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Summary

As a result of the diminished efficacy of Sporgon 50WP, the sole fungicide used to control *Verticillium*, the main objective of this project has been to screen potential fungicides for their effectiveness against this pathogen.

Ten fungicides were tested *in vitro* for their effect on spore germination and mycelial growth of primarily *Verticillium*, but also *Mycogone, Dactylium* and *Trichoderma harzianum* Th2. Their effects on *Agaricus* mycelium were also tested as a measure of likely phytotoxicity. Bavistin DF and Sporgon 50WP were also included in the tests as known materials against which the novel fungicides might be compared.

None of the fungicides appeared outstanding when compared to Sporgon 50WP. One chemical however, Chemical F, had considerable effect against both *Verticillium* mycelium and spore germination, making it a potential candidate for further screening. However, its apparent phytotoxicity against *Agaricus, in vitro,* would need to be evaluated under standard cropping conditions (*in vivo*).

The search for an alternative chemical to Sporgon 50WP should continue.

ACTION POINTS FOR GROWERS

Until a new chemical is found, if *Verticillium* control is not being achieved despite the use of Sporgon 50WP, a number of factors should be considered.

- Under high inoculation pressure control will be difficult to achieve. Every effort should therefore be made to ensure that hygiene measures are effective. Badly infected third flushes should be cooked out or terminated without further picking.

- With the knowledge that Sporgon 50WP levels in casing drop steadily during the cropping period (See HDC report M 14c), it is vitally important to check that the levels of fungicide in the casing after treatment are adequate. In the event of poor control casing samples should be tested for the amount of active ingredient present.

- In the event of unsatisfactory control being achieved, the resistance status of *Verticillium* isolates should be established as it appears that two populations of *Verticillium* are present on British mushroom farms. About 64% of isolates are somewhat resistant to Sporgon 50WP while 30% of isolates are more sensitive, however, the more sensitive isolates appear to be more aggressive pathogens (See HDC report M 14c). Nonetheless, it is likely that in many instances the more resistant isolates will be more difficult to effectively control thus placing greater emphasis on both good hygiene and efficient pesticide application.

SCIENCE SECTION

Screening fungicides for potential control of primarily *Verticillium*, also *Mycogone*, *Dactylium* and *Trichoderma*.

1. Introduction

In the late 1960's and early 1970's, the benzimidazole fungicide Benlate, (active ingredient (a.i.) benomyl), was widely used to control *Verticillium*, however, within a few years of its introduction widespread resistance to this chemical had developed throughout the British *Verticillium* population (Fletcher & Yarham, 1976). In the 1980's, a new chemical, Sporgon 50WP (a.i. prochloraz manganese) was launched to control *Verticillium*, with good results, and this chemical has been relied on heavily since that time to control *Verticillium* outbreaks in Britain (Fletcher *et al.*, 1983). In the early 1990's, an epidemic of cobweb disease, caused by *Cladobotryum* (*Dactylium*) *dendroides*, occurred in Britain and Ireland, and problems in controlling the disease were associated with, among other things, the development of weak or total resistance to the benzimidazole fungicides Bavistin DF (a.i. carbendazim) and Hymush (a.i. thiabendazole) (Grogan *et al.*, 1996; HDC report M 14a). To date, the benzimidazole fungicides are still effective against the other two major fungal pathogens of *Agaricus*, namely *Mycogone perniciosa* and *Trichoderma harzianum* Th2 (Grogan *et al.*, 1996; 1998; HDC reports M 14a and M 14b).

In recent years, the effectiveness of Sporgon 50WP has been called into question as growers claim that, despite Sporgon 50WP use, *Verticillium* outbreaks occur where previously they had been controlled. Recently completed HDC-funded research (Grogan *et al.*, 1998; HDC report M 14b) indicates that a significant proportion (64%) of British *Verticillium* isolates tested were moderately resistant to Sporgon 50WP, and capable of restricted growth at concentrations of prochloraz manganese which would be present in casing. *Cladobotryum* (*Dactylium*) isolates also demonstrated weak resistance to prochloraz manganese thereby reducing the efficacy of this fungicide, particularly if good hygiene practices are not in place to curtail the spread of disease within a mushroom farm. Inoculation studies indicate that Sporgon 50WP will significantly reduce disease losses caused by both *Verticillium* (Grogan *et al.*, 1999; HDC report M 14c) and *Cladobotryum* (Grogan *et al.*, 1998; HDC report M 30) but, under high inoculation loads, it will not give total control.

