CONTRACT REPORT M14

MUSHROOMS
FUNGICIDE RESISTANCE

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CONTENTS

Reference to growers and practical application

Application

The objective of this work is to assess the current situation with regard to resistance to the most effective fungicides of the commonly occurring fungal pathogens of the mushroom crop. Such a survey has not been done since the early 1970s and *Trichoderma* spp, have recently become important problems both as a cause of cap spotting and also as an aggressive coloniser of compost. Also, from time to time growers express doubts about the effectiveness of particular products in specific situations and although there are many explanations of disease control failures, fungicide resistance can be a very important one and should be identified, if it exists, at the earliest possible time.

The results show little change in the situation with regard to *Mycogone perniciosa* and *Verticillium fungicola* since the 1970s. The benzimidazole fungicides (benomyl as Benlate and thiabendazole as Hymush) remain effective for the control of *Mycogone* but *Verticillium* is totally resistant to them, as it has been since 1974-5. Prochloraz manganese (Sporgon) remains effective for the control of *Verticillium*. *Dactylium dendroides* is generally sensitive to benomyl although isolates were found at a number of farms which were resistant to thiabendazole. *Trichoderma* species showed a range of sensitivities to all active ingredients tested but isolates associated with the compost problem (Th2) were generally sensitive to benomyl.

Where disease control failures occur it is important for growers to have isolates checked for their sensitivity to the main fungicides particularly in view of the recent occurrence of resistance to the benzimidazoles in *Dactylium* in Ireland.

Summary

1. All the isolates of *Verticillium fungicola* were sensitive to prochloraz manganese (Sporgon) at 20 ppm but from four farms isolates grew at 2 ppm. This does not indicate the development of resistance but merely the range of sensitivity within the population.

2. None of the isolates of *V. fungicola* was sensitive to benomyl or thiabendazole. This represents no change since the mid 1970s.

3. All isolates of *Mycogone perniciosa* were sensitive to benomyl, thiabendazole and prochloraz manganese although isolates from one farm grew at 2 ppm benomyl and 2 ppm thiabendazole. From another farm one isolate grew at 2 ppm prochloraz manganese. None of these isolates can be said to be resistant.

4. Isolates of *Cladobotryum dendroides* capable of growth at 20 ppm thiabendazole were found on one farm and although some isolates grew at 2 ppm benomyl none grew at 20 ppm. This is in contrast to the recent Irish outbreak where isolates grew well at both 20 ppm benomyl and thiabendazole. Some isolates from five farms made some growth at 20 ppm prochloraz manganese. This may represent a move towards resistance but could also be an indication of the wide range of sensitivity of fungus to this fungicide.

5. Isolates of *Trichoderma* showed variation in sensitivity according to the species and strain but the majority of isolates of Th2 were fungicide sensitive.

6. This survey shows some changes in the sensitivity of pathogen populations since the last survey in particular with *Cladobotryum*. *Trichoderma* was not included in the last survey.

Action points for growers

1. There is no immediate cause for concern with regard to fungicide use and effective disease control of mushroom pathogens but the reports of *Cladobotryum* resistant to the benzimidazoles in Eire and of *Mycogone* with a low level of resistance to thiabendazole in China must serve as a warning to British growers.

2. The *Trichoderma* situation requires careful monitoring particularly if fungicide treatment for control of the compost problem becomes widespread practice. This will exert a selection pressure on the pathogen population which could speed up the development of resistance.

3. Growers should always be careful in their use of fungicides avoiding routine use. This, together with good hygiene to minimise disease risk, will help to ensure that fungicide resistance remains an unimportant problem.

4. In any situation where disease control is not as good as expected growers should have fungal pathogens checked for fungicide resistance.

5. Poor performance of fungicides frequently results from poor application. All aspects of application should be checked as a matter of priority.

Research Report

Introduction

The mushroom industry is very dependent upon a small number of effective fungicides for the control of the three main fungal pathogens, *Verticillium fungicola*, *Mycogone perniciosa* and *Cladobotryum dendroides*. Recently *Trichoderma harzianum* has caused severe cap spotting problems in some crops and its control is also fungicide dependent. Fungicide resistance to benomyl occurred in *Verticillium fungicola* within three years of its introduction (Fletcher, J.T. and Yarham, D.J. (1976). Annals Applied Biology, 84, 343-353) and an ADAS survey in 1974/75 showed resistance to be widespread (Gaze, R.H. and Fletcher, J.T. Mushroom Journal No. 35, November 1975). At that time, isolates of *M. perniciosa* and *C. dendroides* were all sensitive to benomyl. *Trichoderma* species were not tested. The occurrence of benzimidazole resistance resulted in a search for a new fungicide with a different mode of action and prochloraz manganese was found to be very effective against benomyl resistant strains of *Verticillium fungicola* (Fletcher, J.T., Hims, M.J. and Hall, R.J. (1983) Plant Pathology 32, 123-131). Benzimidazole fungicides have continued to be used for the control of *Mycogone* and *Cladobotryum* and there have been no reports of benzimidazole resistance with the exception of one for *Mycogone* from Korea by G.P. Kim (1979) (Research Reports ORD 21: 33-38.) and the recent of occurrence of *Cladobotryum* resistance in Ireland.

