth rate by 50% was lower than for carbendazim at 1- 5 ppm compared with 4 - 8 ppm for carbendazim. However growth of B1 isolates at concentrations of 5 - 20 ppm prochloraz manganese was greater than for carbendazim, with growth rates of up to 20% of the controls being recorded. The theoretical concentration of prochloraz manganese in the casing following a single 120 g (4 oz) dose is in the order of 15 - 20 ppm. We do not know what the actual concentration of active ingredient in the casing is but if the fungicide is uniformly mixed throughout the casing it should be 15 - 20 ppm. In practice it may be present at higher and lower concentrations in localised areas. We also know that the organic content of casing reduces the effective concentration of fungicides due to adsorption so that the effective concentration may be significantly lower than the theoretical concentration of 15 - 20 ppm. There is a HDC funded experiment underway which aims to determine the fate of Sporgon in casing applied in various ways so that we may know the answers to some of these questions regarding theoretical and actual fungicide concentrations in casing. If the actual concentration of fungicide in the casing layer turns out to be lower than the theoretical levels then there will be a real cause for concern and an immediate need to know how the laboratory results, indicating resistance and partial resistance, relate to on-farm conditions.

***Dactylium* species Group A and B2 isolates.**

The remaining 25% of British isolates were much more sensitive to two of the three fungicides tested. A small number of these (4 isolates) were fast growing Group B2 isolates while the remainder consisted of all the slower growing Group A isolates. However, one of the Group B2 isolates had characteristics in common with the Group A isolates so that taxonomically Group B2 isolates may be closer to Group A isolates than to B1 isolates. More work is needed to clarify the taxonomy of the *Dactylium* group.

Thiabendazole (Hymush). All the Group A and Group B2 isolates were only partially resistant to thiabendazole (Hymush) in contrast to the complete resistance found in Group B1 isolates. The concentration of fungicide which caused a 50% reduction in growth varied from 1 - 2 ppm in some isolates to 5 - 10 in others but no isolate grew at a concentration of 20

ppm thiabendazole. This suggests that this chemical would be useful in eradicating cobweb caused by Group A and B2 isolates although isolates should be monitored for further development of resistance.

Carbendazim (Bavistin). All the Group A and Group B2 isolates were sensitive to Carbendazim (Bavistin) with growth rates being reduced to 50% of the controls by concentrations of 0.1 - 0.5 ppm. Little or no growth was observed at concentrations of 2 ppm or above indicating that this chemical should give effective control against Group A and Group B2 *Dactylium* isolates. The behaviour of Carbendazim in casing following its application may, however, lead to a loss of control in later flushes (see part 2 of this report)

Prochloraz manganese (Sporgon). The response of Group B2 isolates to prochloraz manganese was variable but they appear to be slightly more sensitive than Group B1 isolates. The concentration which reduces the growth rate by 50% was less than 1 ppm for all isolates tested although some also grew a little at concentration of up to 50 ppm (Plate 2). Similarly the growth of Group A isolates in response to increasing concentrations of prochloraz manganese was variable with some isolates appearing more resistant than Group B1 isolates while others appeared more sensitive.

The growth of all *Dactylium* isolates in the presence of prochloraz manganese was much more variable than in the presence of either thiabendazole or carbendazim. This is probably a reflection of the mode of action of prochloraz which differs to that of thiabendazole and carbendazim.

Resistance to prochloraz manganese seems to require changes in many genes (multi-gene resistance) which build up slowly with time. Resistance to carbendazim and thiabendazole on the other hand can occur with only one gene change (single gene resistance) and this can happen in a very short space of time. The variability observed within the Group A isolates may indicate that some of these isolates are in a more advanced state of resistance than the Group B1 isolates which were all very similar.

The results from a previous HDC research project in 1993 (Fletcher & Jaffe) indicated that

thiabendazole resistant *Dactylium* was present on only one farm out of 14 surveyed. This present survey finds that 35 farms out of 44 now have thiabendazole resistant *Dactylium*. This may partly explain the recent epidemic although there are many unanswered questions such as are the *Dactylium* isolates different or have they become resistant?

The results from this work will serve as a baseline against which any changes in the future can be compared. We still need to know how laboratory results relate to the behaviour of isolates in fungicide-treated casing. It is strongly advised that this work be done sooner rather than later so that if field resistance is demonstrated, steps can be taken to address the consequences.