The British mushroom grower has a choice of four fungicides to control disease outbreaks:
Bravo (a.i. chlorothalonil) - has a severe mycotoxic effect on *Agaricus*;
Bavistin DF - all *Verticillium* and many *Cladobotryum* (*Dactylium*) isolates are resistant;
Hymush - all *Verticillium* and many *Cladobotryum* (*Dactylium*) isolates are resistant;
Sporgon 50WP - many *Verticillium* and *Cladobotryum* (*Dactylium*) isolates are weakly resistant.

Mushroom growers can no longer afford the financial losses associated with major disease epidemics yet there is a strong drive towards reducing the amount of chemicals used in crop production worldwide. Current research programs seek to understand the factors which affect the severity of disease outbreaks in order to identify better hygiene measures

or crop management techniques which can reduce disease levels. Such work is often costly and time consuming and in the meantime growers need to be able to use chemicals to contain and control a disease situation.

The British mushroom industry is heavily dependent on Sporgon 50WP which has been demonstrated to give good but not total control of either *Verticillium* or *Cladobotryum* (*Dactylium*) and, should further resistance to this product emerge, then disease control will be severely jeopardized. The industry needs to identify potentially useful chemicals long before the effectiveness of existing chemicals is completely undermined as again this work takes time, and new chemicals with different modes of action are continuously being sought for all the major food crops.

If the effectiveness of Sporgon 50WP is compromised any further, the financial impact on the British mushroom industry will be severe. This research report was commissioned to continue the search for a new fungicide effective against mushroom pathogens, in particular *Verticillium*, in order to reduce the heavy reliance and pressure on a single product which carries serious repercussions for the industry in the event of that product failing for whatever reason.

2. Materials and Methods

2.1. Chemicals

Ten fungicidal products, currently being used in agriculture or horticulture, were provided for study by various chemical companies and/or mushroom allied trades for *in vitro* screening. Three of the products are marketed as "biocontrol products" (See Appendix 1 for details). The fungicides Sporgon 50WP and Bavistin DF were also included for comparison. A list of the products and their active ingredients is given in Table 1. Prior to pouring culture media plates, a fresh stock of chemical was prepared at a concentration of active ingredient of 1000 mg/litre, equivalent to 1000 ppm. For the alternative products, BioBooster, Deon and RidoVert, the stock solution was prepared by adding 100 times the standard dose rate to 1 litre of water (based on a single dose being applied in 1 litre of water per m^2 of mushroom bed).

It should be noted that none of the experimental products are approved for use on mushrooms and use on this crop is illegal.

Table 1. Details of chemicals used in *in vitro* screening experiments

Active ingredient (a.i.)	**Product name**	**Manufacturer**	**% a.i.**
Prochloraz-Mn	Sporgon 50WP	AgrEvo	46
Carbendazim	Bavistin DF	BASF	50
Azoxystrobin	Amistar	Zeneca Crop Protection	25
Cinnamaldehyde	Vertigo	Amycel	50
Fenbuconazole	Indar 5EW	Headland	5
Flusilazole	Benocap	Dow AgroSciences Ltd	20
Chemical F	--details withheld--		
Iprodione	Rovral WP	Rhône-Poulenc Agriculture	50
Pyrimethanil	Scala	Promark	40
Biocontrol products:			
See Appendix 1	Biobooster	Shieer Tec B.V.	Appendix 1
See Appendix 1	Deon	Shieer Tec B.V.	Appendix 1
See Appendix 1	RidoVert	Galway homeo-pathics Ltd	Appendix 1

2.2 Media preparation

All *in vitro* testing was done using a Glucose/Gelatin medium (GGM) (Appendix 2). This is a low nutrient growth medium which encourages organisms to utilise their enzyme systems in order to obtain nutrition. Bottles of media were prepared, sterilised by autoclaving at 121°C for 15 minutes, allowed to cool to 50°C prior to incorporating the fungicides and then poured into sterile 90 mm diameter Petri dishes. Each fungicide to be screened was incorporated into the medium to give concentrations of 0, 0.2, 2 and 20 ppm using different volumes of the concentrated (1000 ppm) stock solution. For the

alternative products, three concentrations were also prepared but as the a.i. of these products is not as defined as in mainstream chemicals, the concentrations chosen consisted of x 1, x 10 and x 100 a standard dose/litre of water.

