Horticulture Innovation Australia

Final Report

Facilitation of new/existing products for the mushroom industry

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Media Summary

This project was established to ensure continued access by Australian mushroom growers to products that will assist them to sustainably manage pest and disease problems.

A total of three efficacy growing room studies were completed between July 2009 and December 2009; laboratory efficacy work evaluating imazalil (Magnate 750 WSG®) as a spawn treatment against *Trichoderma* green mould was completed by March 2010. These included two efficacy trials for abamectin (Wizard 18®)against red pepper mites and one trial for Mn prochloraz against cobweb disease There was one laboratory efficacy study against green mould caused by *Trichoderma aggressivum* (Th4) and *T. atroviride* (Th3) using a granular formulation of imazalil.

The efficacy study for the fungicide Mn prochloraz (Octave®) against cobweb disease evaluated three different use patterns; incorporated into the casing at the full rate, at half rate, followed by watering on half rate after the first flush as a split application and finally as a watering on split application, the first application at fresh air, the second after the first flush. Both split applications were the most effective in managing the cobweb disease, with greater incidence of the disease in third flush mushrooms with a single treatment at casing.

Abamectin was selected as a low toxicity alternative for the organophosphate insecticide diazinon. Five residue trials were completed, all necessary residue samples collected and residue analyses undertaken. Preliminary laboratory efficacy work established that abamectin is very active against mushroom mites and this was confirmed in two growing room efficacy studies.

The fungicide imazalil (Magnate750WSG®) was evaluated using a petri dish bioassay as a treatment against green mould, Th4 and Th3. As expected, the Th4 was not controlled by the benzimidazole fungicides, but was by imazalil. Th3 was controlled by all the fungicide treatments, but imazalil was the most effective.

All relevant data, including growing room reports, residue analyses, efficacy data, etc., has been sent to Kevin Bodnaruk (AKC Consulting) for him to prepare submissions to APVMA for permit/registration so that the chemicals covered in this report may be given approval for use on mushrooms.

The implications of this work for the Australian mushroom industry are discussed. This project is on-going in terms of legislative requirements, availability of existing products, management of new and existing pesticide resistance issues and development of new pesticide chemistry, including organic products.

Technical Summary

The Australian mushroom industry is reducing its reliance on pesticides in general and as far as possible is encouraging the use of low-toxicity products. This project was established to ensure continued access by Australian mushroom growers to new or existing products that will assist them to sustainably manage pest and disease problems.

A total of three efficacy growing room studies were completed between July 2009 and December 2009; laboratory efficacy work evaluating imazalil as a mushroom inoculum treatment to suppress Trichoderma green mould was completed by March 2010. These included two efficacy trials for abamectin against the red pepper mite, *Siteroptes mesembrinae* and one trial for Mn prochloraz against cobweb disease, *Cladobotryum mycophilum.* There was one laboratory efficacy study against green mould caused by *Trichoderma aggressivum* (Th4) and *T. atroviride* (Th3) using a water soluble granule formulation of imazalil.

The efficacy study for the fungicide Mn prochloraz (Octave®) against cobweb disease was required to support the permit for a split application against cobweb. The study evaluated three different use patterns; incorporated into the casing at the full rate, incorporated into the casing at half rate, followed by watering-on at half rate after the first flush and as a watering-on split application, the first application at fresh air, the second after the first flush. The purpose of split applications is to ensure higher concentrations of the active ingredient remain in the casing, throughout the life of the crop. The validity of this proposal was confirmed in that the split application provided superior control to a single application at casing.

Abamectin was selected as a low toxicity, non-petroleum based alternative for the organophosphate insecticide diazinon. Five residue trials were completed and all the necessary residue work undertaken. Abamectin acts as an acaricide active against tetranychid or tarsonemid mites and as a nematicide active against veterinary nematodes. Preliminary laboratory efficacy work has established that abamectin is very active against mushroom mites related to tarsonemids and the two growing room studies confirmed the earlier work.