4. Fungicide resistance among *Trichoderma* isolates

A total of 24 samples of green mould were obtained during the survey. Six different species of *Trichoderma* were identified as outlined in Table 5. One sample was identified as *Gliocladium virens* and was included for comparison.

Table 5

Species	Number of Isolates
T. harzianum (mostly Th1)	14
T. viride	4
T. pseudokoningii/longibrachiatum	3
T. hamatum	1
T. koningii	1
Gliocladium virens	1
Total	24

4.1 Growth response of Trichoderma harzianum isolates to fungicides

4.1.1 Thiabendazole (Hymush)

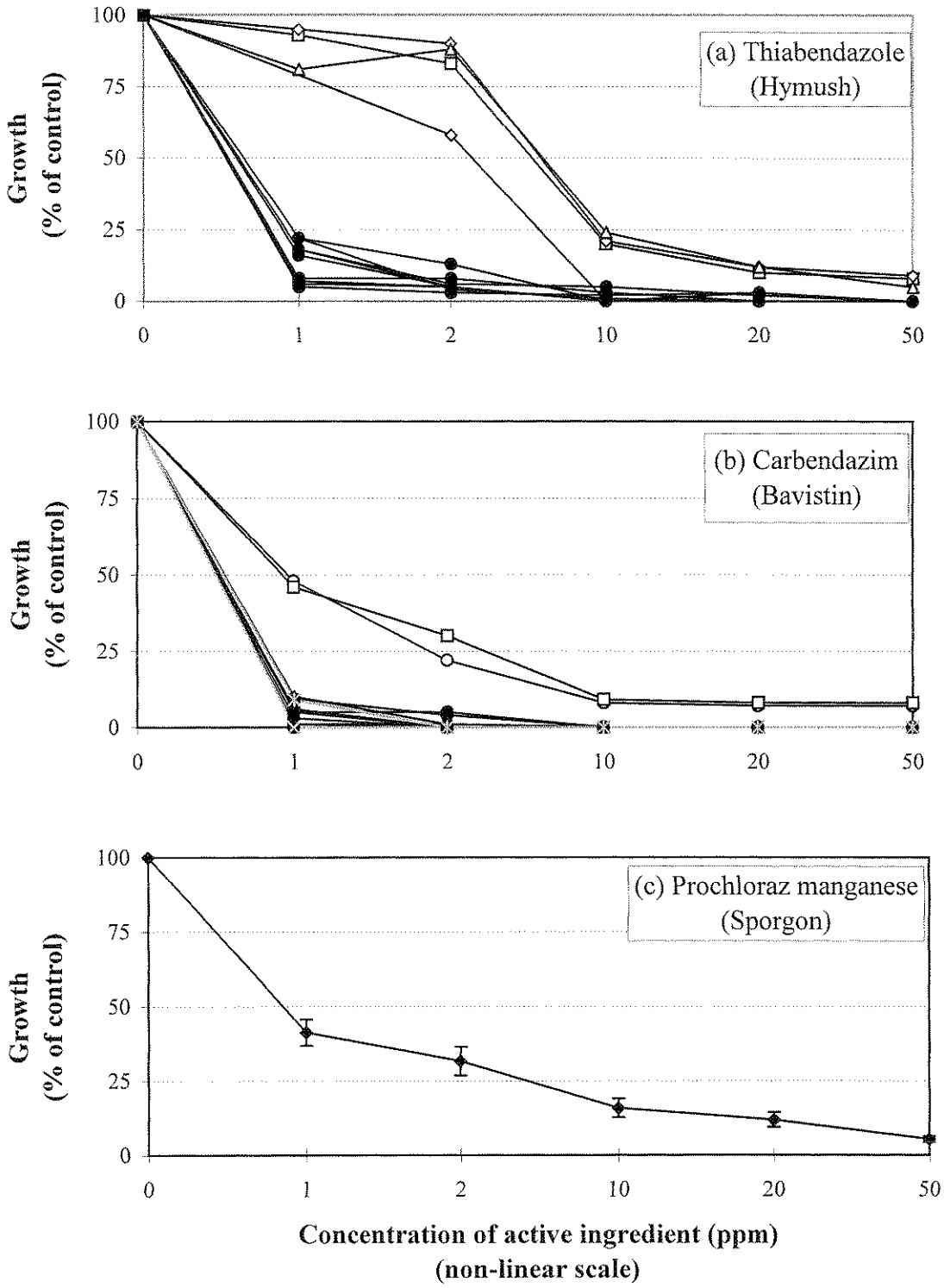
The majority of the *T. harzianum* isolates tested were sensitive to thiabendazole but four isolates were partially resistant, being capable of significant growth at 2ppm (Figure 8a). Two of the four partially resistant isolates were from the same farm (212A and 212B) and one was from France (172).