2.3 Culture preparation

The mushroom pathogens *Verticillium fungicola*, *Mycogone perniciosa*, *Cladobotryum (Dactylium) dendroides*, *Trichoderma harzianum* Th2 as well as *Agaricus bisporus* were used in all *in vitro* tests to determine their sensitivity to the selected chemicals. Stock cultures of the organisms were grown on either Oxoid Potato Dextrose Agar (*Verticillium fungicola* and *Mycogone perniciosa*) or Oxoid Malt Extract Agar (*Cladobotryum (Dactylium) dendroides*, *Trichoderma harzianum* Th2 and *Agaricus bisporus*). Cultures were prepared in advance so that on the day when *in vitro* tests were to be set up, they had a growing margin from which subcultures could be taken.

2.4 *In vitro* tests

Mycelial growth

Culture plugs of each pathogen, and *A. bisporus* were taken from the growing edge of a fresh culture and placed on a series of Petri dishes containing GG medium amended with 0, 0.2, 2 and 20 ppm of a given chemical. Three replicate plates were prepared for each organism at each concentration of active ingredient. Radial growth was measured over a period of up to 14 days, depending on the growth rate of each organism. When growth measurements were completed, the growth of organisms on fungicide amended media was expressed as a percentage of the growth on media containing no fungicide. Results were then compared with those for Sporgon 50WP and Bavistin DF to determine if the test chemicals were more or less inhibitory than these two fungicides, and whether or not they appeared to have potential for further testing.

For the alternative products, BioBooster, Deon and RidoVert, a different method was used as it was obvious that some products appeared to be none-sterile. Incorporation of none-sterile liquid into the sterile culture media would have resulted in excessive bacterial growth within the medium which could have affected the results. As an alternative, sterile 13 mm antibiotic assay filter discs were placed on the left hand side of Petri-dishes containing unamended GG medium. A small volume of each dilution (125 μ ml) was then pipetted onto each disc. A mycelial plug of the desired organism was then placed opposite the product-impregnated disc. Radial growth was recorded in two directions: (i) towards the impregnated discs and (ii) away from of the impregnated discs, with growth in the direction of the disc being expressed as a proportion of the growth away from the disc. These proportions were then expressed as a percentage of the growth-ratio in the controls (which was usually in the region of 1.0).

Spore germination

Spore suspensions of the four pathogens were prepared to give in the region of 1 million spores per ml (1 x 10^6 spores/ml). A dilution series was then prepared to give 1 x 10^2, 1 x 10^4 and 1 x 10^6 spores/ml. A 0.1 ml aliquot of each spore suspension was then plated out

on to GG medium amended with the test chemicals, to give 10, 1000 and 100 000 spores per plate. Two replicate plates were prepared for each spore concentration at each concentration of the test chemical. Percentage germination levels were expressed to the nearest 25% when compared with controls.

For the alternative products, BioBooster, Deon and RidoVert, impregnated filter discs were placed on Petri-dishes of GG medium which had been seeded with 1000 spores per dish. For each concentration of product, two Petri-dishes were prepared, each receiving five product-impregnated filter discs. If germination in the zone around a filter disc was inhibited, then it scored 0 and if germination was uninhibited it scored 1. This gave a possible maximum score of 10 for each concentration, which was converted into a percentage value compared with the control.

Results from both the mycelial growth and spore germination tests were then compared with those for Sporgon 50WP and Bavistin DF to determine if the test chemicals were more or less inhibitory than these two fungicides, and whether or not they appeared to have potential for further testing.

3. Results

None of the chemicals tested gave very encouraging results in terms of a potential new chemical for use in the battle against mushroom pathogens. The results for each chemical are discussed individually.