There have been some reports in the UK during the last year concerning the poor control of *Verticillium* with Sporgon but resistance tests of isolates from such outbreaks have not shown evidence of a change in pathogen sensitivity (Fletcher, J.T., unpublished). In addition, a small number of *Trichoderma harzianum* isolates have been tested and although they have generally been found to be sensitive to benomyl, one was found to be completely insensitive. It was, however, sensitive to prochloraz (Fletcher, unpublished).

The objective of the study was to assess the current position in the industry to make certain that growers are using the most effective fungicides for the appropriate diseases. Results were be obtained by examining representative samples of four fungal pathogens (*V. fungicola*, *M. perniciosa*, *C. dendroides* and *T. harzianum*) measuring their sensitivities to benomyl, thiabendazole and prochloraz Mn. The results are comparable with those obtained in 1974/75 and give some measure of any shifts in sensitivity that have occurred.

Materials and Methods

Isolates of the four fungal pathogens were collected from mushroom farms. This was done by contacting spawn and compost producers, advisers and by contacting all members of the Mushroom Growers Association. Advice was given on the type of sample required and how to package it in order that it arrived at ADAS Wye in good condition.

An initial isolation of the fungal pathogens was made onto potato dextrose agar with streptomycin added (PDA). When 'clean' cultures were obtained 6 mm disks of agar with mycelium from the leading edge of the colony were transferred to fungicide amended PDAs. Initially all isolates were screened at 2 ppm of the three fungicides tested Benlate, 50% (ai benomyl), Hymush (60% ai. thiabendazole) and Sporgon, (50% ai prochloraz manganese). Chlorothalonil was not included in the tests as there are no records of fungicide resistance in any pathogen to this fungicide. Carbendazim was also not included as benomyl hydrolyses to carbendazim. If growth occurred at the 2 ppm concentrations a further test was made at 20 ppm.

Where no growth occurred at 2 ppm the isolate was deemed to be very sensitive to the fungicide. Growth at both 2 ppm and 20 ppm was considered to indicate a level of fungicide resistance which might be expected to result in reduced disease control.

Isolates were received over the whole period of the project although most followed the initial request for samples. Many of the isolates were stored on PDA's slopes at 3°C for future reference.

Results

Table 1 shows the number of isolates examined of each pathogen and the numbers of different farms involved.

Table 1. Number of farms sampled and number of isolates tested of four fungal pathogens of mushrooms

	Numbers	
	***Farm tests**	**Isolates**
Verticillium	11	162
Mycogone	5	17
Cladobotryum	16	94
Trichoderma	52	64

* Samples of different pathogens were sometimes sent from the same farm and occasionally a second sample was sent from a farm some time after the first. This column does not therefore represent the total numbers of different farms sampled

Verticillium fungicola

Samples were received from eleven different farms and a total of 162 isolates examined. All of these isolates were resistant to benomyl and thiabendazole, ie. they all grew well at 2 and 20 ppm of both fungicides. Isolates from 6 farms showed no growth at 2 or 20 ppm prochloraz manganese but from a few farms, isolates grew at 2 ppm but not at 20 ppm. Isolates from one farm divided into two groups, five that did not grow at 2 ppm and six that did. No isolates grew at 20 ppm prochloraz manganese from any of the farms in the survey.

Cladobotryum dendroides

Isolates were received from 12 different farms. The results of growth at 2 and 20 ppm showed differences between the isolates.

Benomyl

Nine isolates from four different farms grew at 2 ppm but none grew at 20 ppm.

Thiabendazole

87 of the 94 *Cladobotryum* isolates grew at 2 ppm and 13 of the 14 isolates from one farm grew at 20 ppm but only one of these 13 isolates grew at 2 ppm benomyl.

Prochloraz manganese

All eight isolates from one farm and seven of 14 isolates from another were completely inhibited at 2 and 20 ppm. All of the other isolates from the second farm (six) grew at 2 ppm. At 20 ppm isolates from five farms made some growth. In all cases, except where single isolates from a farm were examined, a proportion grew at 20 ppm, ie. 12 out of 17, 2 out of five and three out of nine.

Mycogone perniciosa

17 isolates were received from five different farms and tested for fungicide sensitivity.

Benomyl

Three isolates from one farm grew at 2 ppm but not at 20 ppm.

Thiabendazole

Three isolates from one farm grew at 2 ppm but not at 20 ppm. These were the same isolates that grew at 2 ppm benomyl.

Prochloraz manganese

One isolate grew at 2 ppm but none grew at 20 ppm.

Trichoderma species

64 isolates were tested from 51 different locations. Their sensitivities to the fungicides used were variable with 9.4% resistant to benomyl, 14% to thiabendazole and 18.7% resistant to prochloraz manganese (Table 2and 3).