4.1.2 Carbendazim (Bavistin)

The majority of the *T. harzianum* isolates tested were sensitive to carbendazim with very little growth occurring at 1 and 2 ppm (Figure 8b). Two isolates were partially sensitive and grew reasonably well at 1 and 2 ppm.

Figure 8.

The response of *T. harzianum* isolates to three fungicides



4.1.3 Prochloraz Manganese (Sporgon)

The response of *T. harzianum* isolates to prochloraz manganese was more variable than for carbendazim or thiabendazole. Growth of isolates at 1 ppm ranged from 13 to 60% of the control growth rates (Figure 8c). A few isolates appeared to be strongly inhibited by prochloraz but most isolates grew significantly over the range of concentrations tested indicating partial resistance to this fungicide.

The results presented above indicate that by and large *T. harzianum* isolates are sensitive to Hymush and Bavistin but that partially resistant isolates are also present. Some isolates were identified according to their molecular type (Th1/Th2/Th3) and all were of type Th1. However, apart from the sister isolates 212A and 212B, there was no consistent pattern in the behaviour of the partially resistant isolates to suggest that resistance was associated with a distinct group of isolates. The pair of sister isolates were partially resistant to all three fungicides while all remaining isolates were sensitive to Bavistin and either sensitive or partially resistant to Hymush and Sporgon.

T. harzianum isolates are frequently associated with fungal spotting on mushroom caps so it is extremely useful to know what fungicides can be used to control outbreaks of symptoms.

4.2 Growth responses of T. viride and T. koningii to fungicides

The response of *T. viride* and *T. koningii* to the fungicides Hymush, Bavistin and Sporgon were similar (Figure 9 and 10). Both species groups were partially resistant to thiabendazole but neither grew at concentrations of 10 ppm or above. Both were sensitive to carbendazim with little or no growth being recorded at 1 ppm or above. Both species groups were partially resistant to prochloraz manganese and were capable of reduced growth up to a concentration of 50 ppm

These results indicate that the fungicide Bavistin is still very effective in inhibiting the growth of both *T. viride* and *T. koningii*. The fungicides Hymush and Sporgon should also be quite effective although fungicide resistance profiles should be monitored so as to detect any changes in fungicide resistance patterns.

Figure 9.