The fungicide imazalil (Magnate 750 WSG®) was evaluated as a treatment against green mould, *Trichoderma* species. This product is being evaluated in the USA for the same purpose, where industry needs are greater due to fungicide resistance issues. Preliminary efficacy data from a single trial using an emulsifiable concentrates (EC) formulation of the active ingredient confirmed activity. A petri dish bioassay confirmed the effectiveness of the granular formulation mixed with gypsum and mixed with the mushroom inoculum. *T. aggressivum* shows resistance to the benzimidazole fungicides and these fungicides were ineffective, whereas imazalil provided complete protection. *T. atroviride* is susceptible, but imazalil provided superior protection compared to the other fungicides.

All relevant data, including growing room reports, residue analyses, efficacy data, etc., has been sent to Kevin Bodnaruk (AKC Consulting) for him to prepare submissions to APVMA for permit/registration so that the chemicals covered in this report may be given approval for use on mushrooms.

The implications of this work for the Australian mushroom industry are discussed. This project is on-going in terms of legislative requirements, availability of existing products, management of new and existing pesticide resistance issues and development of new pesticide chemistry, including organic products.

Main Report

Introduction

The Australian mushroom industry is reducing its reliance on use of pesticides (Allan and Clift 2004), but it is important to maintain an armoury of pesticides that can be used if necessary. This project originally commenced in 2002 to generate the data necessary to either maintain mushroom industry access to existing pesticides and use patterns or to gain registration for new pesticides or use patterns.

An important aspect of the strategy is, as far as possible, to have at least two pesticides, of differing modes of action, for use against each major mushroom pest or disease. The strategy is important for both resistance management and availability of products to the Australian mushroom industry at any given time. This strategy has been accepted by the APVMA in allowing the mushroom industry continued access to diazinon, the initial permit for use of benomyl (now globally withdrawn from sale) and the permit for carbendazim.

There are three ways a pesticide becomes involved in this project. Firstly, APVMA may request residue data and possibly efficacy data on an existing product. Secondly, development of fungicide resistance (Allan *et al* 2008, Romaine *et al* 2008) may prompt the need for new pesticides with different modes of action. Thirdly, new pesticide chemistry may prompt the introduction of a new product or an organic material may become commercially available. It is important that the mushroom industry has access to the Marsh Lawson Research Unit (MLMRU) to undertake this type of work in a manner compatible with current cultivation procedures.

The main resistances present in the Australian mushroom industry are to the benzimidazole fungicides, carbendazim and thiabendazole, developed by dry bubble (*Verticillium fungicola* and *V. lamellicola*) and by cobweb (*Cladobotryum mycophilum*, but not by *C. dendroides*) (Allan *et al* 2008). At present there is no resistance to Mn prochloraz by any of these species. Even the benzimidazole resistance is variable, with some Australian disease isolates remaining susceptible (Fletcher *et al* 2004, Allan *et al* 2008). The split application of fungicides, although starting out with a lower concentration of the active ingredient provides a higher average concentration over the life of the crop and provides better disease control (Clift *et al* 2004, Fletcher *et al* 2004). It is important that this use pattern is established and retained for as many fungicides as possible (Fletcher *et al* 2004).

At the start of this current project, residue trials had been completed for the insecticides diazinon and cyromazine, the fungicides carbendazim, thiabendazole and Mn prochloraz, the nematicide fenamiphos and the acaricide abamectin. This project was designed to complete the required number of efficacy studies; two growing room trials for abamectin against mushroom mites, one for split application of Mn prochloraz against cobweb disease, and laboratory efficacy for imazalil as a spawn treatment against Trichoderma green mould. The results from these studies are reported here.

Materials and Methods

Growing Room Efficacy Studies

One growing room holds four trolleys, each with three levels holding up to six crates on each level. The treatments were arranged in a balanced randomised complete block design, with each treatment occurring at each level on each side of the trolley.

