Project title	Mushrooms: Inhibiting fungicide degradation in casing, and evaluating fungicides, biopesticides and diseased area covering methods for fungal disease control
Project number:	M 62
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Report:	Final, March 2017
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Location of project:	NIAB EMR, Pershore Centre and grower sites
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Date project commenced:	1 September 2015
Date project completed (or expected completion date):	31 March 2017
Key words:	Mushrooms, Fungicides, Diseases

CONTENTS

GROWER SUMMARY

Headline

- Vivando (a.i. metrafenone) provided good control of dry bubble disease irrespective of the prochloraz resistance of the *Lecanicillium fungicola* isolate
- Vivando and Sporgon (a.i. prochloraz) were equally effective in controlling wet bubble disease but Vivando also provided control of cobweb disease
- Shirlan (a.i. fluazinam) did not affect mushroom yield or produce fluazinam residues and has potential for disease control if tested at a higher rate
- Prochloraz degrades less rapidly in casing than metrafenone or fluazinam; the rate of degradation can be reduced by adding 25% recycled casing to fresh casing
- Glucose solution increased bacterial populations of the casing without causing blotch and may therefore have potential to suppress blotch causing Pseudomonads
- A 70% clay: 30% salt mixture was as effective in suppressing regrowth of pathogens and diseased mushrooms as salt, but with a smaller effect on SMC EC

Background and expected deliverables

Sporgon (a.i. prochloraz-manganese which rapidly dissociates into prochloraz) is the only approved fungicide for the UK mushroom industry. It provides good control of wet bubble (*Mycogone perniciosa*), moderate control of dry bubble (*Lecanicillium fungicola*) and weak or ineffective control of cobweb (*Cladobotryum* species). There has been reported resistance in some *Lecanicillium fungicola* isolates to prochloraz. Vivando (a.i. metrafenone) is approved for use on mushrooms in France and Spain. There is anecdotal evidence that Shirlan (a.i. fluazinam) can give control of cobweb disease.

Biopesticides for control of fungal patens in mushroom crops are restricted to bacterial products, since the mushroom is also a fungus. *Pseudomonas chlororaphis* MA342 (Cedemon or Cedress) has an EU registration for control of fungal diseases on cereals and Serenade Soil (*Bacillus subtilis* QST 713) is registered for a wide range of fungal pathogens on crops.

Fungicide-degrading microbes can metabolise prochloraz and other pesticides into inactive by-products, thereby reducing the efficacy of an applied dose. Inhibition of prochloraz and other fungicide degradation by promotion of a microbial population antagonistic to fungicide degrading organisms could lead to improved disease control.

Information from *in vitro* agar plate tests can provide information on the inhibition of mycelial growth and spore germination of different fungal pathogen isolates to different fungicide concentrations. However, it is unclear how these fungicide concentrations translate into effective concentrations in casing *in vivo*.

Salt is widely used for covering patches of diseased mushrooms on growing beds but this has an adverse effect on the subsequent use of the spent compost in casing or growing media by increasing the electrical conductivity. The uses of mixtures with sand or clay may reduce the salt application to the casing thereby improving the quality of the spent compost.

Summary of the project and main conclusions

1. Mushroom yield was not significantly affected by casing amendments (bark, green waste compost, recycled casing, glucose solution), fungicides (Sporgon, Vivando, Shirlan) or biopesticides (Cedress, Serenade).
2. The application of a dilute glucose solution to the casing resulted in a sustained increase in the *Pseudomonas* and *Bacillus* spp. populations in the casing without stimulating bacterial blotch.
3. During a mushroom experiment in pots, prochloraz in the casing degraded by 46% compared with 81% for metrafenone and 77% for fluazinam.
4. Prochloraz degraded more rapidly when 25% bark was added to casing. Spent casing added at 25% did not affect prochloraz degradation in a pot experiment but reduced degradation in a large scale experiment from 53-71% to 25-33%.
5. Prochloraz was found in first flush mushrooms at levels just above the detection limit but not in mushrooms grown in 25% spent casing. Metrafenone was only detected in mushrooms grown in 25% bark or with Cedress added, and here only at levels close to the detection limit and well below the MRL for mushrooms. Fluazinam was not detected in mushrooms grown in any of the casing samples.
6. Sporgon and Vivando both significantly reduced the number of mushrooms with dry and wet bubble diseases compared with the untreated control. Shirlan also suppressed dry and wet bubble diseases but the effects were not quite significant at $P = 0.05$.
7. Cedress suppressed wet bubble disease (effect not quite significant at $P = 0.05$) but did not significantly affect dry bubble or cobweb diseases. It did not cause blotch.
8. A *Lecanicillium fungicola* isolate that showed resistance to prochloraz in agar plate tests was also resistant to Sporgon in a pot culture test. However, inhibition of pathogen mycelial growth rate or spore germination on agar plates did not fully reflect potential disease control with fungicides in pot culture tests.

9. The use of a 70% clay:30% salt mixture was as effective as 100% salt in covering diseased areas and prevent regrowth of pathogens or diseased mushrooms; using clay and salt instead of salt reduced the EC of the final spent casing.

Financial and environmental benefits

- Less dependency on a single fungicide (Sporgon a.i. prochloraz) assuming Vivando (a.i. metrafenone) is registered for use in the UK in 2017
- Control of dry bubble disease caused by prochloraz resistant *Lecanicillium fungicola* isolates, wet bubble disease and of cobweb disease using Vivando
- Reduced fungicides residues in spent compost for metrafenone compared with prochloraz
- Recycling 25% casing reduces dependency on peat and may reduce the degradation of prochloraz in casing following Sporgon application
- Stimulation of a non-pathogenic population of Pseudomonads in the casing, either by adding Cedress (*Pseudomonas chlororaphis*) or a dilute glucose solution may provide antagonism to pathogenic *Pseudomonas* species that cause blotch
- Using a clay:salt mixture for disease covering was as effective in preventing regrowth of pathogens or diseased mushrooms as salt, but with a smaller and less detrimental effect on the EC of the spent casing

Action points for growers

- Look out for the UK registration of Vivando to augment disease control using Sporgon
- Investigate use of recycled casing to reduce prochloraz degradation in casing
- Use a mixture of 70% clay and 30% salt instead of salt to reduce EC in spent SMC

SCIENCE SECTION

Introduction

Sporgon (a.i. prochloraz-manganese which rapidly dissociates into prochloraz) is the only approved fungicide for the UK mushroom industry. It provides good control of wet bubble (*Mycogone perniciosa*), moderate control of dry bubble (*Lecanicillium fungicola*) and weak or ineffective control of cobweb (*Cladobotryum* species) if used at an individual dose of 120 g/100 m^2 (Fletcher & Gaze 2008). The current maximum dose is 100 g/ 100 m^2 (BASF/Sylvan Spawn). Since the 1990s there has been reported resistance to prochloraz in some *Lecanicillium fungicola* isolates (Grogan et al 1999; Gea et al 2005). Prochloraz is classed as an endocrine disrupter which threatens the long-term availability of Sporgon (Lyons, 1999). Vivando (a.i. metrafenone) is approved for use on mushrooms in France and Spain. There is anecdotal evidence that Shirlan (a.i. fluazinam) can give control of cobweb disease. Shirlan is approved for control of potato blight and has an extension of authorisation for minor use (EAMU, previously specific off-label approval SOLA) for root rot in cane fruit. The owners of fluazinam (ISK) and manufacturers of Shirlan (Syngenta) have agreed for Shirlan to be tested for mushroom disease control in this project. Metrafenone and fluazinam do not have known activity as an endocrine disrupter (Anon. 2000, 2007; Ewence et al 2013). The EU MRLs for fluazinam, metrafenone and prochloraz in mushrooms are 0.05, 0.4 and 3 mg/kg respectively.

Biopesticides for control of fungal pathogens in mushroom crops are restricted to bacterial products, since the mushroom is also a fungus. *Pseudomonas chlororaphis* MA342 (Cedemon) has an EU registration for control of fungal diseases on cereals and may therefore control fungal diseases in mushrooms. It may also suppress bacterial blotch in the same way as previous products based on non-pathogenic *Pseudomonas* spp. (Fahy et al 1981). The owners of Cedemon (Lantmannen Bioagri) have agreed to supply Cedemon for mushroom disease testing in this project and provide relevant toxilogical data if needed for an EAMU application. Serenade Soil (*Bacillus subtilis* QST 713) is registered for a wide range of fungal pathogens on crops.

Fungicide-degrading microbes can metabolise prochloraz and other pesticides into inactive by-products, thereby reducing the efficacy of an applied dose (Hollrigl-Rosta et al. 1999). Populations of such microbes can develop in spray tanks and in mushroom casing, thus resulting in a rapid degradation of active ingredient (Grogan et al. 2008). Lack of an antagonistic microflora in peat-based casing materials may enable such populations to develop rapidly and unopposed. Amendment of soils with composts and other organic materials has been shown to inhibit the microbial degradation of some pesticides (Dungan

et al. 2001; Siad-Pullicino et al. 2004). Defra and HDC funded work has shown that the casing bacterial population can be modified by different casing types and additives (Noble et al. 2003; Noble et al. 2009). Addition of casing nutrient supplements such as glucose may have a similar effective in modifying the casing microbiota (Masaphy et al. 1989; Nair et al. 1993). The bacterial biopesticide *Bacillus subtilis* (registered as Serenade Soil) may not be effective in suppressing fungal pathogens, but may be antagonistic to fungicide degrading bacteria in the casing. Inhibition of prochloraz and other fungicide degradation by promotion of a microbial population antagonistic to fungicide degrading organisms could lead to improved disease control.

Information from *in vitro* agar plate tests can provide information on the inhibition of mycelial growth and spore germination of different fungal pathogen isolates to different fungicide concentrations. Mycelial growth of *Mycogone perniciosa* and sensitive *Lecanicillium fungicola* isolates can be controlled by less than 10 ppm prochloraz whereas resistant *Cladobotryum dendroides* and *Lecanicillium fungicola* isolates can tolerate at least 20 ppm prochloraz (Fletcher & Jaffe 1993; Grogan et al. 1998). However, it is unclear how these prochloraz concentrations translate into effective concentrations in casing *in vivo*. Prochloraz concentrations of 10 and 100 ppm in agar controlled the mycelial growth of two *Lecanicillium fungicola* isolates, whereas two Sporgon applications, each equivalent to 17-20 ppm prochloraz in the casing, resulted in 94% and 75% control of dry bubble disease in a mushroom crop using the same two isolates (Grogan & Gaze 1997; Grogan et al 1999). The level of disease will depend on the pathogen isolate, inoculum concentration applied to the casing, the mushroom strain and other cropping factors. Clearly, Sporgon or other fungicides should not be used if the prevailing pathogen isolate(s) have sufficient fungicide resistance to make application ineffective; this can only be established rapidly using *in vitro* agar plate tests.