Response of *T. viride* isolates to three fungicides

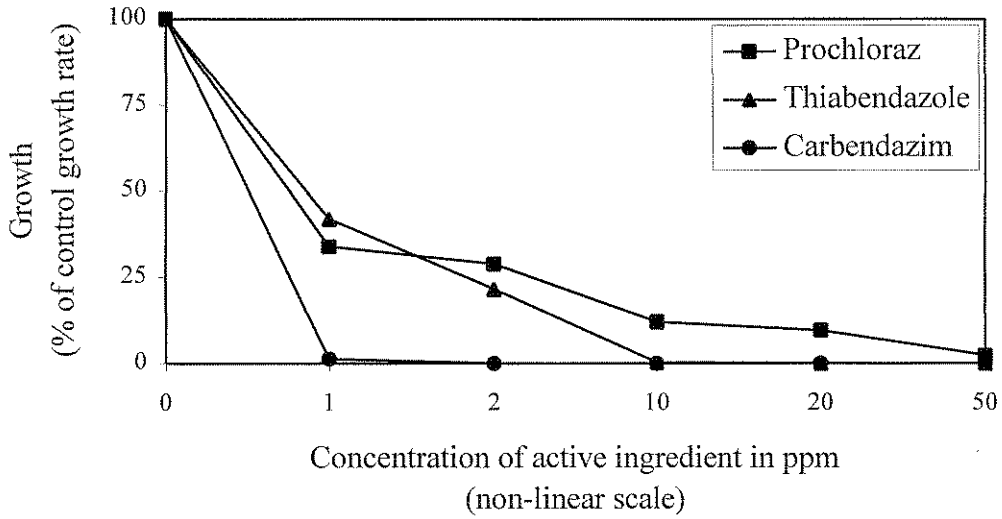
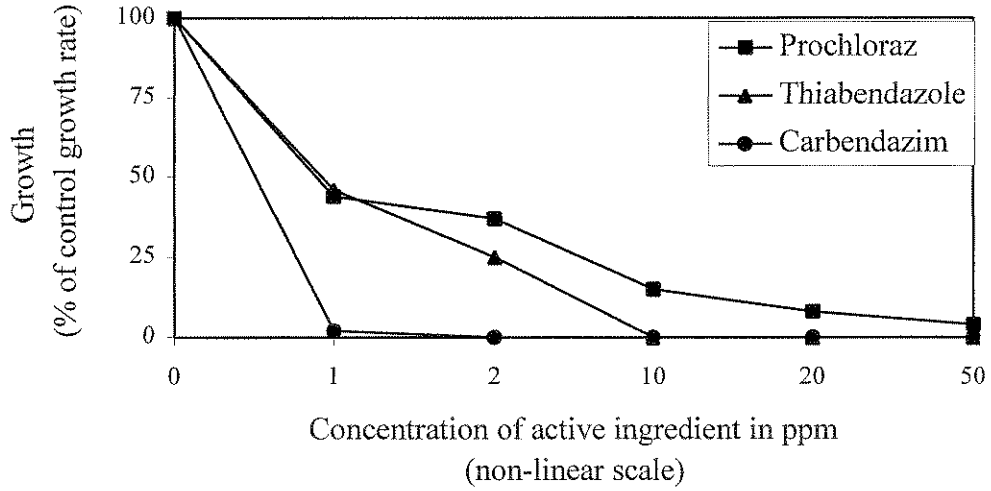


Figure 10.

The response of one *T. koningii* isolate to three fungicides



4.3 Growth responses of T. pseudokoningii/longibrachiatum and T. hamatum to fungicides

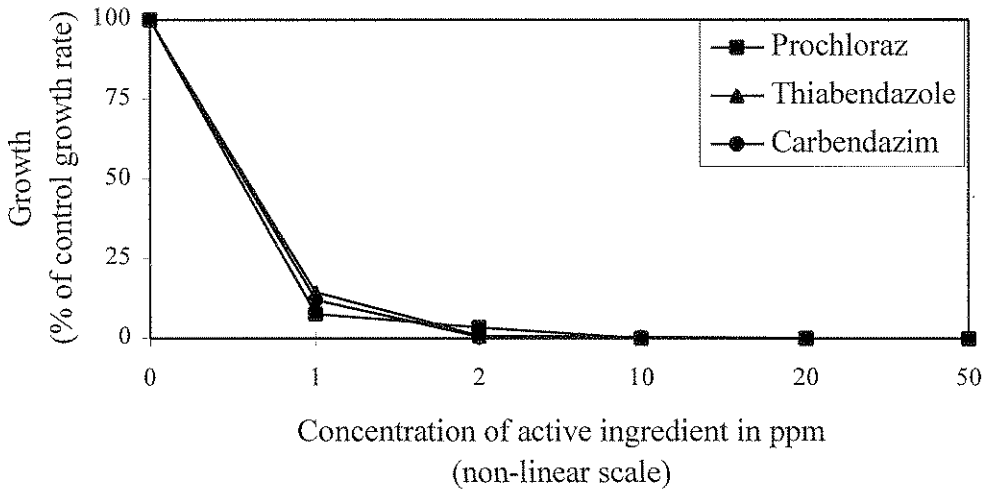
The responses of *T. pseudokoningii/longibrachiatum* and *T. hamatum* to the fungicides Hymush, Bavistin and Sporgon were very similar with all isolates tested being sensitive to all three fungicides (Figure 11, data for *T. hamatum* not shown).

4.4 Growth response of Gliocladium virens to fungicides

The single isolate of *G. virens* tested was largely sensitive to the three fungicides Hymush, Bavistin and Sporgon. A small amount of growth occurred at 1 ppm prochloraz (Sporgon), 37% growth was recorded at 2 ppm thiabendazole (Hymush) while no growth at all was recorded in the presence of Carbendazim (Bavistin) at 1 ppm (data not presented in graph format).