Table 2 Sensitivity of 64 isolates of *Trichoderma* species to benomyl, thiabendazole and prochloraz manganese

	Fungicide concentrations in ppm					
	benomyl		**thiabendazole**		**prochloraz Mn**	
	2	**20**	**2**	**20**	**2**	**20**
Number of isolates	18*	6	45	9	26	12
Percent	28.1	9.4	70.3	14.1	40.6	18.7

* numbers of isolates growing at this concentration

The individual isolates showed considerable variation in terms of sensitivity to the three fungicides. For instance, ten isolates were sensitive to all three at the lowest concentration of 2 ppm and two isolates grew at 2 and 20 ppm of all three fungicides, ie. they were insensitive (or resistant) to all three fungicides tested at 20 ppm. The remainder showed a variety of combinations (Table 3).

Table 3. Sensitivity of 64 isolates of *Trichoderma* species according to their reactions to benomyl, thiabendazole and prochloraz manganese

Numbers of isolates	Fungicide concentrations in ppm					
	benomyl		thiabendazole		prochloraz manganese	
	2	20	2	20	2	20
10	-	-	-	-	-	-
1	+	-	-	-	-	-
20	-	-	+	-	-	-
6	-	-	-	-	+	+
4	+	-	+	-	-	-
3	-	-	+	-	+	-
1	-	-	+	+	-	-
2	-	-	+	-	+	+
7	+	-	+	-	+	+
2	-	-	-	-	+	+
2	-	-	+	+	+	-
3	+	+	+	+	+	-
1	+	+	+	+	-	-
2	+	+	+	+	+	+

Key: + growth
- no growth

45 of the 64 isolates have been identified to species and strain level by characterisation of their DNA (Dr Peter Mills, Queens University, Belfast).

All of the isolates other than *T. harzianum* were sensitive to all of the fungicides with the exception of one isolate of *T. longibrachiatum* which was insensitive to thiabendazole at 20 ppm. Of the isolates of *T. harzianum*, three were resistant to benomyl (one Th2 and two Th3), five to thiabendazole (three Th2 and two Th3 and five to prochloraz manganese (three Th1 and two Th3) all making growth at 20 ppm of the appropriate fungicide (Table 4).

Table 4 **The sensitivity of isolates of *Trichoderma* species and strains to benomyl, thiabendazole and prochloraz manganese**

Species/strain and number of isolates ( )	Fungicide concentration at ppm					
	benomyl		thiabendazole		prochloraz manganese	
	2	20	2	20	2	20
T. hamatum (4)	1*	0	2	0	1	0
T. pseudokoningii (2)	0	0	2	0	0	0
T. longibrachiatum (2)	0	0	1	1	2	0
T. harzianum Th1 (12)	4	0	6	0	7	3
T. harzianum Th2 (19)	3	1	13	3	2	0
T. harzianum Th3 (5)	3	2	4	2	5	2
T. viride (1)	0	0	1	0	0	0

* Number of isolates able to grow at this concentration

When the Th2 is considered only one isolate of the 19 tested grew at 20 ppm benomyl and three grew at 20 ppm thiabendazole.

Conclusions

This survey of the sensitivity to fungicides of isolates of fungal pathogens from mushroom crops shows that some changes have occurred since the last survey which was completed in the early 1970s (Fletcher and Yarham, 1976). Fortunately, *Mycogone perniciosa* and *Verticillium fungicola* remain sensitive to the benzimidazoles and prochloraz manganese respectively. The greatest change has occurred with *Cladobotryum dendroides* where resistance to thiabendazole but not benomyl was detected on one farm. There is a considerable range in the sensitivity of isolates of this fungus to prochloraz manganese although some isolates (15) were completely inhibited by the lowest concentration of all three fungicides. But isolates from five farms made some growth at 20 ppm. It is not clear whether this is truly fungicide resistance or just a large range in the sensitivity of isolates from different populations. The effects of this range has been seen by growers during the last few years where on some farms poor control of cobweb disease has been achieved with this fungicide.

Trichoderma species were not included in the last survey and the results here indicate a range of variation in sensitivity particularly within the forms of *T. harzianum*. The significance of such variation could be considerable if the industry relies upon the use of fungicides for the control of the weed mould problem in compost. So far very little resistance to benomyl (and therefore carbendazim) has been detected which indicates that the fungicidal treatment of compost or spawn should be effective. Prochloraz manganese is an alternative material but is likely to be more mycotoxic than the benzimidazoles so may not be as satisfactory for spawn application.

The numbers of farms sampled in this survey represents a fairly small proportion of the total and the numbers of isolates of individual pathogens are also relatively small. The sample was not taken at random and was biased toward those farms that had disease problems at the time of requesting material. Nonetheless, the survey gives an indication of the present status of the sensitivity of the fungal pathogens of the mushroom crop. It is of interest that none of the *Cladobotryum* isolates had the degree of resistance to the benzimidazoles shown by those recently found in Eire. Neither did any of the *Mycogone* isolates show reduced sensitivity to thiabendazoles as recently found in China.

The results indicate that with careful use the existing approved fungicides should continue to be effective but the situation needs constant monitoring as changes in fungicide sensitivity can occur very rapidly.

References

All references are included in the text.