Due to the accumulation of mainly potassium salts, spent mushroom compost (SMC) typically has an electrical conductivity (EC) of around 2 mS cm^{-1} which restricts its application rate as a soil amendment or growing medium component (Szmidt & Chong 1995). Measurements by Desremaux et al. (2004) showed that by adding 1.25 kg salt/m^3 casing (equivalent to a typical salted disease patch every 20 m^2 casing surface) the EC was increased by around 0.5 mS cm^{-1}. This, together with the high sodium ion concentration would render the SMC useless as a growing medium component (Szmidt & Chong 1995). Adie et al. (2006) used wetted paper for covering cobweb diseased areas. This reduced the spread of *Cladobotryum* spores, but the paper was covered with salt to prevent the regrowth of the pathogen on the casing. A diseased area covering method which reduces the use of salt would improve the value of the SMC.

Materials and methods

Disease pot experiments

General cropping procedure

A series of experiments were conducted to assess the effects of different fungicides, biopesticides and casing additives on fungal disease control, mushroom yield and fungicide residues and degradation. Mushrooms were grown in plastic pots, 230 mm diameter x 220 mm depth, each containing 4 kg of Phase III compost, spawn-run with the mushroom strain Sylvan A15. The pots were cased with 1.3 L of casing materials (moist mixtures of peat and sugar beet lime, and other materials in some treatments). Spent casing, bark and green waste compost were wetted before incorporation into the casing mix. Caccing (spawn-run compost of the strain A15) was added to casing at 2.3 kg/m^3. The pots were watered and kept in a growing room at 25°C for 6 days; the room was then 'aired' and the air temperature reduced to 18°C. Three flushes of mushrooms were picked from the pots.

In all experiments, there were four replicate pots of each factorial treatment. The number of healthy and diseased mushrooms, disease type, and yield of healthy mushrooms were recorded.

Application of pathogen isolates to pots

Inocula of pathogen isolates of known propagule concentration (colony forming units, cfu/ mL) were applied in 100 mL water per pot to the casing in specified treatments in some of the experiments as described. Unless otherwise stated, pathogen isolates used in the experiments were obtained from recent disease outbreaks on English mushroom farms. The same volume of water was applied to untreated control pots. Pots with or without pathogen inocula were kept in separate areas of the room to reduce cross contamination.

Application of fungicides and biopesticides to pots

The following fungicide and biopesticide treatments were applied to pots, three days after casing was applied, at the rates shown unless otherwise stated:

(a) Shirlan (Syngenta, a.i. 38.4% fluazinam)
0.018 mL in 1.8 L water/m^2; 1µL in 100 mL water per pot

(b) Sporgon (BASF, a.i. 46% w/w prochloraz, as 50% prochloraz-manganese)
1 g/m^2 in 1.8 L water/m^2; 55.6 mg in 100 mL water per pot

(c) Vivando (BASF, a.i. 25.2% w/w/ metrafenone)
1mL/m^2 in 1.8 L water/m^2; 55.6 uL in 100 mL water per pot

(d) Cedress *Pseudomonas chlororaphis* (Lantmannen, a.i. 20% w/w *Pseudomonas*

chlororaphis MA342,10^9 -10^{10} cfu/mL)
18 mL/m^2; 1 mL in 100 mL water per pot.

(e) Serenade Soil (Bayer, a.i. 13.9 g/L *Bacillus subtilis* QST 713, 1.042 x 10^9 cfu/mL)
1.4 mL/m^2 ; 0.08 mL in 100 mL water per pot.

(f) Control, 100 mL water per pot.

Determination of bacterial populations in casing

The casing populations of *Pseudomonas* and *Bacillus* species at the start and end of the experiments were determined by plating casing extracts. *Pseudomonas* sp. were determined by using Pseudomonas isolation agar (PIA). The heat tolerance of *Bacillus* sp. spores was used to kill vegetative forms of bacteria. The extracts were first heat treated at 100ºC for 10 min followed by plating on Mossel agar plates (mannitol + yolk + polymyxin, MYP) and LB media. *B. subtilis* were identified as yellow-cream coloured colonies without a halo.

Experiment 1.1 Effect of casing additives on fungicide degradation and fungicide residues in mushrooms

The following casing additives were applied to peat casing at the volume inclusion rates shown, or to the surface of pots with peat casing in 100 mL water:

(a) Cooked-out spent casing (25 %v/v)

(b) Bark (12.5 %v/v) and green waste compost (12.5 %v/v)

(c) *Bacillus subtilis* QST 713 (Serenade Soil, AgraQuest) at 1.4 mL/m^2

(d) *Pseudomonas chlororaphis* MA342 (Cedress, Lantmannen) at 18 mL/m^2

(e) Glucose at 20 g/L in water

(f) Control (none).

Treatment pots (a), (b) and (e) received the same volume of water (100 mL) as the other sprayed treatments.

The following fungicide treatments were applied to the above pots, four days after the casings or casing additives were applied:

(a) Sporgon @ 1 g/m^2 in 1.8 L water/m^2; 55.6 mg in 100 mL water per pot

(b) Vivando @ 1mL/m^2 in 1.8 L water/m^2; 55.6 uL in 100 mL water per pot

(c) Shirlan @ 0.018 mL/m^2 in 1.8 L water/m^2; 1 uL in 100 mL water per pot

(d) Control, 100 mL water per pot.

Samples of casing (100 g) and mushrooms (100 g) from the first and second flushes were analysed by GC-MS and LC-MS for residues of prochloraz, metrafenone, and fluazinam. The analyses were conducted by Groen Agro Control, Delfgauw, The Netherlands.

Experiment 1.2 On-farm tests of casing additive on mushroom yield and Sporgon residues

Separated spent mushroom casing (Harte Peat casing) was obtained after the third flush of mushrooms had been picked and was cooked out at 60ºC for four hours. No Sporgon had been applied to the crop. The separated, cooked out casing was then incorporated at 25% v/v with fresh casing (Harte Peat casing) at the start of the crop at two commercial mushroom farms. Sporgon was applied at 0.16 g/m^2 (Farm A) and 1 g/m^2 (Farm B) to the crops on day 3 after filling. Samples of casing from areas with and without recycled casing (two replicates per crop) were then analysed for prochloraz residues after the third flush. The residues were analysed by SAL, Bar Hill, Cambs. Mushroom yields and disease incidence on areas with and without recycled casing were recorded. The samples were also tested for pH and electrical conductivity.

Experiment 2.1 Effect of fungicides and biopesticides on wet and dry bubble and cobweb diseases

The experiment was conducted as three consecutive sub-experiments (2.1.1, 2.1.2 and 2.1.3). In each of these sub-experiments, the following fungicide and biopesticide treatments were applied to pots, three days after casing was applied:

(a) *Pseudomonas chlororaphis* MA342 (Cedress, Lantmannen) at 18 mL/m^2
1 mL in 100 mL per pot.

(b) Shirlan 0.01 mL per L (0.018 mL in 1.8 L water per m^2)
1 µL in 100 mL water per pot

(c) Sporgon 1 g/m2 in 1.8 L water/m2
55.6 mg in 100 mL water per pot

(d) Vivando 1mL/m2 in 1.8 L water/m2
55.6 uL in 100 mL water per pot

(e) Control, 100 mL water per pot.

Preliminary tests were conducted with isolates of *Lecanicillium fungicola*, *Mycogone perniciosa* and *Cladobotryum dendroides* to determine the concentrations of inoculum that were required to obtain some disease without over-dosing. The highest inoculum concentration was required for *Cladobotryum* and the lowest for *Mycogone* (Table 1). Spore

inocula were prepared on plates, washed with sterile distilled water and diluted to produce the spore concentrations in Table 1. The pathogen suspensions (1 mL) were diluted in 2L water and applied in 100 mL water, six days after the pots were cased. In each sub-experiment, water (100 mL) was also applied to uninoculated controls. Pots with and without pathogen inoculum were kept in separate areas of the growing room and inoculated pots were picked first on each harvest date. Four replicate pots of each fungicide x pathogen treatment were prepared in each sub-experiment.

Table 1. Pathogen applied inoculum treatments (cfu mL^{-1}) used in Experiments 2.1 and 3.2

Pathogen	Isolate		Applied inoculum concentration, cfu/ml	
	Expt. 2.1	Expt. 3.2	Expt. 2.1	Expt. 3.2
Lecanicillium fungicola	A	A, B, C	2.9×10^2	2.0×10^2
Mycogone perniciosa	A	A, B, C	1.5	2.0
Cladobotryum dendroides	A	A, B, C	1.7×10^4	2.5×10^4

Experiment 3.1 Fungicide sensitivity of fungal pathogen isolates and Agaricus in vitro

Plates with potato dextrose agar (PDA) containing prochloraz, metrafenone or fluazinam at a range of concentrations from 0 to 200 ppm were prepared for testing the tolerance of mycelial growth and spore germination of three isolates each of *Lecanicillium fungicola*, *Mycogone perniciosa* and *Cladobotryum dendroides* and *Agaricus bisporus* (Sylvan A15) to the fungicides. For each test (mycelial growth or spore germination) three replicate plates were prepared for each isolate at each fungicide concentration, including a zero control. All the plates were incubated at 25ºC.

To test the effect of fungicide concentration on mycelial growth rate, a plug of each test isolate was plated into the centre of each plate. The mycelial front was then recorded at 1-2 day intervals until the plate was fully colonized and the radial growth rate was then calculated. To test the effect of fungicide concentration on spore germination, PDA plates with a range of fungicide concentrations were prepared as described above. Spore suspensions of the three isolates of *Lecanicillium fungicola* (5×10^6 to 6×10^6 spores/ml), *Mycogone perniciosa* (2.2×10^6 to 2.8×10^6 spores/ml) and *Cladobotryum dendroides* (3.8×10^6 to 4.3×10^6 spores/ml) were prepared. The suspensions (100 µL) were then plated on to three replicates of each fungicide concentration of the above agar plates. Spore germination was scored as positive if any of the three replicate plates of each pathogen isolate showed pathogen growth after incubation.

Experiment 3.2 Fungicide sensitivity of pathogen isolates and Agaricus in vivo

Mushroom culture pots were prepared as previously described. The above three isolates of *Lecanicillium fungicola*, *Mycogone perniciosa* and *Cladobotryum dendroides* were applied to casing at the propagule concentrations in Table 1, together with uninoculated controls. Sporgon or Vivando were applied to the casing at the recommended rate for an individual application (1 g/m^2 for Sporgon; 1 mL/m^2 for Vivando in 1.8L/m^2). The application rate of Shirlan was increased from 0.01 mL per L in Experiment 1.1 to 0.02 mL per L (0.036 mL in 1.8 L water per m^2) since no mushroom yield reduction or fluazinam residues were detected in mushrooms in Experiment 1.1. The same application rate of water (1.8L/m^2) was used a control. The occurrence of disease symptoms and mushroom yield from the pots was recorded in three flushes of mushrooms.

Experiment 4.1Comparison of disease covering methods on-farm

The following covering methods were used on diseased areas with dry bubble, wet bubble, and cobweb, and on mushrooms without disease symptoms:

(a) Paper, pre-soaked in saturated salt solution

(b) Paper, pre-soaked in water

(c) Clay (90 to 50%):salt (10% to 50%) mixtures

(d) Sand (90 to 50%): salt (10% to 50%) mixtures

(e) Salt (100%).

The treated areas were examined for disease one week later and samples taken from the edges and tested for *Lecanicillium*, *Mycogone* and *Cladobotryum* regrowth. Samplers of the covering materials before use and of spent casing taken from treated areas were tested for pH and electrical conductivity, and compared with samples taken from untreated areas.