The active ingredient, formulated product used and types of application included were:

- Mn prochloraz: Octave WP Fungicide® 460 g/Kg, either incorporated as an aqueous suspension into the casing material, 3.0 g product per square metre or watered onto the crates at fresh air and again after first flush as a split application, each 1.5 g product per square metre (Figure 1), tested against a virulent benzimidazole resistant isolate of the cobweb pathogen *Cladobotryum mycophilum*.
- Abamectin: Wizard 18® EC, using 10 mL product in 3 L water applied at 250 mL per growing crate, watered onto the crates against swarming red pepper mites at fresh air and if necessary again after first flush as a split application

Evaluation of Mn prochloraz against cobweb disease.

One trial was done, with six treatments, six replicates per treatment arranged in a randomised complete block design, with each treatment occurring once at each level on each side of the growing room.

Treatment 1	Uninfested untreated control
Treatment 2	One application of Mn Prochloraz as the casing material is being prepared in the ribbon mixer, 3.0 g product per square metre growing crates.
Treatment 3	First application of Mn Prochloraz as the casing material is being prepared in the ribbon mixer, 1.5 g product per square metre growing crates. Second application, 1.5 g product in 1 L water per square metre made within 48 hours after the first flush has been picked.
Treatment 4	First application of test item at pinning, 1.5 g product in 1 L water per square metre, usually 7 -9 days after application of the casing layer (Fig 1). Second application, 1.5 g product in 1 L water per square metre made within 48 hours after the first flush has been picked.
Treatment 5	Infested untreated control, 20,000 spores m^{-2} of the cobweb pathogen *C. mycophilum* .applied at pinning.
Treatment 6	One application of Mn Prochloraz as the casing material is being prepared in the ribbon mixer, 3.0 g product per square metre growing crates, then 20,000 spores m^{-2} of the cobweb pathogen *C. mycophilum* applied at pinning.

Disease assessment was made prior to each harvest, recording both healthy and diseased yield. Statistical analysis was done on healthy yield, diseased yield and percent infection.

Fig 1 Afsheen Shamshad watering on Mn prochloraz fungicide as an aqueous suspension at pinning (Treatment 4).

Evaluation of abamectin

Two efficacy trials were done, each with the following four treatments:

Treatment 1 Uninfested untreated control, six replicates

Treatment 2 *T atroviride* spawn added at 80 g per crate on the day of delivery (Fig 2), water control on the swarming red pepper mites, 9 replicates.

Treatment 3 *T atroviride* spawn added at 80 g per crate on the day of delivery, abamectin watered on at pinning at the rate of 10 mL product in 3 L water, applied at 250 mL per 0.25 m^2 on the swarming red pepper mites, 9 replicates.

Treatment 4 *T atroviride* spawn added at 80 g per crate on the day of delivery, diazinon watered on at pinning at the rate of 3 mL product in 3 L water, applied at 250 mL per 0.25 m^2 on the swarming red pepper mites, 9 replicates.

Fig 2 Alan Clift adding the *Trichoderma* inoculum to compost delivered that day for the first abamectin efficacy trial.

In both trials, the growing crates were regularly examined three times per week after casing and mite infestation assessed as percentage of each crate covered in swarming red pepper mites associated with the *T. atroviride*. Following mite assessment at pinning, shortly after fresh air at 6 days since casing, the drenches were applied as specified and mite assessments continued throughout the trial. Diazinon was used as the existing product for comparative purposes.

Laboratory evaluation of imazalil granules as a spawn treatment

A water soluble granular formulation of imazalil, (Magnate 750 WSG®) was used for efficacy studies involving spawn treatment. Three benzimidazole fungicides with similar use patterns in the UK, Canada or the USA were included for comparative purposes

Spawn inoculation and/or treatment were with a method based on Romaine *et al* (2008). A spore suspension was prepared from an agar slant culture of either a benzimidazole resistant isolate of *Trichoderma aggressivum* (IPDM 100) or a susceptible isolate of *T. atroviride (IPDM 002)*, both from Australian mushroom farms. The spore suspension was diluted and the spore density determined using a haemocytometer, then 1 ml containing 10^6 spores per mL was added to 20 g gypsum, mixed evenly in a mortar and pestle then allowed to dry at ambient conditions (Figure 3).