Figure 11.

Responce of *T. pseudokoningii/longibrachiatum* to three fungicides

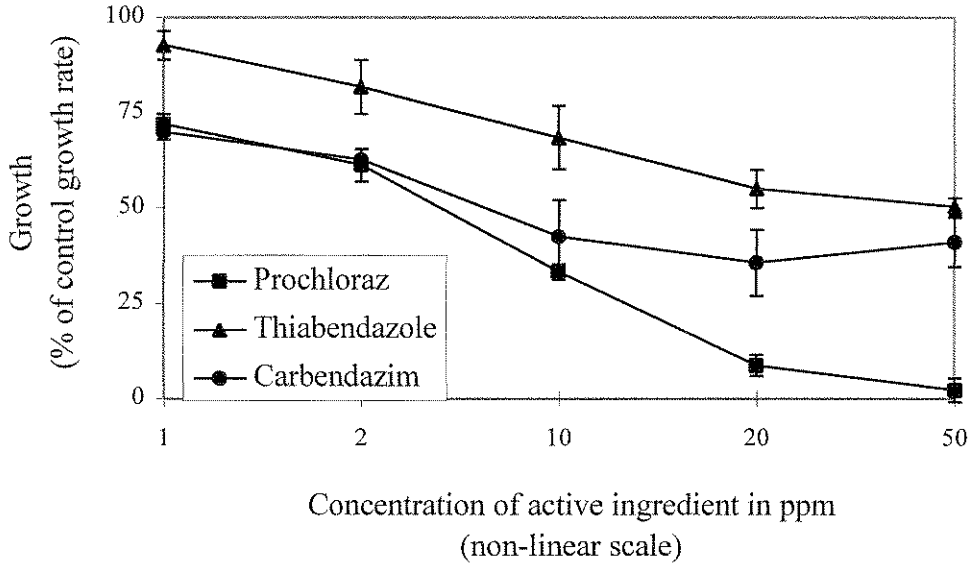


5. Fungicide resistance among *Aphanocladium album* isolates

Four isolates of *Aphanocladium album* were obtained from spotted mushroom samples from around England. All four isolates were resistant to both Hymush and Bavistin, growing quite well over the range of concentrations tested. Sporgon inhibited growth at concentrations of 20 and 50 ppm but isolates grew quite well at 1, 2 and 10 ppm indicating partial resistance (Figure 12).

Figure 12.

Response of *Aphanocladium album* to three fungicides.



6. Conclusions

Resistance to fungicides under laboratory conditions varied considerably from one group of isolates to another and also among isolates within groups but some conclusions can be drawn.

1. All *Dactylium* isolates were capable of *reduced* growth at low concentrations of Sporgon though some seemed more or less sensitive than others.

2. A significant number of *Dactylium* isolates (15) were completely sensitive to Bavistin but the majority of isolates (41) were capable of good growth at low concentrations.

3. The majority of *Dactylium* isolates (41) were fully resistant to Hymush but a significant number of isolates (15) were sensitive at concentrations expected in casing.

4. The majority of *Trichoderma* isolates tested (21 out of 23) were sensitive to Bavistin. Two *T. harzianum* isolates from one farm exhibited partial resistance.

5. The majority of *T. harzianum* isolates tested were sensitive to Hymush but four partially resistant isolates were also recorded.

6. The majority of *T. harzianum* isolates tested were capable of reduced growth in the presence of Sporgon indicating partial resistance.

7. *Trichoderma viride* and *T. koningii* isolates tested were partially resistant to Hymush and Sporgon but sensitive to Bavistin.

8. *T. longibrachiatum/pseudokoningii* and T. hamatum were sensitive to all three fungicides Hymush, Bavistin and Sporgon.

9. *Aphanocladium album* isolates were resistant to Hymush and Bavistin but Sporgon reduced growth significantly at high concentrations.

Part II - Degradation of Carbendazim in Casing

1. Introduction

Work by Fletcher *et al.*, (1980) indicated that Benlate was failing to control *Mycogne* although isolates of that organism were sensitive to benomyl, the active ingredient. They went on to show that benomyl was being degraded by bacteria present in the casing and that the active ingredient could disappear either very quickly or more slowly depending on the history of fungicide use on a farm. The active ingredient benomyl breaks down to carbendazim in the casing which is also the active ingredient in Bavistin. In view of the similarity of these two active ingredients it was decided to examine whether or not Bavistin-derived carbendazim would also break down in casing and also to see whereabouts in the casing the active ingredient was concentrated following different methods of application.