Results

Experiment 1.1 Effect of casing additives on fungicide degradation and fungicide residues in mushrooms

Mushroom yield

Mushroom yield in three flushes was not significantly affected by the casing additive and biopesticide treatments (Fig. 1) or fungicides applications (Fig. 2).

No disease symptoms (fungal or bacterial) were observed on mushrooms in the experiment.

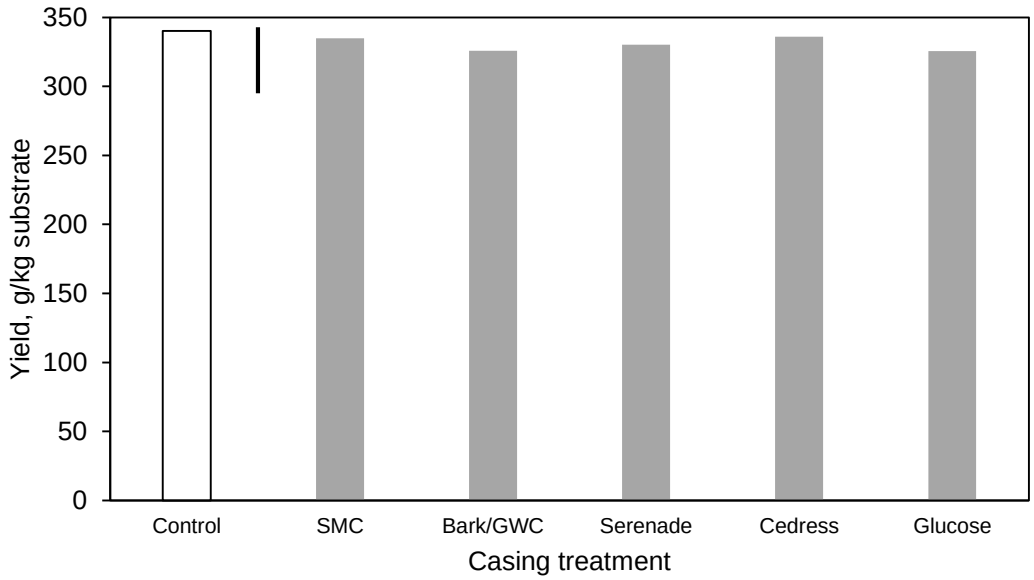

Figure 1. Effect of casing additive and biopesticide treatments on mushroom yield, Expt. 1.1. Values are the means of four fungicide treatments and four replicates; bar shows LSD (*P* = 0.05).

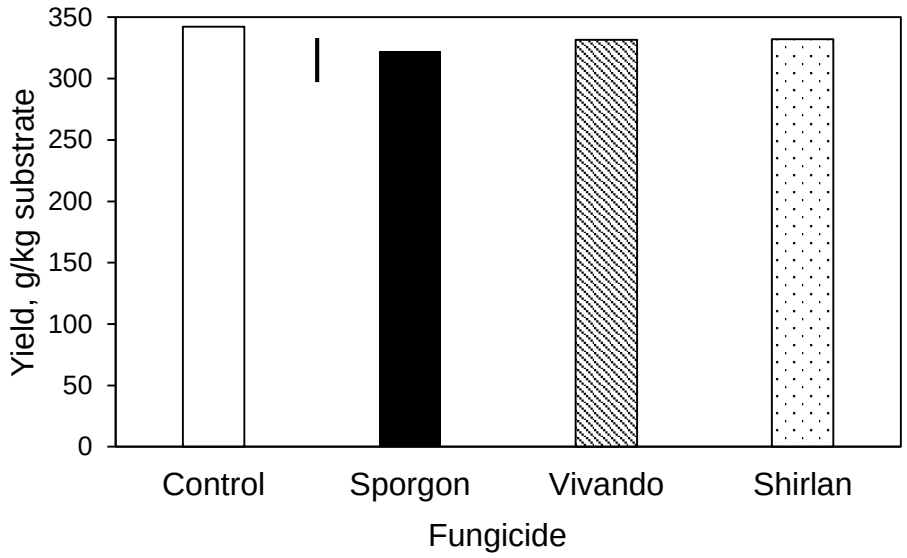

Figure 2. Effect of fungicide treatments on mushroom yield, Expt. 1.1. Values are the means of six casing additive or biopesticide treatments and four replicates ; bar shows LSD (*P* = 0.05).

Bacterial populations in casing

Colony counts of *Bacillus subtilis* on MYP and LB media were similar. Following application of Serenade to casing, the population of *Bacillus* species in the casing remained higher than in the untreated control throughout the crop; the casing population remained at around 10^6

cfu/g until the first flush, followed by a decline. At the end of the third flush, the *Bacillus* species population in the casing was significantly higher following application of Serenade, except where both Serenade and Sporgon were applied (Figs. 3 and 4). Here, the difference between the Serenade treatment and the control (natural background *Bacillus* species population) was not significantly different. This indicates that Sporgon was toxic to *Bacillus subtilis* Q173 in the Serenade rather than to the background *Bacillus* species population. The effects of adding Vivando or Shirlan fungicides to the casing on the *Bacillus* species population was not significant (Fig. 4). Adding glucose solution to casing slightly increased the *Bacillus* spp. population in the casing compared with the untreated control (Fig. 3).

Following the application of Cedress, the population of *Pseudomonas* spp. in casing was about tenfold higher than in the control and other treatments (Fig. 5). The application of a glucose solution to the casing increased the natural *Pseudomonas* sp. population above the untreated control until the end of the crop. By the time of the third flush, the populations of *Pseudomonas* spp. in the different casing and biopesticide treatments were similar. Applications of Sporgon, Vivando or Shirlan did not significantly affect the casing population of *Pseudomonas* spp. compared with the untreated control (data not shown).

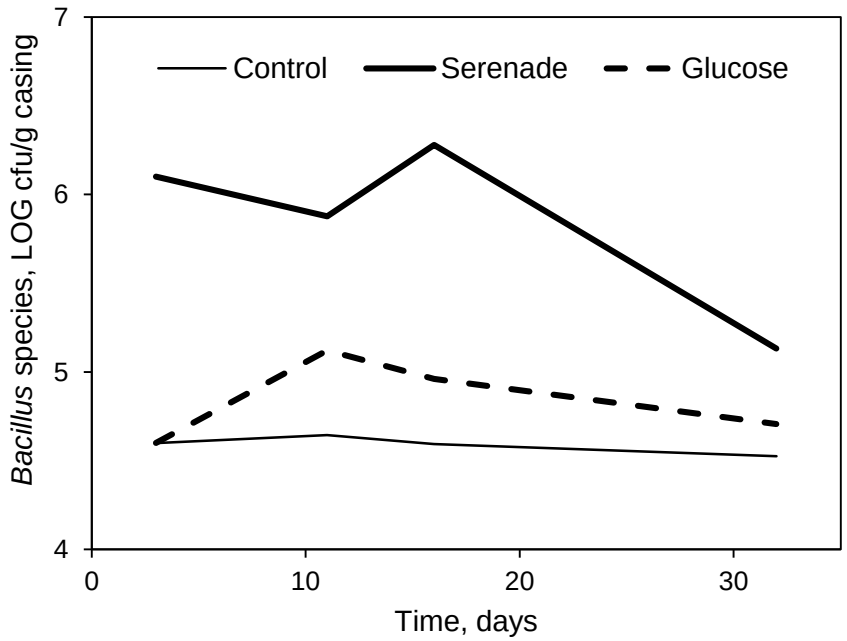

Figure 3. Population of *Bacillus* species in casing following application of Serenade or glucose, Expt. 1.1. Values are means of two replicate casing samples and two determinations per sample.

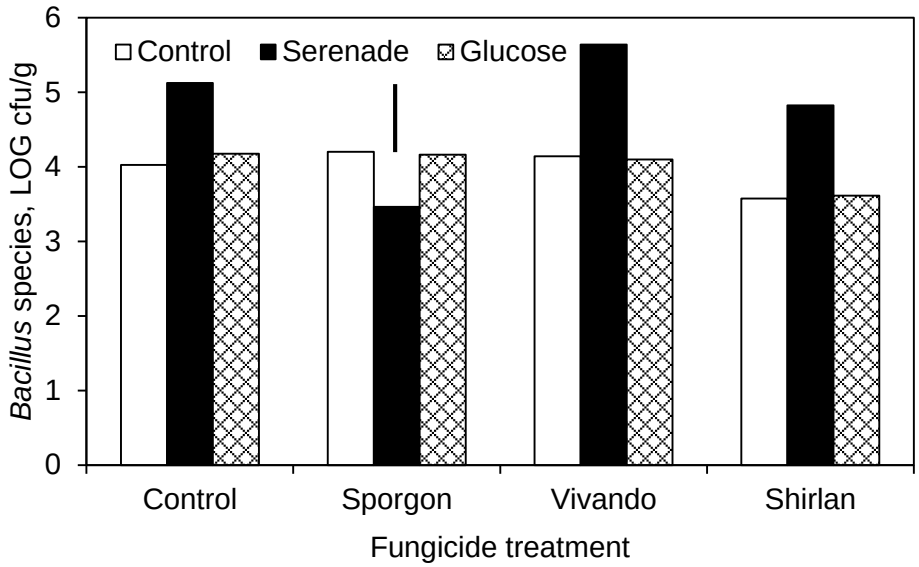

Figure 4. Population of *Bacillus* species in third flush casing with and without application Serenade or glucose, in combination with fungicide treatments; Expt. 1.1. Values are means of two replicate samples and two replicate *Bacillus* species counts made on LB and MYP media.

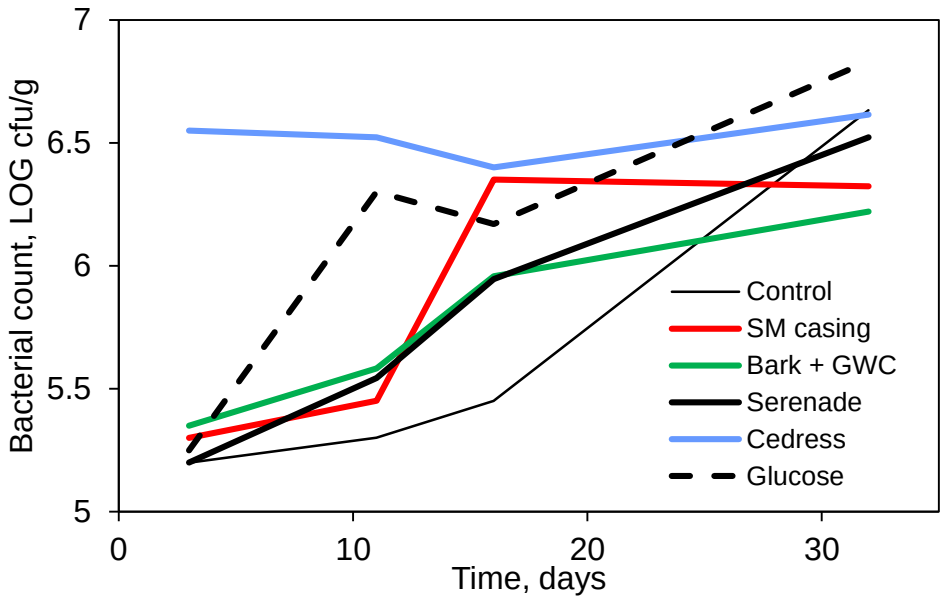

Figure 5. Population of *Pseudomonas* species in different casing and biopesticide treatments, Expt. 1.1. Values are means of two replicate casing samples and two determinations per sample.