The recommended fungicide rates are provided in Table 1. Two rates, the full rate and half this were tested. When both spores and fungicide were to be applied, the spores were applied first as described above, then after drying, fungicide-treated gypsum was added. Each 20 g gypsum was thoroughly mixed with 200 g spawn, ensuring an even coating, then 30 spawn grains were randomly selected, 10 in each of three replicates. Each replicate consisted of a labelled plastic petri dish, with 2 mm thick 2% tap water agar on which the 10 spawn grains were placed and incubated at 25 ^{o}C for five days. Each spawn grain was assessed for infection by the appropriate species of *Trichoderma* and the results expressed as a proportion out of 10. The original results were transformed using Inverse sin prior to statistical analysis. The means were then back-transformed, expressed as a proportion and tabulated.

Table 1 Fungicide rates tested as spawn treatments

Fungicide		Rate used
Active ingredient	Product	
imazalil	Magnate 750 WSG	1.33 g Magnate ground in 20 g gypsum, mixed with 200 g spawn
thiophanate-methyl	Topsin 750WP	0.5 g Topsin ground in 20 g gypsum, mixed with 200 spawn
carbendazim	Farmoz Howzat Systemic Fungicide	0.25 mL Howzat in 1 mL water, mixed with 20 g gypsum, mixed with 200 g spawn
thiabendazole	Storite Flowable Fungicide	0.25 mL Storite Flowable in 1 mL water, mixed with 20 g gypsum, mixed with 200 g spawn

Fig 3 Alan Clift using a mortar and pestle to evenly mix imazalil granules with gypsum to treat grain spawn prior to use in laboratory evaluation as a spawn treatment.

Results

Evaluation of Mn prochloraz against cobweb disease.

Cobweb disease established well in the two treatments involving direct infection (Treatments 5, 6, Fig 4). The mean weights of healthy and diseased mushrooms are presented in Figure 5.   Healthy yield was lowest for the two infected treatments and it is clear the fungicide provided limited protection. The infections in the other treatments were due to cross contamination from the sporulating infections, with the two watering on treatments providing superior control compared to the single initial application.

Fig 4. Cobweb in infected crate, Treatment 5.

