CONTRACT REPORT M13

Sensitivity of mushroom pathogens to new fungicides.

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Reference to growers and practical application

Application

Since 1970 a large number of fungicides has been introduced into agriculture largely for use on extensive field grown crops such as cereals and potatoes. Many of these have resulted from long research programmes by chemical companies and new chemistry has been developed. Products from different companies are sometimes related and major fungicide groups are recognised with the products within these groups having the same, or a similar mode of action. Many new generation fungicides are often very specific in their site of action perhaps affecting one physiological process within the target species. Such a specific effect has the weakness that it requires only a small change on the part of the fungal pathogen to result in changes in sensitivity and the development of fungicide resistance. Mushroom growers have not benefited from the most recent fungicide developments although some of the original site specific materials such as those in the benzimidazole group (Benlate, Bavistin, Hymush) have done good service for a long time. Their effectiveness has been diminished to some extent by the development of resistance which was seen most dramatically in the dry bubble pathogen *Verticillium fungicola*. The industry has yet to benefit from the azole or morpholine groups. Recently work on a number of other products has reached advanced experimental stages within a number of companies, eg. those in the group based on the fungal metabolite strobilurine and these may have potential benefit for use on the mushroom crop.

At present, the industry relies upon a few very effective products. But fungicide resistance can develop quickly and sometimes very unexpectedly. It is therefore important to know which of the new fungicides have potential for use in the industry. The work reported here is an examination by screening of major pathogens of the crop on fungicide amended agar. The results give some indication of fungicides with potential for disease control and also for those which may be useful as tray disinfectants. Promising products must be tested *in vivo* and residue results obtained so that where necessary or worthwhile, applications for Off-label registration can be made.

Summary

In order to identify new potential fungicides for use in the mushroom industry, 22 fungicides were examined in agar plate tests, and compared with those already used in the industry. The fungicides were incorporated at 2 and 20 ppm into potato dextrose agar and the growth on four pathogens *Mycogone perniciosa*, *Cladobotryum dendroides*, *Verticillium fungicola* var *fungicola* and *Trichoderma harzianum* [Th2] and also *Agaricus bisporus* strain 609 was measured.

Only 2 new products (ISK Biotech 66825 and BASF 49004F) appear to merit further investigation. The ISK Biotech product was the most promising. Growth of mushroom mycelium at 2 and 20ppm was not inhibited to a significant extent but it was toxic to the 4 pathogens used. The main reason for fungicide rejection was the level of mycotoxicity that most displayed toward *A. bisporus*. Some of the products, eg. fenpropimorph, propiconazole, flusilazole and metconazole were toxic to all the organisms tested including *Agaricus bisporus* and may have potential as tray dips.

Action points for growers

1. There is no immediate concern that the common diseases of mushrooms cannot be controlled with existing products although fungicide resistance is found to some extent (see report on Contract M14 - Mushrooms - Fungicide Resistance).

2. The recent withdrawal for commercial reasons of the recommendation for the use of Benlate on the mushroom crop highlights the precarious position of the industry with regard to successful disease control.

3. Similarly the development of fungicide resistance in the cobweb pathogen in Ireland is a cause for concern and growers must be vigilant and have tested outbreaks of any pathogen where fungicides have been used correctly and appear not to work.

4. Screening fungicides highlights the narrow margin between disease control and toxicity to the crop shown by some products and no margin at all with others. It is vitally important to apply fungicides according to label recommendations, not to exceed these rates for fear of crop damage, but also not to under dose, which may result in poor disease control and in some cases, favour the development of fungicide resistance.

5. Of the products examined, one in particular shows considerable promise (ISK Biotech ASC 66825). Growers should support further work on this fungicide in order to develop the necessary data to enable Off-label registration to be obtained at the earliest opportunity.

6. ISK Biotech 66825 is not yet registered in the UK for use on any crop (it is in the USA) and the industry therefore has time to generate the necessary data.

7. Further investigations should be made of those fungicides highly toxic to the mushrooms as well as its pathogens as these could be developed into very effective tray, net, wood etc., dips.

Research Report

Introduction

In order to control the main fungal pathogens of mushrooms, including *Mycogone perniciosa*, *Verticillium fungicola*, *Trichoderma harzianum*, a few fungicides have been used including prochloraz manganese and the benzimidazoles mainly benomyl Recently Dupont have removed the label recommendation for the use of this chemical. on the mushroom crop. The use of so few fungicides is partly because of the difficulty of finding products which are not mycotoxic to mushroom strains but are toxic to the pathogens (Gandy and Spencer 1978). The incentive for manufacturers to develop fungicides specifically for the mushroom market is not great as the market size, even internationally, is relatively small. The industry must therefore rely upon the use of fungicides developed for other markets possibly obtaining off-label registration for their use on mushrooms.