Fungicide residues

No fungicides were detected in any of the casing materials that did not have fungicides applied. Following applications of Sporgon, Vivando and Shirlan respectively, there was an average degradation of prochloraz in the casing of 46% during the cropping period compared with 81% for metrafenone and 77% for fluazinam (Tables 2 – 4). Prochloraz degraded more rapidly when 25% bark was added to casing, but degradation was similar in the other casing treatments (Table 2). Following application of Sporgon, prochloraz was found in first flush mushrooms at levels just above the detection limit but not in mushrooms grown in 25% spent casing (Table 2). In pots treated with Vivando, metrafenone was only detected in mushrooms grown in 25% bark or with Cedress added, and here only at levels close to the detection limit and well below the MRL for mushrooms. Fluazinam was not detected in mushrooms grown in any of the casing samples following application of Shirlan.

Table 2. Residues of prochloraz in casing samples at the start and end of the crop, and in first flush mushrooms, mg/kg, following application of Sporgon to casing. MRL for mushrooms = 3 mg/kg

Casing treatment	Casing start	Casing end	Mushrooms
Control	18	12	0.011
+ spent casing	19	11	<0.010
+ bark	26	8	0.014
+ Serenade	20	11	0.012
+ Cedress	19	11	0.010
+ glucose	22	14	0.013

Table 3. Residues of metrafenone in casing samples at the start and end of the crop, and in first flush mushrooms, mg/kg, following application of Vivando to the casing. MRL for mushrooms = 0.4 mg/kg

Casing treatment	Casing start	Casing end	Mushrooms
Control	13	4.5	<0.010
+ spent casing	11	3.0	<0.010
+ bark	17	2.5	0.011
+ Serenade	14	1.8	<0.010
+ Cedress	16	1.6	0.010
+ glucose	11	2.3	<0.010

Table 4. Residues of fluazinam in casing samples at the start and end of the crop, and in first flush mushrooms, mg/kg, following application of Shirlan to the casing. MRL for mushrooms = 0.05 mg/kg

Casing treatment	Casing start	Casing end	Mushrooms
Control	0.15	0.027	<0.01
+ spent casing	0.11	0.031	<0.01
+ bark	0.15	0.050	<0.01
+ Serenade	0.12	0.034	<0.01
+ Cedress	0.11	0.028	<0.01
+ glucose	0.16	0.017	<0.01

Experiment 1.2 On-farm tests of casing additives and supplements

Incorporation of 25% v/v recycled casing did not significantly affect mushroom yield at either farm compared with the untreated casing control (Table 5). No diseased mushrooms were observed on any of the test areas. Due to the higher (x 6.25) Sporgon application rate at Farm B compared with Farm A, residues of prochloraz were much higher after the third flush at Farm B (Table 5). However, at both farms, the residues of prochloraz were higher in the casing containing 25% recycled casing. Since the recycled casing did not contain Sporgon, this indicates that the degradation or loss of prochloraz during the crop was reduced by the incorporation of recycled casing. The rate of prochloraz degradation was reduced from 53-71% in unamended casing to 25-33% in casing containing 25% recycled casing.

Casing EC was higher at Farm A than at Farm B. The inclusion of 25% recycled casing increased casing EC (Table 5). The final pH of the casings (7.4 to 7.6) was not significantly different between farms or casing treatments.

Table 5. Initial casing moisture and electrical conductivity, mushroom yields and prochloraz residues in casing after the third flush from plots cased with unamended casing or casing containing 25% separated recycled casing, following application of Sporgon. Values are the means of two replicates.

Farm	Mushroom yield, kg/m^2		Casing moisture % w/w		Casing EC, µS/cm		prochloraz, mg/ g casing	
	Control	+Recycled	Control	+Recycled	Control	+Recycled	Control	+Recycled
A	25.4	25.8	85.8	83.7	334	720	0.76	2.00
B	26.1	26.3	84.2	84.1	314	516	9.40	13.50

Experiment 2.1 Effect of fungicides and biopesticides on wet and dry bubble and cobweb diseases

Typical symptoms of dry bubble, wet bubble and cobweb diseases in the pot experiments are shown in Picture 1, Appendix. Cobweb disease first appeared as cap spotting followed by typical whitish-pink mycelium growing over the surface of mushrooms and casing. Symptoms of dry bubble and wet bubble were characteristic of those previously described (Fletcher & Gaze, 2008).

Sporgon and Vivando both significantly reduced the number of mushrooms with dry bubble disease compared with the untreated control (Fig. 6). Shirlan also suppressed dry bubble but the effect was not quite significant at $P = 0.05$. The effect of Cedress on dry bubble disease was not significant. In Expt. 2.1.1, none of the fungicide or biopesticide treatments significantly affected the number of healthy mushrooms (Fig. 7) or mushroom yield (Fig. 8).

Sporgon and Vivando significantly reduced the number of mushrooms with wet bubble disease compared with the untreated control (Fig. 9). Shirlan and Cedress also suppressed the amount of wet bubble disease although the effects were not quite significant at $P = 0.05$. The *Mycogone* inoculum significantly reduced the number of healthy mushrooms (Fig. 10) and the mushroom yield (Fig. 11) across all the fungicide and biopesticide treatments. Differences in the numbers of healthy mushrooms and mushroom yields between the fungicide and biopesticide treatments were not significant.

Vivando significantly increased both the number of healthy mushrooms and mushrooms with cobweb disease symptoms per pot (Fig. 12). The increase in the number of mushrooms with cobweb disease symptoms resulting from Vivando was due to more mushrooms surviving to a pickable size, whereas in other treatments, the pots became overwhelmed by cobweb mycelium. The *Cladobotryum* inoculum resulted in a large reduction in the healthy mushroom

yield (Fig. 13). However, mushroom yield from pots inoculated with *Cladobotryum* was higher from the Vivando treatment than from the control, Cedress and Shirlan treatments (Fig. 13). Differences in mushroom yield between the Sporgon, Shirlan and Cedress treatments and the untreated control were not significant, irrespective of the *Cladobotryum* inoculum.

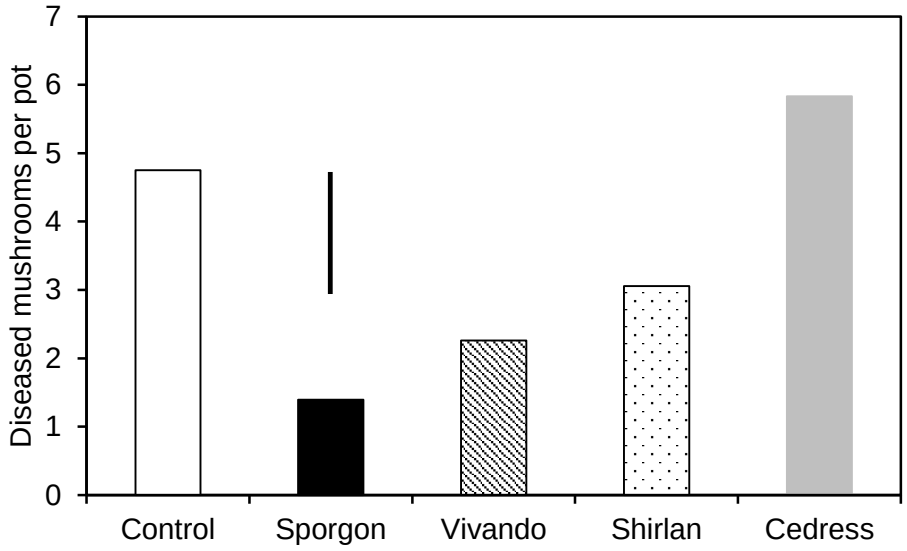

Figure 6. Effect of fungicide and biopesticide treatments on the number of mushrooms with dry bubble per pot in *Lecanicillium* inoculated pots, Expt.2.1.1. Each value is the mean of four replicate pots; bar shows LSD (*P* = 0.05).

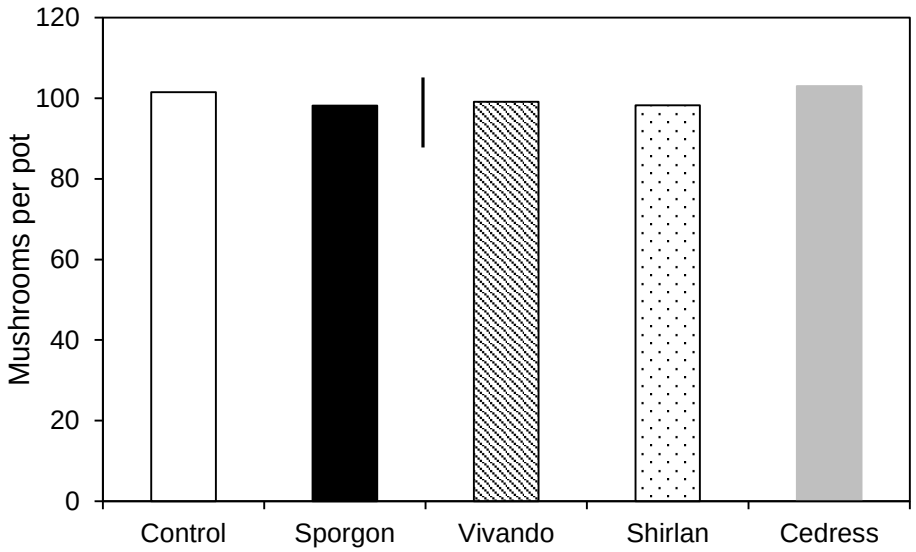

Figure 7. Effect of fungicide and biopesticide treatments on the number of healthy mushrooms per pot, Expt.2.1.1. Values are the means of pots with and without *Lecanicillium* inoculum, and four replicate pots; bar shows LSD (*P* = 0.05).

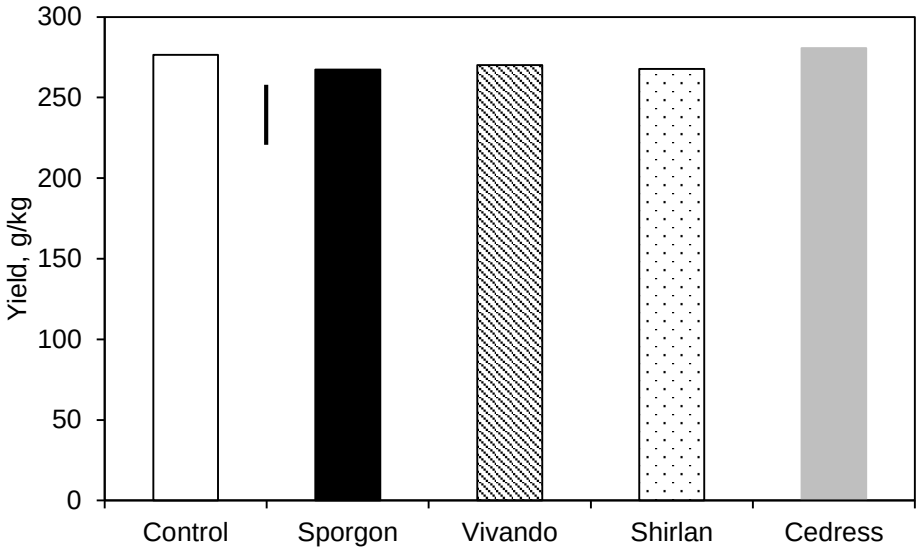

Figure 8. Effect of fungicide and biopesticide treatments on mushroom yield, Expt.2.1.1. Values are the means of pots with and without *Lecanicillium* inoculum, and four replicate pots; bar shows LSD (*P* = 0.05).