3.1 Sporgon 50WP (prochloraz manganese)

Mycelial growth: Figure 1 shows the growth responses of the four pathogens and *A. bisporus* to prochloraz manganese. *Agaricus* is unaffected at concentrations of 0.2 and 2 ppm but is inhibited at 20 ppm whereas all pathogens, except *Verticillium*, show a good degree of inhibition, *in vitro*, at concentrations of 0.2 ppm upwards. The *Verticillium* isolate chosen was one identified as being weakly resistant to prochloraz, but which is none the less significantly controlled by it. This chemical is thus still relatively effective in controlling all major mushroom pathogens while being not very toxic to *Agaricus* at the recommended rate of use. **This set of growth response curves, or better, is therefore considered to be a prerequisite of any new chemical to be considered for further tests.**

Spore germination: Spore germination of all pathogens, except *Verticillium*, was completely inhibited by prochloraz manganese at both 2 and 20 ppm (Table 2). *Verticillium* spores were completely inhibited at 20 ppm only but at 2 ppm 75% germination occurred. Subsequent growth of those spores which germinated however was significantly reduced, with colony diameters being only 30% of the controls.

3.2 Bavistin DF (Carbendazim)

Mycelial growth: Figure 2 shows the growth responses of the four pathogens and *A. bisporus* to carbendazim, another approved and effective chemical for some pathogens. *Agaricus* is unaffected by this fungicide at concentrations of 0.2 and 2 ppm but is inhibited at 20 ppm. *Verticillium* grew well at all concentrations and *Dactylium* grew well at all concentrations except 20 ppm. The isolates of these two pathogens which were used in this test were known to be resistant to benzimidazole fungicides to varying degrees, and were chosen for that reason, so any chemicals which gave growth responses similar to these would be of no interest for these organisms. Both *Trichoderma harzianum* and *Mycogone* perniciosa were inhibited at concentrations of 2 ppm or above, and Bavistin DF is still used to control these pathogens.

Spore germination: Carbendazim completely inhibited germination of both *Trichoderma harzianum* and *Mycogone* spores at 2 and 20 ppm. *Verticillium* spores germinated well at both concentrations while *Dactylium* spores germinated well at 2 but not 20 ppm. These results reflect the sensitivities of these organisms to carbendazim.

3.3 Amistar (azoxystrobin)

Mycelial growth: Figure 3 shows the growth responses of the four pathogens and *A. bisporus* to azoxystrobin. None of the organisms tested were inhibited to any significant degree over the range of concentrations tested.

Spore germination: Only *Mycogone* spores were completely inhibited at 2 and 20 ppm. Between 50 and 100% of spores of all other pathogens germinated at these concentrations. However, the subsequent growth of *Verticillium* spores was significantly reduced to about 10-25% of the controls.

3.4 Vertigo (cinnamaldehyde)

Mycelial growth: None of the organisms tested showed any inhibition at concentrations of cinnamaldehyde of up to 2 ppm, and only *Dactylium* and *Mycogone* were significantly inhibited at 20 ppm, and then not less than a level of 50%.

Spore germination: Spores of all pathogens germinated at 2 ppm cinnamaldehyde but *Verticillium* and *Mycogone* spores were inhibited at 20 ppm.

Vertigo (cinnamaldehyde) is an approved fungicide for the control of *Verticillium* in mushrooms in the USA where the chemical Sporgon 50WP is not available for use. The *in vitro* data (and personnel communications with USA research workers) suggest that Sporgon 50WP is the more effective chemical. It may however, be a useful alternative should greater resistance to Sporgon 50WP develop. **It may therefore be worthwhile to compare the efficacy of these two chemicals at some time in the future.**

3.5 Indar 5EW (fenbuconazole)

Mycelial growth: *Verticillium fungicola* was the least inhibited by this chemical, followed by *Agaricus bisporus* (Figure 5). *Trichoderma harzianum* was moderately inhibited while *Dactylium* and *Mycogone* were more significantly inhibited.