One problem with the use of so few fungicides is that the pathogens are constantly subjected to the same chemicals and the probability of a pathogen developing resistance through mutation is increased, This is particularly the case where the mechanism of action is very specific and resistance may be controlled by a single gene. Since the introduction of such fungicides to the mushroom industry fungicide resistance has occurred. For instance the initial use of the benzimidazole fungicides for the control of fungal pathogens resulted in a sharp decline in fungus disease incidence but *V. fungicola* became resistant to these within three years, (Fletcher and Yarham, 1976). Recent investigations at ADAS, Wye show that *Trichoderma* species vary in their sensitivity to thiabendazole, benomyl and prochloraz manganese. Also a strain of *Cladobotryum dendroides* has been found in Ireland which is resistant to all the benzimidazoles.

In an attempt to identify potential new fungicides for the industry the four major fungal pathogens and *A. bisporus* strain 609 were screened for their sensitivities to an array of existing and experimental fungicides.

Materials and Methods

Solutions of each fungicide were made up in sterile distilled water and appropriate amounts added to either potato dextrose agar (PDA), for the fungal pathogens, or malt agar (MA), for the *A. bisporus* tests, to give concentrations of each active ingredient of 2 or 20 ppm. The fungicides were added to the agar after autoclaving just prior to pouring into 85mm petri dishes.

Two lines were drawn on the bases of each petri dish which bisected each other at right angles in the centre. 6mm plugs of the pathogens and the *A. bisporus* 609, which had been grown on PDA and MA respectively, were placed on the bisecting point and incubated at 25°C. Controls using agar without fungicide added were done concurrently. There were 4 replicates for each combinations of fungal pathogen, fungicide and fungicide concentrations.

The length of time between this procedure and growth measurements depended upon the growth rate of the isolates. *T. harzianum* and *C. dendroides* were measured after 3 days whilst *M. perniciosa* and *V. fungicola* had slower growth rates and measurements were made after 7 days. *A. bisporus* had the slowest growth rate and measurements were made after 9 days. Two diameter measurements were taken from each petri dish. One along each of the bisecting lines. The measurements included the 6mm diameter of the plug. The measurements from the two diameters per replicate and from the four replicates were meaned. In all, 22 fungicides were tested.

Table 1. Fungicides Used

PRODUCT NAME	ACTIVE INGREDIENT	% ACTIVE INGREDIENT
Sporgon	Prochloraz manganese	50.0
Benlate	benomyl	50.0
Hymush	thiabendazole	60.0
PP450	flutriafol	12.5
Systhane flo	myclobutanil	6.0
Patrol	fenpropidin	82.4
Corbel	fenpropimorph	79.5
Bavistin DF	carbendazim	50.0
Filex	propamocarb hydrochloride	66.5
Sulphur flowable	sulphur	58.8
Tilt	propiconazole	21.7
Rubigan	fenarimol	12.0
ISK Biotech ASC.66825	not known	50.0
Punch C	flusilazole + carbendazim	23.0
Topas	propiconazole	10.6
Fungaflor	imazalil	18.8
Alto 100 SI	cyproconazole	8.8
Fytospore	cymoxanil	5.25
Bayer UK 443d	tebuconazole	25.0
Shell SF07573	metconazole	6.7
BASF 49004F	not known	50.0

Results

The results at 2ppm fungicide concentration (Table 2) show that ten of the tested fungicides had very little mycotoxic effects on the growth of *A. bisporus*. Of these, four are standard products already in use in the industry. At 20 ppm (Table 3) only four products in addition to the standards show a low level of mycotoxicity. These four were propamocarb hydrochloride (Filex), sulphur (Sulphur Flowable), ISK Biotech 66825 and BASF 49004F. When growth of the pathogens at 2 ppm fungicide concentration is considered (Table 2) there are a number that are as toxic or more toxic than the standards. For instance myclobutanil, fenpropimorph, propiconazole, fenarimol, ISK Biotech 66825, flusilazole and penconazole are all very toxic to *Cladobotryum* and *Mycogone*. For *Verticillium*, flusilazole, imazalil, cyproconazole and BASF 49004F were as toxic as the standard prochloraz manganese. Metconazole, propiconazole and fenpropimorph were as toxic as benomyl to *Trichoderma*. Unfortunately, all of these products with the exception of ISK Biotech 66825 and BASF 49004F were toxic to *A. bisporus*. At 20 ppm many of the tested fungicides were toxic to the pathogens. Fenpropimorph, propiconazole, flusilazole and metconazole were almost totally inhibitory to all of the organisms tested including *A. bisporus*.