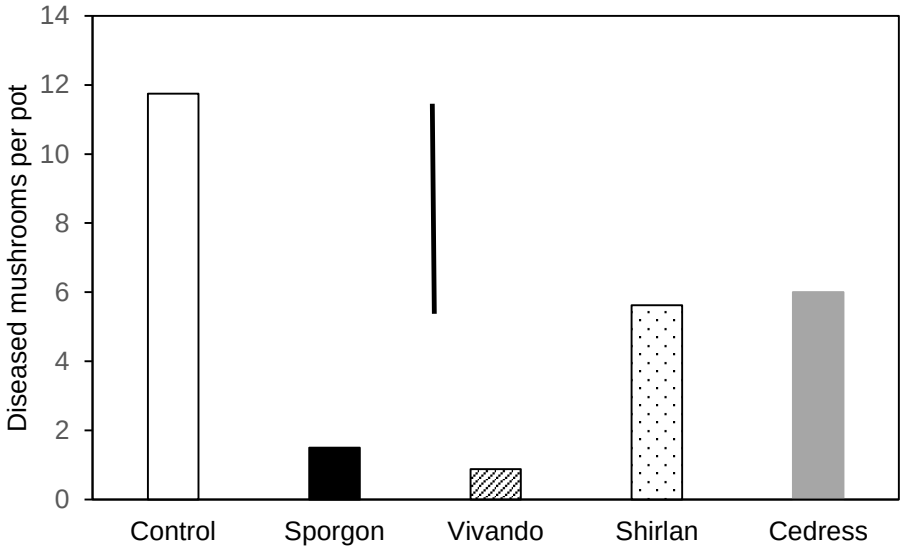

Figure 9. Effect of fungicide and biopesticide treatments on the number of mushrooms with wet bubble per pot in *Mycogone* inoculated pots, Expt.2.1.2. Values are means of four replicate pots; bar shows LSD (*P* = 0.05).

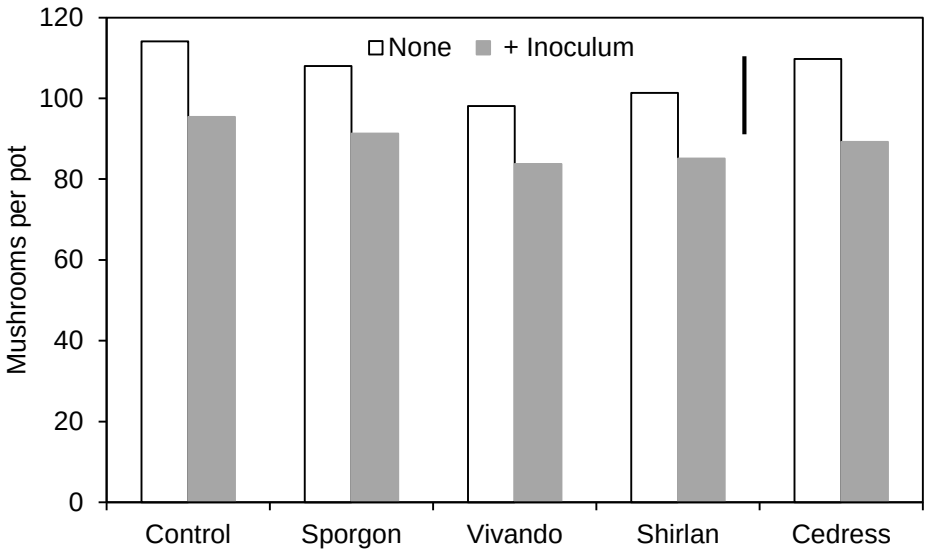

Figure 10. Effect of *Mycogone* inoculum and fungicide and biopesticide treatments on the number of healthy mushrooms per pot, Expt.2.1.2. Values are means of four replicate pots; bar shows LSD (*P* = 0.05).

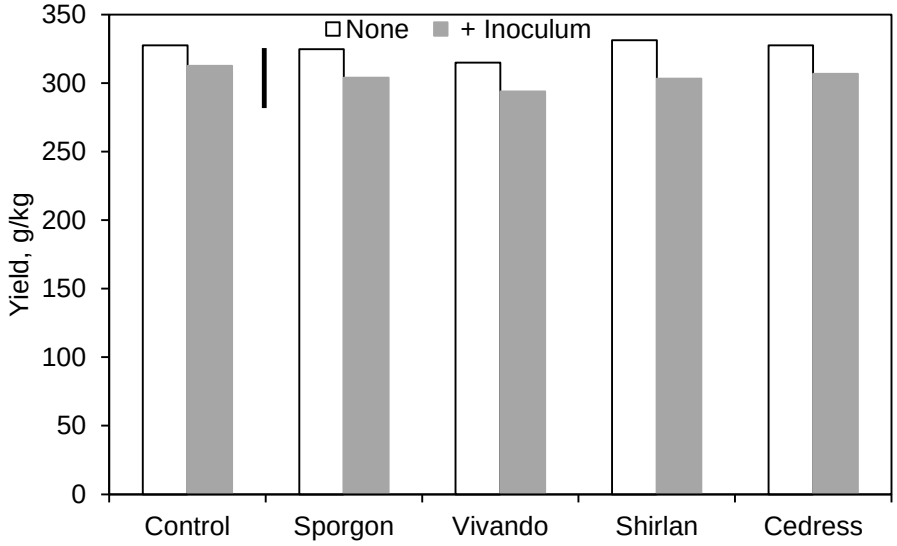

Figure 11. Effect of *Mycogone* inoculum and fungicide and biopesticide treatments on the yield of mushrooms, Expt.2.1.2. Values are means of four replicate pots; bar shows LSD (*P* = 0.05).

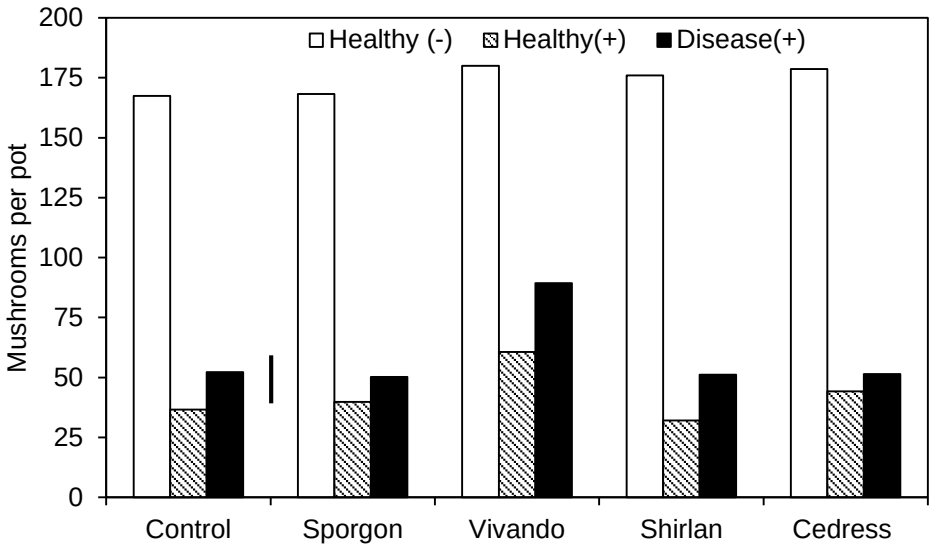

Figure 12. Effect of *Cladobotryum* inoculum (+/-) and fungicide and biopesticide treatments on the numbers of healthy and cobweb diseased mushrooms per pot, Expt.2.1.3 Values are means of four replicate pots; bar shows LSD (*P* = 0.05).

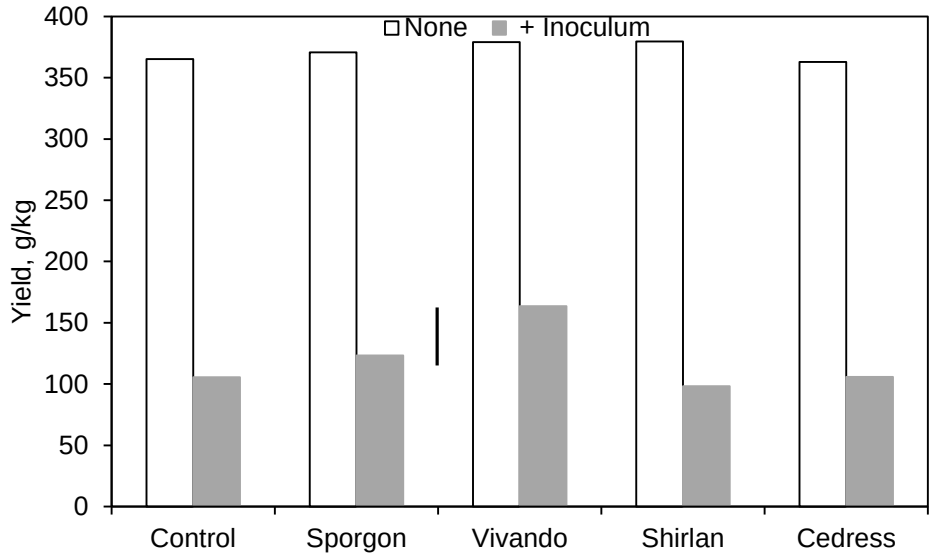

Figure 13. Effect of *Cladobotryum* inoculum and fungicide and biopesticide treatments on the yield of mushrooms, Expt.2.1.3. Values are means of four replicate pots; bar shows LSD (*P* = 0.05).

Experiment 3.1 Fungicide sensitivity of fungal pathogen isolates and Agaricus in vitro

Agaricus bisporus

Fluazinam reduced mycelial growth rate of *Agaricus* slightly less than prochloraz with a significantly greater ED_{50} value (Fig. 14; Table 6). There was a significantly smaller effect of metrafenone on mycelial growth of *Agaricus*, with a concentration of 100 ppm not reducing mycelial growth below 50% of the untreated control.