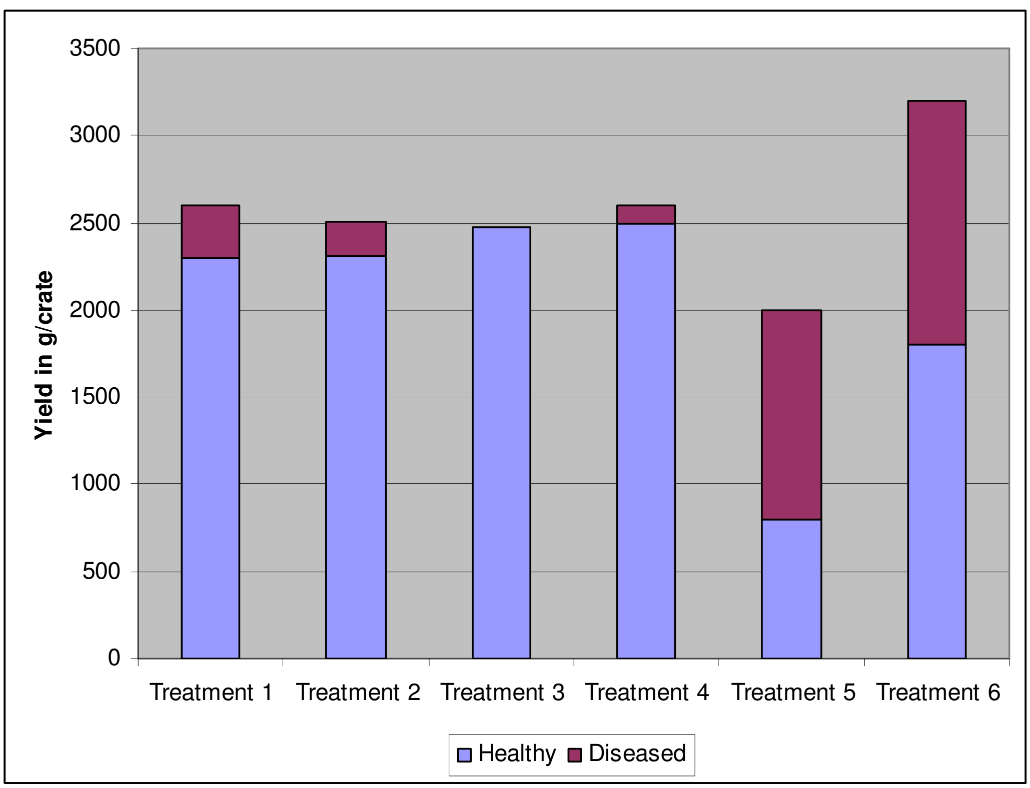

Fig 5. Healthy and diseased yields from the cobweb and Mn prochloraz trial.

Treatment 1 Uninfested, untreated control, Treatment 2 One application of MnProchloraz as the casing material is being prepared in the ribbon mixer, Treatment 3 First application of Mn Prochloraz as the casing material is being prepared in the ribbon mixer. Second application made within 48 hours after the first flush has been picked. Treatment 4 First application of test item at pinning, usually 7 -9 days after application of the casing layer. Second application made within 48 hours after the first flush has been picked, Treatment 5 Infested untreated control, 20,000 spores m^{-2} of the cobweb pathogen *C. mycophilum* .applied at pinning, Treatment 6 One application of Mn Prochloraz as the casing material is being prepared in the ribbon mixer, then 20,000 spores m^{-2} of the cobweb pathogen *C. mycophilum* applied at pinning.

Evaluation of abamectin

The mean infestation levels observed in the two trials are presented as Table 2. Both products suppressed the red pepper mite populations, although the mites were swarming over the first flush mushrooms, Fig 6.

Table 2 Efficacy of abamectin and diazinon against swarming red pepper mites[1]

Trial ID	Treatment[2]	Stage of crop[3]		
		Fresh air	Pinning	1st Flush
MRU09_05	Water control	32	31	1
	abamectin	35	0	0.4
	diazinon	27	0	0
MRU09_06	Water control	62	36	52
	abamectin	36	0	0
	diazinon	45	0	2

[1] Percentage of treated area infested with mites;
[2] mite infestation assessed at Fresh air, then treatments applied as a drench;
[3] Fresh air 4-7 days after casing, Pinning 8-10 days, 1st Flush 11-21 days, 2nd Flush >21 days

Fig 6. Red pepper mites swarming over button mushrooms, early in first flush on Control crate.

Laboratory efficacy for spawn fungicide treatments against green mould

The observed percentage infections by both species of pathogen are presented as Table 3. The benzimidazole fungicides were clearly not effective against the resistant isolate of *T. aggressivum*, whereas imazalil was highly effective against both isolates.

Table 3 Percent spawn infection by two species of *Trichoderma* using four Fungicides

Fungicide	Rate	Pathogen	
		T. atroviride	*T. aggressivum*
NIL	nil	98.5	98.5
Imazalil	full	0.0	0.0
	half	0.0	0.0
Thiophanate-methyl	full	26.5	73.8
	half	28.4	76.5
Carbendazim	full	9.0	85.8
	half	48.5	90.5
Thiabendazole	full	11.5	93.5
	half	19.5	94.8

Discussion

The Australian mushroom industry is less reliant on pesticides than it was ten years ago (Allan and Clift 2004) and the pesticides it does use are regularly reviewed by the government regulator, AVPMA. Resistance is also a factor in pesticide use (Allan *et al* 2008). Mushroom cultivation is a labour intensive industry with concerns about pesticide toxicity, and mushrooms can be eaten fresh with minimum preparation. Hence the industry pays great attention to toxicity and residue issues (Allan and Clift 2004).