Spore germination: Fenbuconazole effectively inhibited spore germination of *Mycogone*, and to a lesser extent *Dactylium* and *Trichoderma harzianum* (Table 2). All *Verticillium* spores germinated at 2 and 20 ppm but at 20 ppm, subsequent growth was inhibited to about 20-25% of the controls.

3.6 Benocap (flusilazole)

Mycelial growth: *Verticillium fungicola* was the least inhibited by this chemical (Figure 6). *Agaricus bisporus* growth was significantly inhibited at relatively low concentrations as were *Trichoderma*, *Dactylium* and *Mycogone*.

Spore germination: Flusilazole effectively inhibited spore germination of *Mycogone* at 2 and 20 ppm, and reduced germination levels in *Dactylium* and *Trichoderma harzianum*

(Table 2). All *Verticillium* spores germinated at 2 and 20 ppm but at 20 ppm, subsequent growth was inhibited to about 10% of the controls.

3.7 Chemical F.

Mycelial growth: *Agaricus bisporus* and *Verticillium* were both significantly, but not totally, inhibited over the range of concentrations tested whereas remaining pathogens were only slightly inhibited (Figure 7).

Spore germination: Chemical F completely inhibited germination of *Verticillium* spores at 20 ppm and reduced germination to 50 % at 2 ppm. In addition, subsequent growth at 2 ppm was reduced to less than 10 % of the controls. **This feature may make it worthwhile to test this chemical under cropping conditions to see if spore germination, and subsequent mushroom infection could also be inhibited significantly by this chemical. However, *Agaricus* growth was also inhibited by this chemical but cropping experiments would be needed to establish if there was any significant phytotoxic effect *in vivo*.**

3.8 Rovral WP (iprodione)

Mycelial growth: *Verticillium fungicola* was the least inhibited by iprodione, followed by *Agaricus bisporus* (Figure 8). *Trichoderma harzianum* and *Dactylium* were significantly inhibited at 2 and 20 ppm while *Mycogone* was more tolerant at 2 but inhibited at 20 ppm.

Spore germination: Iprodione inhibited spore germination of all pathogens at 20 ppm but not at 2 ppm (Table 2).

3.9 Scala (pyrimethanil)

Mycelial growth: Pyrimethanil had little or no inhibitory effect on *Verticillium*, *Dactylium* or *Trichoderma* over the range of concentrations tested, and was only moderately inhibitory to *Mycogone* at 20 ppm (Figure 9).

Spore germination: Pyrimethanil had no inhibitory effect on *Verticillium*, *Dactylium* or *Trichoderma* (Table 2). (There was insufficient time to carry out spore germination tests for *Mycogone* but based on the mycelial growth response, it is likely that this chemical would not have completely inhibited the germination of *Mycogone* spores).