2. Materials and Methods

2.1 Fungicide

The commercially produced fungicide Bavistin DF was used in this experiment. The concentration of the active ingredient, carbendazim, is 50% w/w. It was applied to casing either by (a) incorporation into the casing at mixing at the standard rate of 250g/100 sq metres of bed area or (b) in the first watering after casing at the rate of 250g in 200 litres of water per 100 sq metres of bed area.

2.2 Casing

Commercially available, ready-mixed casing produced by Nooyen was used in the experiment.

2.3 Experimental set up

Commercial compost in bags was used for this study. Twenty replicate bags were prepared for each of the two fungicide treatments and the untreated control treatment. Bags were spawn run, cased, case run, aired and cropped according to standard procedures at HRI mushroom unit.

2.4 Sampling

Casings were sampled 1, 5, 11, 18, 27 and 33 days after casing. For each treatment, 5 replicate bags were chosen at random for sampling. From each bag 2 casing samples were taken, one from the top centimetre of casing and the other from the bottom centimetre of casing next to the compost.

2.5 Carbendazim determination.

The presence of carbendazim in casing samples was ascertained using a U.V. spectrophotometer as described by Fletcher *et al.* (1980). A sample of casing weighing 10 grams (fresh weight) was placed in a 250 ml conical flask to which was added 50 mls of ethanol. Five replicate flasks were prepared for each fungicide treatment at each casing depth. Flasks were sealed with parafilm to prevent evaporation of the ethanol, and then incubated for 1 hour on a rotary shaker at 200 revs/minute at 25°C. The incubated samples were then filtered through a glass fibre filter using a suction pump and the ethanol casing extract was collected and placed in a screw capped bottle.

Preliminary analyses indicated that the concentration of carbendazim in the initial extracts was too high for accurate detection so each extract was diluted at a rate of 1 part extract : 1 part ethanol.

For spectrophotometry analysis 4ml samples of extract were scanned at 287 nanometres - the absorbance wavelength of carbendazim. Each sample was paired with a control casing extract

containing no fungicide so that the readings obtained were directly related to carbendazim content. Casing extract itself contains substances which also absorb UV light at 287 but by using a non-fungicide treated casing extract in the spectrophotometer reference cell this background absorbance is taken into account and the resultant reading relates to the carbendazim content in the test cell. Means and standard errors were calculated for each set of 5 replicates and the results presented in graph format.