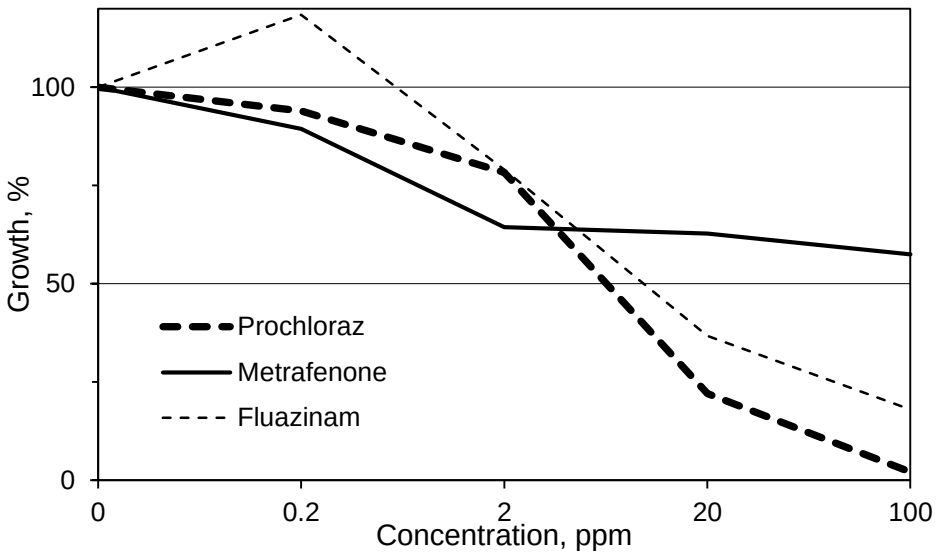

Figure 14. Effect of prochloraz, metrafenone and fluazinam concentrations in potato dextrose agar on the mycelial growth rate of *Agaricus bisporus* A15. Each value is the mean of three replicate samples.

Lecanicillium fungicola

Of the three pathogens, *Lecanicillium fungicola* was the most tolerant to the three fungicides tested in terms of mycelial growth rate, with significantly higher ED_{50} values than for *Cladobotryum dendroides* and *Mycogone perniciosa* (Fig. 15; Table 6). A fungicide concentration of at least 2 ppm was required to reduce the mycelial growth rate of the three *Lecanicillium fungicola* isolates tested to below 50% of the untreated control. *Lecanicillium fungicola* isolate B was significantly more tolerant of prochloraz than isolates A and C, with significantly higher ED_{50} values (Table 6). Isolates B and C had significantly higher ED_{50} values for metrafenone than isolate A. A fluazinam concentration of 2 ppm reduced the mycelial growth rate of all the *Lecanicillium fungicola* isolates to 50% of the untreated control.

Spores of *Lecanicillium fungicola* isolate B germinated at 2 ppm prochloraz whereas isolates A and C did not. All *Lecanicillium fungicola* isolates germinated at 200 ppm metrafenone and 20 ppm fluazinam but not at 200 ppm fluazinam (Table 7).

Table 6. ED_{50} values of mycelial growth rate of pathogen isolates and *Agaricus bisporus* A15 on agar plates containing different concentrations of three fungicides. Values are the means of three replicate samples.

Micro-organism	Isolate	prochloraz ppm	metrafenone ppm	fluazinam ppm	LSD (*P*=0.05)
Cladobotryum dendroides	A	1.21	0.20	0.11	
Cladobotryum dendroides	B	1.54	0.38	0.13	
Cladobotryum dendroides	C	1.43	0.18	0.12	
Cladobotryum dendroides	Mean	1.38	0.25	0.12	0.12
LSD (P = 0.05) between isolates with the same fungicide = 0.21					
Lecanicillium fungicola	A	1.85	1.93	1.82	
Lecanicillium fungicola	B	12.71	13.06	2.00	
Lecanicillium fungicola	C	1.58	12.57	1.83	
Lecanicillium fungicola	Mean	5.38	5.95	1.87	0.26
LSD (P = 0.05) between isolates with the same fungicide = 0.44					
Mycogone perniciosa	A	0.13	1.09	0.16	
Mycogone perniciosa	B	0.16	1.15	0.15	
Mycogone perniciosa	C	0.12	1.03	0.14	
Mycogone perniciosa	Mean	0.14	1.09	0.15	0.06
LSD (P = 0.05) between isolates with the same fungicide = 0.11					
LSD (P = 0.05) between pathogen means with the same fungicide = 1.51					
Agaricus bisporus	A15	11.06	>100	14.28	1.07

Table 7. Spore germination of fungal pathogens after plating of spore suspensions on to agar plates containing different concentrations of fungicides.

Pathogen	Isolate	prochloraz			metrafenone		fluazinam		
	ppm	0.2	2	20	20	200	0.2	20	200
Cladobotryum dendroides	A	+	+	0	+	+	+	+	0
Cladobotryum dendroides	B	+	+	0	+	+	+	+	0
Cladobotryum dendroides	C	+	+	0	+	+	+	+	0
Lecanicillium fungicola	A	+	0	0	+	+	+	+	0
Lecanicillium fungicola	B	+	+	0	+	+	+	+	0
Lecanicillium fungicola	C	+	0	0	+	+	+	+	0
Mycogone perniciosa	A	0	0	0	+	+	+	0	0
Mycogone perniciosa	B	+	0	0	+	+	+	0	0
Mycogone perniciosa	C	0	0	0	+	+	+	0	0

Mycogone perniciosa

At low fungicide concentrations (2 ppm and below), there were no significant differences in fungicide sensitivity (ED_{50}) between the three *Mycogone perniciosa* isolates tested (Fig. 15; Table 6). However, isolate A was more tolerant of higher metrafenone concentrations than isolates B and C (Fig. 15). A 0.2 ppm concentration of prochloraz or fluazinam reduced the mycelial growth rate of *Mycogone perniciosa* to below 50% of the untreated control. However, a 2 ppm concentration of prochloraz completely suppressed mycelial growth whereas *Mycogone perniciosa* continued to grow at a concentration of 20 ppm fluazinam or 100 ppm metrafenone. The ED_{50} value for metrafenone was significantly greater than those for prochloraz or metrafenone (Table 6). Spores of *Mycogone* isolate B germinated at 0.2 ppm prochloraz but not isolates A or C (Table 7). All three *Mycogone perniciosa* isolates germinated at 200 ppm metrafenone and at 20 ppm fluazinam but not at 200 ppm fluazinam.

Cladobotryum dendroides

There were no significant differences in fungicide sensitivity (ED_{50}) between the three *Cladobotryum dendroides* isolates tested (Fig. 15; Table 6). Fluazinam and prochloraz had the strongest and weakest effects respectively on the mycelial growth rate of *Cladobotryum dendroides*, with significantly smaller and larger ED_{50} values than those for metrafenone (Table 6). All three isolates of *Cladobotryum dendroides* germinated at 2 ppm prochloraz, 200 ppm metrafenone and 20 ppm fluazinam but not at 20 ppm prochloraz or 200 ppm fluazinam (Table 7).

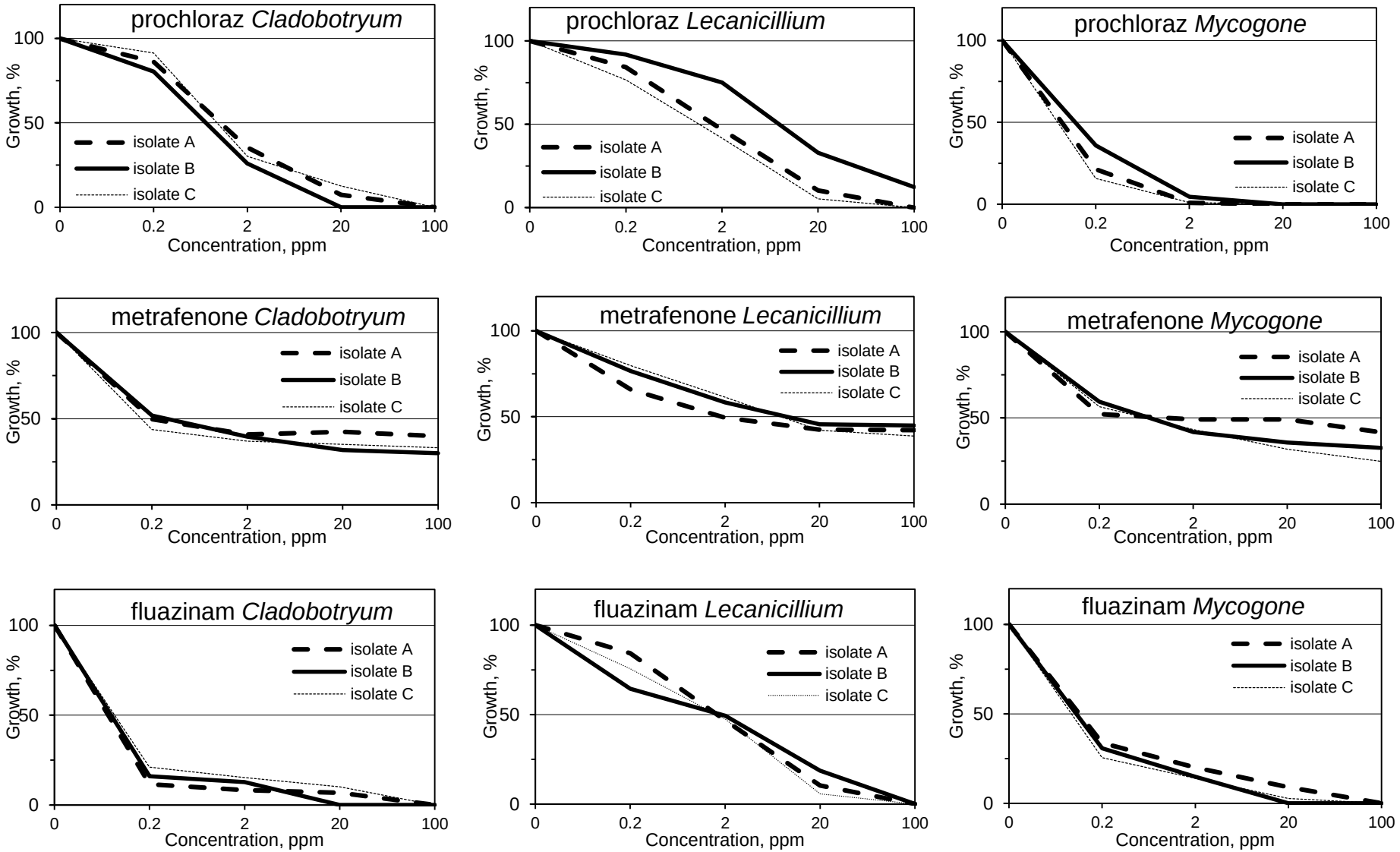

Figure 15. Effect of prochloraz, metrafenone and fluazinam concentrations in potato dextrose agar on the mycelial growth rate of pathogens.

Experiment 3.2 Fungicide sensitivity of pathogen isolates and Agaricus in vivo

A very small number of uninoculated control pots produced diseased mushrooms. Without pathogen inoculum, there were no significant differences between fungicide treatments and the untreated pots in the number of diseased mushrooms or in mushroom yield (Tables 8 and 9).

Control of dry bubble disease

In control pots without fungicide treatment, *Lecanicillium fungicide* isolate C produced significantly more diseased mushrooms than isolates A and B (Table 8). Sporgon resulted in a significant reduction in dry bubble in isolates A and C but not in isolate B. Vivando significantly reduced dry bubble from all three *Lecanicillium fungicola* isolates. The effect of Shirlan on dry bubble was not significant.