Figure 1. Prochloraz manganese

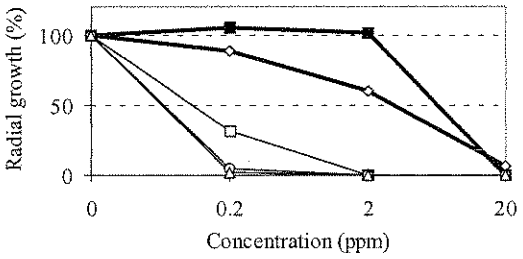


Figure 2. Carbendazim

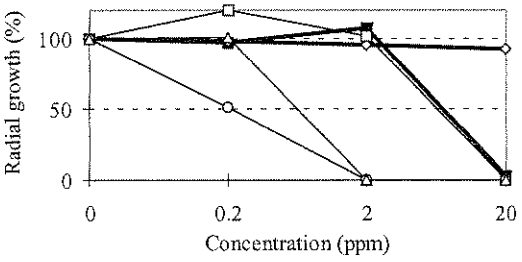


Figure 3. Azoxystrobin

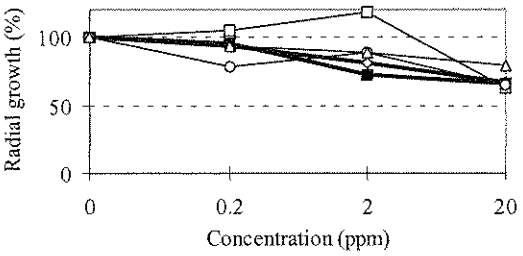


Figure 4. Cinnamaldehyde

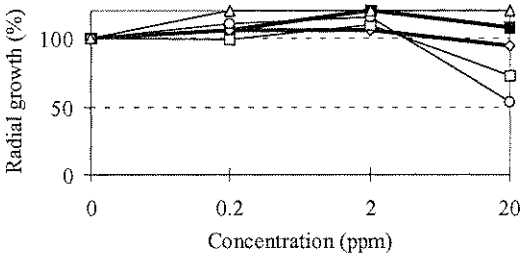


Figure 5. Fenbuconazole

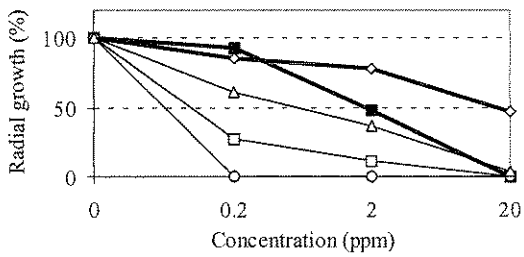


Figure 6. Flusilazole

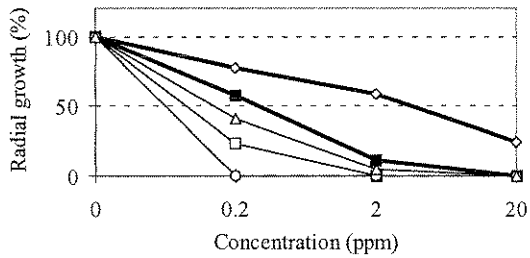


Figure 7. Chemical F

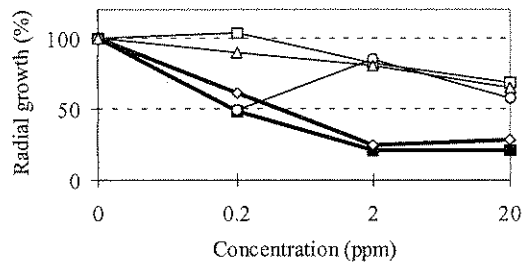


Figure 8. Iprodione

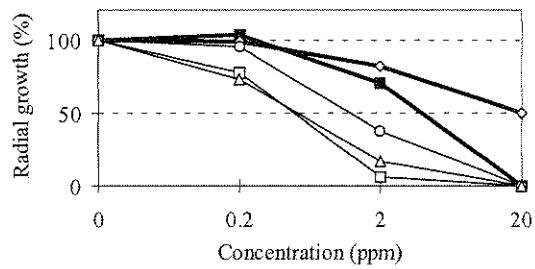
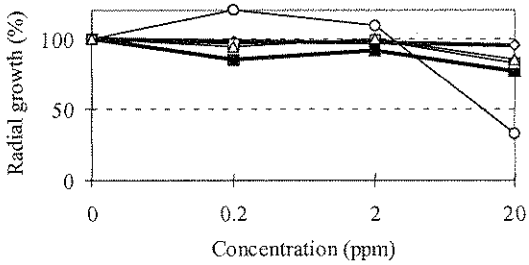


Figure 9. Pyrimethanil



Legend

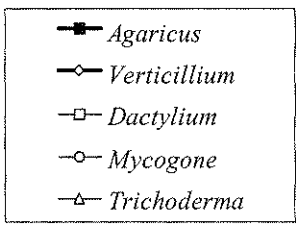


Table 2. Percentage spore germination of four pathogens in the presence of 12 chemicals at concentrations of 2 and 20 ppm.