Table 2 Growth of *A. bisporus* 609 and four fungal Pathogens at 2 ppm Fungicide Concentration.

Fungicides	609 strain	*C.dendroides*	*M.perniciosa*	*V.fungicola*	*T.harzianum*
Control	20	46	24	19	37
Prochloraz	18	13	7	6	10
Benomyl	20	15	6	19	9
Carbendazim	21	6	6	18	8
Thiabendazole	20	28	6	18	10
Flutriafol	19	15	10	13	35
Myclobutanil	12	11	8	15	23
Fenpropimorph	6	8	6	17	9
P.hydrochloride	19	40	23	18	56
Sulphur	20	36	17	15	51
Propiconazole	6	7	6	14	9
Fenarimol	15	11	7	14	24
ISK Biotech 66805	19	8	8	11	26
Flusilazole	10	7	6	7	10
Penconazole	10	8	9	9	44
Imazalil	14	14	7	7	22
Cyproconazole	6	8	10	7	28
Cymoxanil	19	28	12	10	85
Fenpropidin	10	19	6	12	28
Tebuconazole	6	28	6	16	28
Metconazole	6	25	6	11	9
BASF 49004F	16	62	9	9	85

Figures in the tables represent the average diameter per treatment in mm of the isolates. 6mm is the base value indicating no growth from the plug.

TABLE 3 Growth of *A. bisporus 609* and four fungal pathogens at 20 ppm fungicide concentration

Fungicides	609 strain	*C.dendroides*	*M.perniciosa*	*V.fungicola*	*T.harzianum*
Control	20	46	24	19	37
Prochloraz	6	6	6	6	6
Benomyl	16	6	6	7	6
Carbendazim	20	6	6	7	6
Thiabendazole	17	6	6	12	6
Flutriafol	8	6	6	8	33
Myclobutanil	6	6	6	9	15
Fenpropimorph	6	6	6	8	6
P.hydrochloride	20	85	17	9	85
Sulphur	18	85	15	9	85
Propiconazole	6	6	6	7	6
Fenarimol	6	6	6	10	17
ISK Biotech 66805	14	6	6	10	27
Flusilazole	6	6	6	7	6
Penconazole	6	6	6	8	46
Imazalil	6	6	6	6	18
Cyproconazole	6	6	6	6	64
Cymoxanil	6	6	6	8	60
Fenpropidin	6	6	6	9	20
Tebuconazole	6	6	6	9	12
Metconazole	6	6	6	9	6
BASF 49004F	15	62	6	9	76

Discussion

In order to be of value to the mushroom industry a fungicide must show good toxicity to the pathogens whilst being safe to use on the crop. The standard products such as the benzimidazoles and prochloraz manganese show such differential toxicity. In the case of carbendazim the difference is in the order of a 100 fold magnitude. The result of the testing of fungicides on agar reported here show that many of the new products especially the triazole and morpholine fungicides are toxic to the mushroom pathogens but also equally toxic to the mushroom. But two products ISK Biotech 66825, and an experimental BASF material, do show the required differential toxicity. The results with ISK Biotech 66825 are particularly promising. In a preliminary very small cropping experiment ISK Biotech 66825 has given a level of control of *Mycogone* comparable with that of carbendazim (Bavistin DF). This product (sold in the USA as Francide) would appear to have considerable potential for use on the mushroom crop providing residues in the treated mushrooms are at acceptable levels. Further in vivo work needs to be done with this fungicide as it is chemically unrelated to anything at present available for mushroom growers and could be invaluable especially if resistance to the existing products becomes a problem. ISK Biotech are at present pursuing registration of this fungicide on other crops in the UK and when it is approved, the mushroom industry might be able to obtain an off-label approval providing residue data is available.

One additional aspect of this work of considerable relevance to the mushroom industry is the toxicity shown by a number of products, fenpropimorph (Corbel), propiconazole (Tilt), flusilazole (Sanctas) and metconazole, to all the organisms tested. Such properties are those required of an effective tray dip at the end of cropping to eliminate both viable mushroom mycelium and fungal pathogens. It is possible that formulations of these products could be developed as tray dips and would be more environmentally friendly than sodium pentachlorophenate, or the phenolic materials, at present in use.

Conclusions

Only ISK Biotech 66825 and BASF fungicides were shown to be toxic to mushroom pathogens and not to *A. bisporus*. They merit further investigation with the ultimate aim of off-label registration for use in the mushroom industry.

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