In *Lecanicillium fungicola* inoculated pots, mean mushroom yields were significantly lower from untreated pots and pots treated with Shirlan than from pots treated with Sporgon or Vivando (Table 9). In pots treated with Sporgon, yield was significantly lower from pots inoculated with *Lecanicillium fungicola* isolate B than with isolates A or C. In pots treated with Vivando or Shirlan or in untreated pots, there was no significant difference in mushroom yield between pots inoculated with the different *Lecanicillium fungicola* isolates.

Control of wet bubble disease

In *Mycogone perniciosa* inoculated pots, Sporgon or Vivando resulted in fewer mushrooms with wet bubble disease and higher mushroom yields than pots treated with Shirlan or untreated pots (Tables 8 and 9). The reduction in wet bubble disease resulting from Shirlan was not quite significant at $P = 0.05$ but was significant at $P = 0.10$. Differences in the number of diseased mushrooms and mushroom yields between pots treated with different *Mycogone perniciosa* isolates were not significant.

Control of cobweb disease

In *Cladobotryum dendroides* inoculated pots, Vivando produced significantly higher mushroom yields than pots treated with Sporgon or Shirlan, or untreated pots (Table 9). However, the number of cobweb diseased mushrooms was significantly higher from Vivando treated pots due to more mushrooms surviving to a pickable stage of development (Table 8).

Table 8. Number of diseased mushrooms from pathogen inoculated pots and uninoculated pots treated with three different fungicides or water (control). Values are the means of four replicate pots.

Pathogen inoculum	Isolate	Sporgon prochloraz	Vivando metrafenone	Shirlan fluazinam	None (control)	LSD (*P*=0.05)
Cladobotryum dendroides	A	49.5	85.3	50.8	52.0	
Cladobotryum dendroides	B	41.3	45.0	46.5	39.3	
Cladobotryum dendroides	C	17.5	41.8	22.3	18.5	
Cladobotryum dendroides	Mean	36.1	57.3	39.8	36.6	12.1
LSD (P = 0.05 between isolates with the same fungicide = 20.9						
Mean		36.1	57.3	39.8	36.6	
Lecanicillium fungicola	A	2.5	3.5	4.0	6.5	
Lecanicillium fungicola	B	6.5	3.3	6.0	8.0	
Lecanicillium fungicola	C	2.3	3.0	16.5	18.8	
Lecanicillium fungicola	Mean	3.8	3.3	8.8	11.1	2.5
LSD (P = 0.05 between isolates with the same fungicide = 3.1						
Mycogone perniciosa	A	1.8	1.0	5.8	7.4	
Mycogone perniciosa	B	3.8	0.8	6.3	9.3	
Mycogone perniciosa	C	1.5	1.8	5.5	8.5	
Mycogone perniciosa	Mean	2.3	1.2	5.8	8.4	2.7
LSD (P = 0.05 between isolates with the same fungicide = 3.3						
None (control)	Mean	0.3	0.5	0.0	0.3	0.6
LSD (P = 0.05 between pathogen means with the same fungicide = 10.4						

Table 9. Yield (g/kg compost) of three flushes of mushrooms from pathogen inoculated pots and uninoculated pots treated with three different fungicides or water (control). Values are the means of four replicate pots.

Pathogen inoculum	Isolate	Sporgon prochloraz	Vivando metrafenone	Shirlan fluazinam	none (control)	LSD (*P*=0.05)
Cladobotryum dendroides	A	120	166	94	105	
Cladobotryum dendroides	B	152	189	153	157	
Cladobotryum dendroides	C	224	263	211	216	
Cladobotryum dendroides	Mean	165	206	153	159	27.0
LSD (P = 0.05) between isolates with the same fungicide = 46.7						
Lecanicillium fungicola	A	282	271	270	264	
Lecanicillium fungicola	B	241	297	246	232	
Lecanicillium fungicola	C	303	301	255	246	
Lecanicillium fungicola	Mean	275	292	257	247	19.7
LSD (P = 0.05) between isolates with the same fungicide = 34.1						
Mycogone perniciosa	A	300	290	236	225	
Mycogone perniciosa	B	256	321	262	247	
Mycogone perniciosa	C	287	294	240	214	
Mycogone perniciosa	Mean	281	302	246	229	19.5
LSD (P = 0.05) between isolates with the same fungicide = 33.7						
None (control)	Mean	340	344	345	345	13.6
LSD (P = 0.05) between pathogen means with the same fungicide = 23.3						

Relationships between in vitro agar plate tests and in vivo mushroom disease and cropping tests

All three fungicide tested had higher ED_{50} values for *Agaricus bisporus* A15 than for the three pathogens. This corresponded with a lack of mushroom yield loss resulting from fungicide applications when used in the absence of pathogen inoculum.

Where Sporgon was used, the greater prochloraz resistance of *Lecanicillium fungicola* isolate B in agar plate tests corresponded with the greater amount of dry bubble disease resulting from this isolate in pot tests, compared with the prochloraz sensitive isolates A and C (Table 8). The resistance of *Cladobotryum dendroides* isolates and sensitivity of *Mycogone perniciosa* to prochloraz in agar plate tests also corresponded with poor control of cobweb and good control of wet bubble with Sporgon in pot tests. Metrafenone had a significantly lower ED_{50} value than prochloraz for the mycelial growth of *Cladobotryum dendroides* and this corresponded with better control of cobweb disease. However, prochloraz was more inhibitory than metrafenone to spore germination of *Cladobotryum dendroides*. Good control of dry bubble disease was achieved with Vivando (metrafenone) in spite of this fungicide having similar or greater ED_{50} values to prochloraz for all *Lecanicillium* isolates.

Fluazinam was very inhibitory to the mycelial growth of *Cladobotryum dendroides* and *Mycogone perniciosa*, indicated by small ED_{50} values, but this did not translate into good control of cobweb or wet bubble with Shirlan when used at 0.036 ml/m^2. However, Shirlan did suppress both dry and wet bubble diseases when applied at 0.036 ml/m^2 although the effect was not quite significant at $P = 0.05$ (significant at $P = 0.10$).

Experiment 4.1 Comparison of disease covering methods on-farm

Covering diseased areas with clay and salt (10%) or sand and salt (10 to 30%) were not effective in preventing the regrowth of diseased mushrooms, compared with using salt (Pictures 2, 3 and 4, Appendix). However, mixtures of clay (70%) and salt (30%) or sand and salt (50%) were as effective as salt in preventing the regrowth of diseased mushrooms and mycelium (Pictures 3 and 4, Appendix). The clay/salt mixture was easier to apply than the sand/salt mixture because it was more cohesive when applied as a patch. Covering diseased areas with paper soaked in water or saturated salt solution did not prevent regrowth of diseased mushrooms. However, the technique was useful for reducing spore dispersal before the diseased patch was covered with salt.

The EC of salt at the end of the test was 110 mS/cm compared with a 70% clay + 30% salt mixture of 40 mS/cm (Table 10). If the final SMC was treated with 1% w/w salt, this would increase the EC of the spent casing from 0.9 to 2 mS/cm. However, if the same quantity of 70% clay + 30% salt was used for disease covering instead, the final SMC of the spent casing would only increase to 1.3 mS/cm.

Table 10. Analysis of disease covering materials and of spent casing after third flush following use of covering materials.

Covering materials	Mix at start		Mix at end	
	pH	EC, mS/cm	pH	EC, mS/cm
100% salt	7.41	146.6	7.59	109.9
100% sand	8.07	0.5	8.24	0.7
100% clay	8.19	0.2	8.29	0.4
70% sand: 30% salt	7.60	55.6	8.22	50.6
70% clay: 30% salt	8.01	46.3	8.14	40.2
Casing	7.51	0.3	7.38	0.9

Discussion

The average ED_{50} values obtained for prochloraz on mycelial growth rate of *Lecanicillium fungicola* isolates (5.38 ppm) and *Cladobotryum dendroides* isolates (1.38 ppm) are within the ranges reported by Grogan et al (1996; 2001) of 0.3 – 6 ppm for *Lecanicillium fungicola* isolates and 0.1 – 5 ppm for *Cladobotryum dendroides* isolates. The average ED_{50} value obtained for prochloraz on *Mycogone perniciosa* isolates (0.14 ppm) is greater than that reported by Gea et al (2010) for Spanish *Mycogone perniciosa* isolates (0.029 ppm). The latter work was conducted with malt extract agar rather than PDA.

Previous work has often relied either on *in vitro* plate cultures (Gea et al 2005; HDC Report M33a) or on *in vivo* disease experiments (Pyck et al 2016) for testing the fungicide sensitivity of mushroom pathogens. Here, the resistance of *Lecanicillium fungicola* isolates to prochloraz in agar plates and their corresponding resistance to Sporgon in pot culture tests agrees with previous results in HDC reports M14b and M14c (Grogan et al 1999). Gea et al (2010) found that four fungicides with low ED_{50} values for *Mycogone perniciosa* mycelial growth also gave good control of wet bubble disease, unlike iprodione which had a significantly higher ED_{50} value. Although *in vitro* agar plate tests are easy and rapid to perform, this work has shown that the results do not always correspond with results obtained *in vivo* from disease experiments in pot cultures. Metrafenone was less inhibitory to the mycelial growth and spore germination of all three mushroom pathogens investigated but gave better control of cobweb, equivalent or better control of dry bubble, and better control of wet bubble diseases than Sporgon. This work agrees with the results of Pyck et al (2016) in that Vivando can provide good control of dry bubble and cobweb diseases without adverse

effects on mushroom yields or exceeding the EU MRL for metrafenone in mushrooms. Pyck et al (2016) found that Vivando was more effective than Sporgon in controlling dry bubble and cobweb diseases.

A potential source of discrepancy between results obtained from *in vitro* plate tests for pathogen sensitivity to fungicides and their efficacy in disease experiments is the degradation of the active ingredient in the casing. The degradation of prochloraz in casing is well established (Grogan et al. 2008). However, a less rapid degradation of prochloraz than metrafenone or fluazinam was observed in this work. This could result in poor disease control with Vivando in the third flush following a late infection of the crop, since second applications with fungicides is no longer permitted. Results from the large scale tests indicated that amendment of fresh casing with 25% recycled casing can reduce prochloraz degradation. Amendment of soils with composts and other organic materials has been shown to inhibit the microbial degradation of some pesticides by promoting a microbiota that antagonises the fungicide degrading organisms (Dungan et al. 2001; Siad-Pullicino et al. 2004). However, it is not clear whether recycled casing reduces prochloraz degradation by microbial antagonism or by changing the physical or chemical properties of the casing. The results confirm earlier results obtained in HDC project M 60 that amendment with 25% recycled casing does not adversely affect mushroom cropping.

The suppressive, but not quite statistically significant effect of Shirlan on dry and wet bubble diseases, good inhibition of pathogen growth in agar plates, lack of effect on *Agaricus* and absence of fluazinam residues in mushrooms indicate that Shirlan may be effective if used at a higher rate than 0.018 to 0.036 mL/m^2. This requires further experimentation.