	Pathogen							
	Verticillium		*Dactylium*		*Trichoderma*		*Mycogone*	
Chemical (a.i.) Conc. (ppm)	***2***	***20***	***2***	***20***	***2***	***20***	***2***	***20***
Prochloraz	75*	0	0	0	0	0	0	0
Carbendazim	100	75*	100	0	0	0	0	0
Azoxystrobin	100*	100*	100	50	100	100*	0	0
Cinnamaldehyde	100*	0	100	100*	100	100*	100	0
Fenbuconazole	100	100*	100*	0	50	25*	0	0
Flusilazole	100*	100*	50	0	50	0	0	0
Chemical 'F'	50*	0	100	50	100	100*	100	100
Iprodione	100	0	100	0	100*	0	100	0
Pyrimethanil	100	100	100	100	100	100	n.t.#	n.t.
Biocontrol agents: Conc.	***x10***	***x100***	***x10***	***x100***	***x10***	***x100***	***x10***	***x100***
BioBooster	100	100*	100	100*	100	100	0	0
Deon	100	100	100	100	100	100	100	100*
RidoVert	100	100	100	100	100	100	100	100

* growth significantly inhibited

\# n.t. = not tested

3.10 Biobooster

Mycelial growth: BioBooster significantly inhibited the growth of *Agaricus* and *Mycogone* but had little effect on the remaining pathogens (Figure 10).

Spore germination: BioBooster appeared to prevent germination of *Mycogone* spores at x 10 and x 100 the standard dose but all other pathogens germinated at 100% of the controls (Table 2). Subsequent growth of *Verticillium* and *Dactylium* was marginally reduced.

3.11 Deon

Mycelial growth: None of the pathogens were inhibited to any degree by Deon over the range of concentrations tested whereas *Agaricus* was very significantly inhibited (Figure 11). There was significant bacterial growth associated with this product.

Spore germination: Deon had virtually no inhibitory effect on spore germination by any of the pathogens (Table 2).

3.12 RidoVert

Mycelial growth: RidoVert had virtually no inhibitory effects on the growth of any of the pathogens whereas *Agaricus* growth was inhibited by about 60% (Figure 12).

Spore germination: RidoVert had virtually no inhibitory effect on spore germination by any of the pathogens (Table 2).

Figure 10. BioBooster

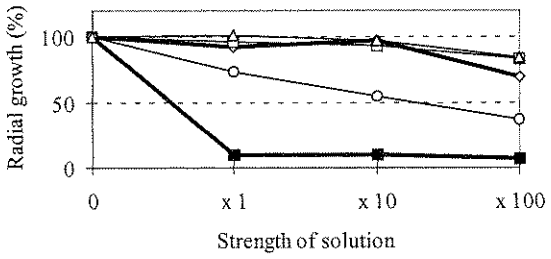


Figure 11. Deon

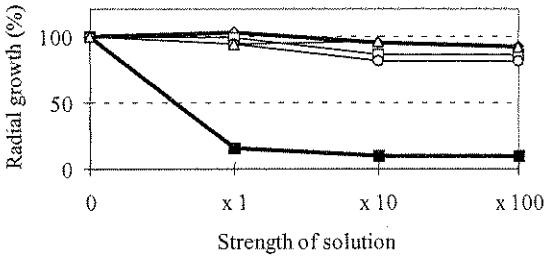
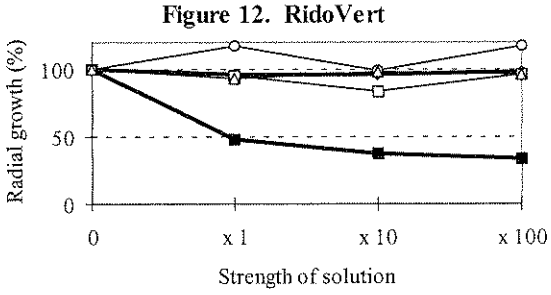
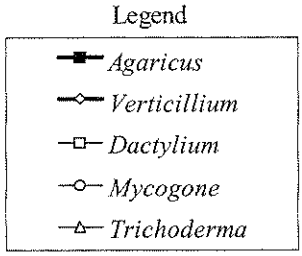


Figure 12. RidoVert





4. Conclusions

- Any chemical which is to give better control of *Verticillium* than Sporgon 50WP, would be expected to inhibit mycelial growth by at least 50% at 2 ppm, and by at least 75% at 20 ppm. Spore germination should also be significantly inhibited at 2 and 20 ppm. In addition, *Agaricus* growth must not be totally inhibited over this range of concentrations.

- No chemical completely inhibited the growth of *Verticillium* at 20 ppm with most chemicals having little or only moderate inhibitory effects.