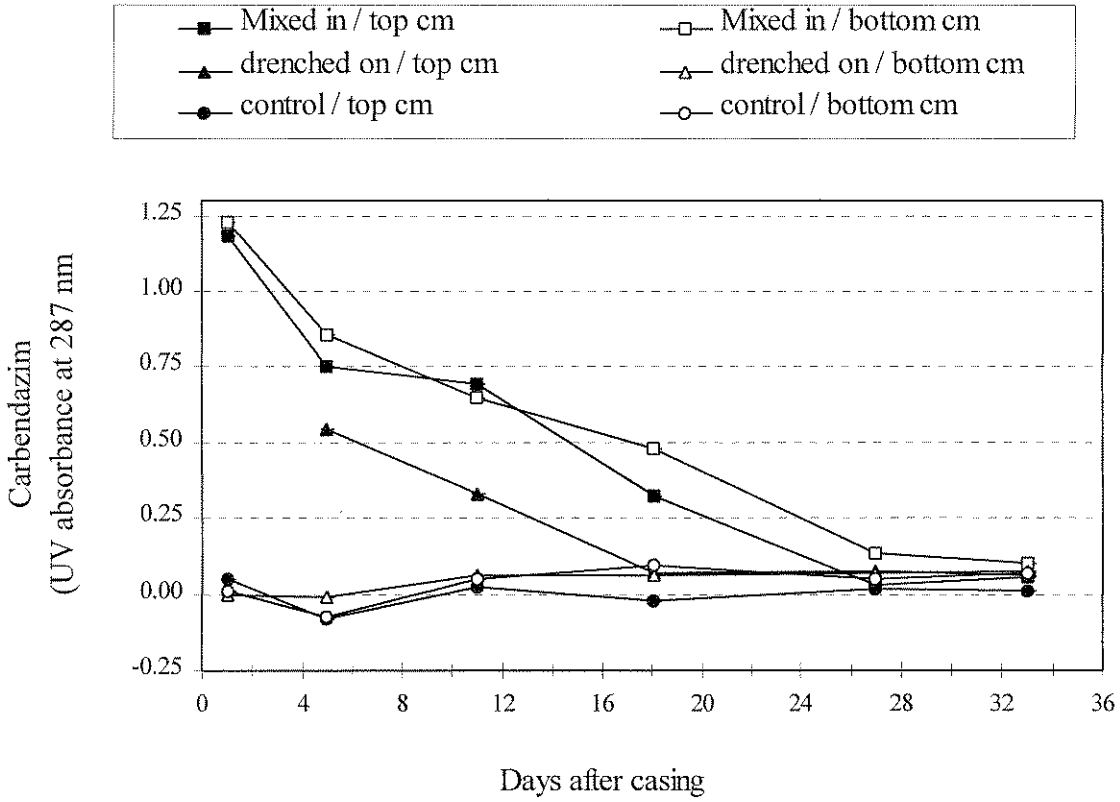
3. Results

There was a significant decline with time in the amount of carbendazim detected in casing following both methods of application (Figure 13). Very little carbendazim was detected 27 days after casing. When the fungicide was mixed into the casing during casing preparation there was very little difference between the amount of carbendazim detected in either the top or bottom of the casing layer. The results shown in Figure 13 also suggest that carbendazim may be being lost more quickly from the top layer compared to the bottom layer but further experimentation would be necessary to prove this conclusively.

When the fungicide was drenched onto the casing surface the fungicide remained in the top layer. In addition it appeared that significantly less fungicide was detected in total compared with the "mixed-in" treatment. This was contrary to what was expected and further experimentation is essential to ascertain what lies behind this anomaly. Since all of the fungicide is theoretically present in the top layer of casing, one would expect a much higher concentration of fungicide in this layer compared with the "mixed-in" treatment. Factors which could have an effect include (a) incorrect rate of use (b) inadequate mixing in the tank (c) inadequate rate of application (d) uneven spraying (e) loss of fungicide onto floor and sides of containers (f) greater de-activation of the active ingredient in the surface layers of the casing. Factors (a) to (e) were minimised during this experiment. Further experimentation is required to establish if there is greater deactivation of the active ingredient in the surface layers.

Figure 13.

Presence of carbendazim (Bavistin) in top and bottom layers of casing following two methods of application



4. Discussion

The results presented above for the disappearance of carbendazim from casing with time is similar to what was reported by Fletcher *et al.* (1980) for Carbendazim (derived from benomyl 1) in casings which were not routinely treated with benomyl. They succeeded in isolating bacteria from the casing which were capable of degrading carbendazim and it is most probable that bacteria are responsible for the decline in carbendazim levels illustrated in Figure 13. Very rapid degradation was not recorded.

With respect to the lower levels of carbendazim detected in drenched casing it is very important to ascertain whether this phenomenon is a standard feature. Drenching is the preferred method of fungicide application within the industry so it is of the utmost importance to know whether or not it is an effective method.

In view of the fact that carbendazim levels in casing drop with time it might be worthwhile exploring the possibility as to whether or not the label recommendations for the use of Bavistin on mushroom crops can be changed to take this information into account.

The single permitted application rate of 250g/100m^2 at casing would appear to be ineffective in controlling diseases which occur after the first flush. In view of the evidence obtained during this experiment a weekly application might be more effective but this is a question which the HDC panel would have to discuss in conjunction with the fungicide manufacturer and with MAFF as a new label or off label would be required.

There is evidence to suggest that thiabendazole (Hymush) is more persistent in casing than carbendazim (Fletcher *et al.* 1980) so that where either of these two chemicals can be used, the former should be chosen for the control of pathogens in later flushes.

5. Conclusions

1. Carbendazim, the active ingredient in Bavistin, disappears from the casing with time with little remaining 18-27 days after casing.

2. Carbendazim stays in the top layer of casing when drenched on but is uniformly distributed throughout the casing if it is mixed into the casing during its preparation.

3. Drenching onto casing may result in a quicker loss of activity compared with mixing into the casing but further experiments are required to establish this.

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