The *Mycogone perniciosa* isolates used in this work were all highly pathogenic to mushrooms, similar to the most pathogenic isolates tested by Szumiga-Tarnowska et al (2015). However, all were effectively controlled by Sporgon and Vivando, and were also suppressed by Shirlan. The biopesticide Cedress also showed some suppression to *M. perniciosa*.

The effect of glucose solution on the Pseudomonad population in the casing confirms the result of Masaphy et al (1989) who also found a significant increase. The glucose solution also increased the *Bacillus* spp. population in the casing. No blotch resulted from the glucose solution application which therefore indicates that the stimulated *Pseudomonas* and *Bacillus* spp. populations may have the potential to antagonise pathogenic Pseudomonads such as *Pseudomonas tolaasii*.

The use of salt for covering diseased areas on mushroom beds is a well established practice for disease control (Fletcher & Gaze, 2008). However, this work has shown that a mixture containing 70% clay: 30% salt is as effective in suppressing regrowth of mushroom

pathogens and diseased mushrooms, with a smaller effect on the final EC of the spent casing, thereby improving its subsequent reuse value.

Conclusions

1. Mushroom yield was not significantly affected by casing amendments (bark, green waste compost, recycled casing, glucose solution), fungicides (Sporgon, Vivando, Shirlan) or biopesticides (Cedress, Serenade).
2. The application of a glucose solution to the casing resulted in a sustained increase in the *Pseudomonas* and *Bacillus* spp. populations in the casing without stimulating bacterial blotch.
3. Applications of Sporgon, Vivando or Shirlan did not significantly affect the casing population of *Pseudomonas* spp. compared with the untreated control.
4. There was an average degradation of prochloraz in the casing of 46% during the cropping period compared with 81% for metrafenone and 77% for fluazinam.
5. Prochloraz degraded more rapidly when 25% bark was added to casing. Spent casing added at 25% did not affect prochloraz degradation in a pot experiment but reduced degradation in large scale experiments.
6. Prochloraz was found in first flush mushrooms at levels just above the detection limit but not in mushrooms grown in 25% spent casing. Metrafenone was only detected in mushrooms grown in 25% bark or with Cedress added, and here only at levels close to the detection limit and well below the MRL for mushrooms. Fluazinam was not detected in mushrooms grown in any of the casing samples.
7. Sporgon and Vivando both significantly reduced the number of mushrooms with dry and wet bubble diseases compared with the untreated control. Shirlan also suppressed dry and wet bubble diseases but the effects were not quite significant at $P = 0.05$.
8. Cedress suppressed wet bubble disease, although the effect was not quite significant at $P = 0.05$) but it did not significantly affect dry bubble or cobweb diseases.
9. Fluazinam reduced mycelial growth rate of *Agaricus bisporus* slightly less than prochloraz, with a significantly greater ED_{50} value. There was a significantly smaller effect of metrafenone on mycelial growth of *Agaricus*, with a concentration of 100 ppm not reducing mycelial growth below 50% of the untreated control.
10. Inhibition of pathogen mycelial growth rate or spore germination on agar plates did not fully reflect potential disease control with fungicides in pot culture tests.
11. A *Lecanicillium fungicola* isolate that showed resistance to prochloraz in agar plate tests was also resistant to Sporgon in a pot culture test. However, metrafenone was less inhibitory to pathogen mycelial growth and spore germination than prochloraz in agar

plate tests whereas Vivando provided better disease control than Sporgon in a pot culture test.

12. The use of a 70% clay:30% salt mixture was as effective as 100% salt in covering diseased areas and prevent regrowth of pathogens or diseased mushrooms; using clay and salt instead of salt reduced the EC of the final spent casing.
13. Clay was easier to apply to diseased areas than sand; a higher proportion (50% w/w) of salt was needed with sand than with clay in order for the mixture to be effective in suppressing regrowth of the pathogen or diseased mushrooms.

Glossary

cfu colony forming units

EAMU extension of authorisation for minor use

SOLA specific off-label approval

EC Electrical conductivity

LSD Least significant difference

MRL Maximum residue level

MYP mannitol + yolk + polymyxin agar

PDA Potato dextrose agar

PIA Pseudomonas isolation agar

SBL Sugar beet lime

SMC Spent mushroom compost

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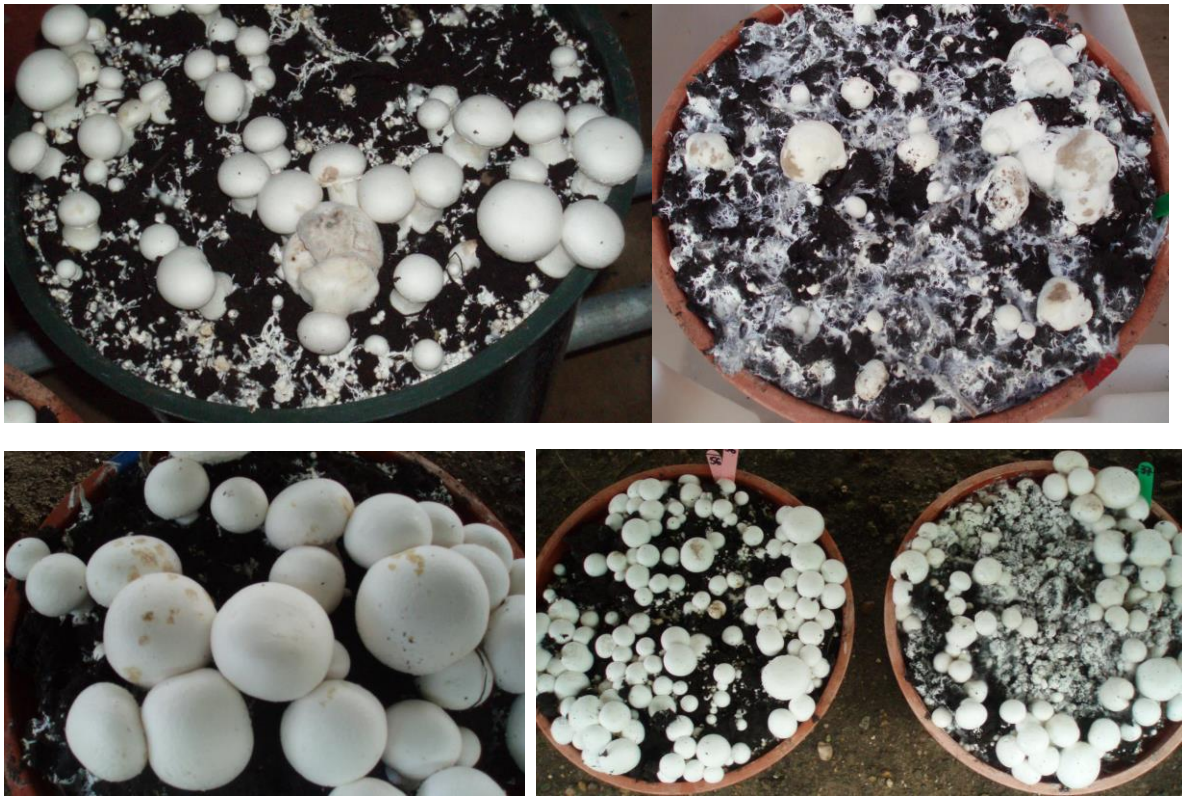
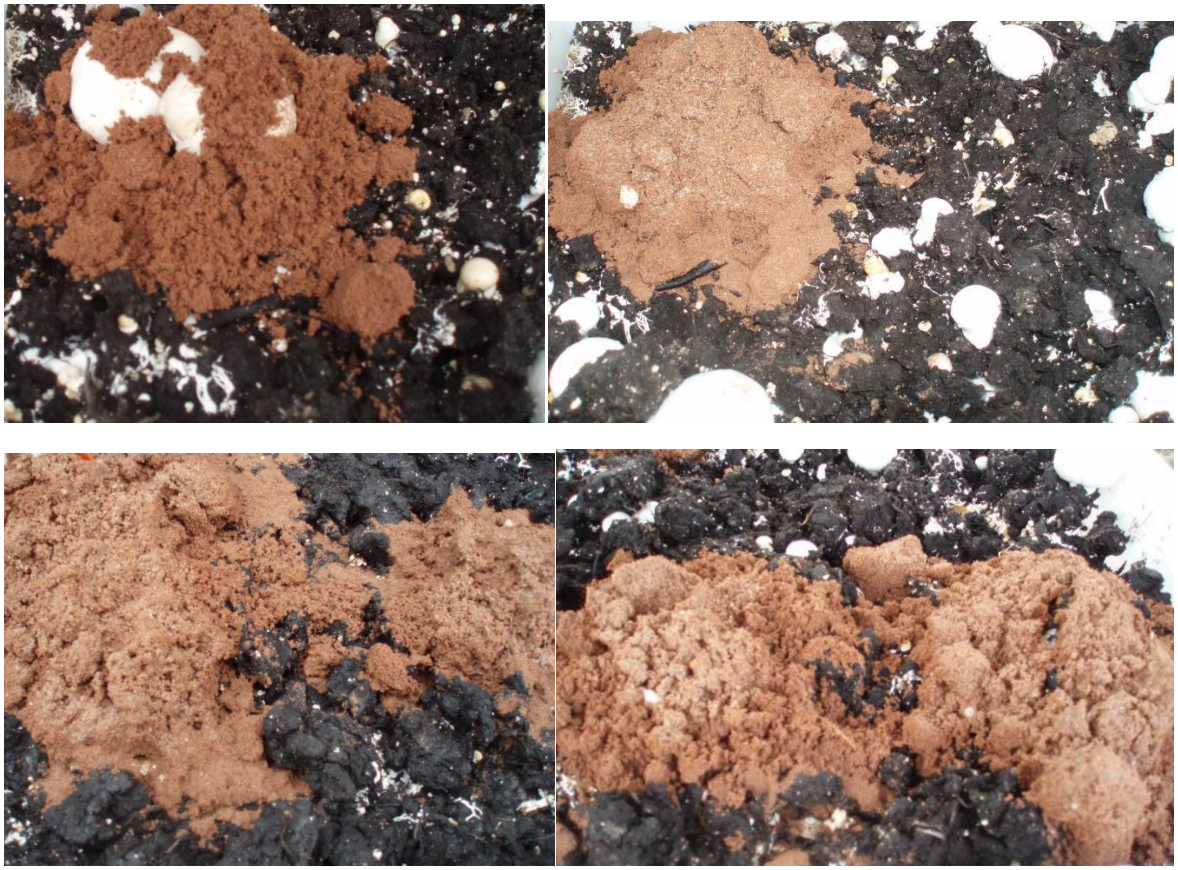
Appendix Photographs



Picture 1. Symptoms of dry bubble (top left), wet bubble (top right) and cobweb (bottom left and right) diseases in the pot culture experiments.

Picture 2. Cobweb (left) and dry bubble (right) diseased areas treated with salt

Picture 3. Top, dry bubble disease treated with clay and salt at 10% (left) and 30% (right); Bottom, wet bubble (left) and cobweb (right) disease treated with clay and 30% salt.



Picture 4 Top: wet bubble disease treated with sand and salt at 10% (left) and 30% (right); Bottom, cobweb (left) and dry bubble (right) disease treated with sand and 50% salt