- Chemical F was the most inhibitory chemical against *Verticillium*. Total inhibition was not observed over the range of concentrations tested, but mycelial growth was reduced by 75% at both 2 and 20 ppm. Spore germination was also significantly inhibited at these concentrations. Although *Agaricus* was also significantly inhibited, this chemical might be worth examining further in small scale cropping experiments.

- None of the biocontrol agents appeared to have any significant effect on *Verticillium,* or the other pathogens tested (except BioBooster against *Mycogone*) under the conditions of these experiments.

- Fenbuconazole and Flusilazole were very inhibitory to *Mycogone* and, to a lesser extent, *Dactylium*, while being only moderately inhibitory to *Agaricus*. These chemicals could merit further investigation at the cropping level if existing chemicals fail to control these pathogens.

- The search for an alternative chemical to Sporgon 50WP must continue.

5. References

Fletcher, J.T. & Yarham, L.J. (1976). The incidence of benomyl tolerance in *Verticillium fungicola, Mycogone perniciosa* and *Hypomyces rosellus* in mushroom crops. *Annals of Applied Biology*, **84**, 343-353.

Fletcher, J.T., Hims, M.J. & Hall, R.J. (1983). The control of bubble disease of mushrooms with prochloraz. *Plant Pathology*, **32**, 123-131.

Grogan, H.M., Gaze, R.H. & Scruby, A. (1996). HDC report M 14a: 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. pp33.

Grogan, H.M., Gaze, R.H., Amey, R. & Scruby, A. (1998). HDC report M 14b. Survey of fungicide resistance in the mushroom pathogens *Verticillium fungicola* and *Mycogone perniciosa.* pp18.

Grogan, H.M., Gaze, R.H., Amey, R. & Scruby, A. (1998). HDC report M 30. Relationship of laboratory resistance to practical control of *Dactylium*. pp26.

6. Appendices.

Appendix 1.

RidoVert - Label information:

RidoVert liquid
Disease treatment for Verticillium and Mycogone on mushroom crops
A natural product, chemical free, safe for user, safe for consumer

Should be stored at room temperature out of extreme cold and heat, away from strong odours and direct sunlight.

Supplier:

Galway homeopathics Ltd
Chapel Lane
Tuam
Co Galway
Ireland
Tel/Fax:
IRL 093 25495

23, Chance Croft
Queensway,
Oldbury
West Midlands
B68 0JY
England
Tel/Fax: UK 0121 421 3210

Ingredients:

Staph 30c
Carbo/Veg 30c
Aconite 30c
Ethanol
Verticillium

Application:

0.5 litres to 152 m^2 850 bags 3.3 cc/m^2.
0.75 litres to 204 m^2 1150 bags 3.7 cc/m^2.

One application in case run
One application after first flush
One application after 2nd flush

Appendix 1 (continued)

Products: **Deon, BioBooster**
(Information provided by a commercial spawn representative)

Supplier:

Shieer Tec B.V.
Ohmweg 11
3442 AA Woerde
Nederland

Tel: 00 31 34 84 34 337
Fax: 00 31 34 84 31 372

Products:	**Details:**	**Rate of use:**
Deon:	Fights cobweb and green mould and insects	3.0 cc/m^2
BioBooster:	Fights off everything except mycelium	1.5 cc/m^2

Timing:

Full dose	1st day of casing
Full dose	4 days later
Full dose	last water/ruffle
.....	
3 waterings	one third each day
same	with 2nd
same	with third
.....	

6 doses

Appendix 2.

Recipe for glucose Gelatin Medium (GGM)

Glucose	4.0 grams
Gelatin	4.0 g
K_2HPO_4	1.75 g
$MgSO_4.7H_2O$	0.75 g
H_2O	1.0 litre
Agar Technical (No. 3, OXOID)	10.0 g

- Weigh out all dry ingredients and place all, except the Agar, into a conical flask.
- Add the water and dissolve ingredients using a magnetic stirrer and hotplate.
- Place agar into a media bottle and then add the dissolved solution, mixing gently.
- Autoclave for 15 minutes at 121°C.
- Cool to 50-55 °C before incorporating fungicides.