The two main insecticides/acaricides recently considered are cyromazine, an Insect Growth Regulator (IGR) and abamectin, an avermectin, both of low mammalian toxicity. The fungicides being evaluated, Mn prochloraz and imazalil are both of low mammalian toxicity. However, the industry is very conscious of the need to be responsible in pesticide use (Allan and Clift 2004).

The reasoning behind split applications for all fungicides being evaluated is that multiple applications at initially lower rates provide superior control in the latter part of the crop. This is especially important if a disease is first noticed in the third flush, when any applications made at casing preparation are no longer effective (Fletcher *et al* 2004). Mites can swarm during harvesting and it is important to be able to harvest and then water on abamectin to prevent further contamination while still maintaining a short withholding period..

The main policy is to have at least two products of different modes of action available for use against major pests and diseases. As far as possible, low toxicity products are selected for evaluation in this project. To date, emphasis has been on collecting residue data but more efficacy work is now required. Products like abamectin, which are low toxicity and have few residue concerns need to be tested against all likely targets to develop the most appropriate use patterns. The reported residue trials included likely use patterns, so the necessary data is available.

Residue and efficacy reports generated in this project and its predecessor (MU06013) are being used by Kevin Bodnaruk (AKC Consulting) in the preparation of submissions to APVMA for registration and/or permit issue for a number of chemicals. These activities are not included as part of the work schedule for this project but a summary of the current progress with chemicals is:

Prochloraz:
All reports are with Kevin Bodnaruk. He will prepare and forward application to APVMA. He will enquire about the use of the Category 25 option for this usage.

Imazalil:
All reports and data are with Kevin Bodnaruk. He will contact APVMA and ascertain whether permit or Category 25 are the most appropriate route to pursue.

Abamectin:
Kevin Bodnaruk to seek approval for usage from APVMA (permit or Category 25).

Thiobendazole:
This application is for usage as a split application.

Kevin Bodnaruk will contact APVMA re: the most appropriate route for forwarding an application.

Thiophanate methyl:
A commercial company has shown interest in Australian registration of the product based on the use of United States residue data. Kevin Bodnaruk is contacting the company in order to forward this matter.

Kevin Bodnaruk will advise the MLMRU Management Committee of outcomes with the various chemicals.

In the immediate future, carbendazim is being reviewed by APVMA and an alternative benzimidazole fungicide will need to be evaluated. The most likely product at this time is thiophanate-methyl and more efficacy data may be required. The issue of pesticide usage in the mushroom industry is on-going and includes a watching brief to continually review existing concerns and likely solutions. These solutions should include both natural and synthetic materials.

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Technology Transfer

The results of this project are regularly presented to the industry at the annual National Conference and articles are published in the quarterly mushroom industry Journal. As products become available by registration or by permit, this information is included into the Knowledgebase module of the industry's Agora website and notification provided in the industry journal.

Recommendations

Residue and efficacy data have now been generated for a group of pesticides and reports are being prepared to support the following use patterns:

- Split application of Mn prochloraz (Octave®): This would be a most valuable treatment for the Australian industry. Bayer Pty Ltd is apparently no longer opposed to this proposed use pattern and may support a registration application. The existing permit (Per10407) for the split application refers only to cobweb disease, so now there is both residue and efficacy data to support use against the two pathogens, cobweb and dry bubble
- Carbendazim split application, as well as spawn treatment for use against *Trichoderma* green mould: Because of general concerns about carbendazim, the mushroom industry will probably find it difficult to retain access to this fungicide. The residue data has already been generated and laboratory efficacy against Trichoderma green mould has been determined.
- Thiabendazole watering-on between flushes: The residue work has been completed and fortunately this fungicide does not have the same health concerns as carbendazim, and efficacy data as a spawn treatment against *Trichoderma* green mould has been generated to support the application as this is a new use pattern.
- Abamectin watering on against mites during cropping: Both residue and efficacy work have been completed.
- Imazalil spawn treatment and also casing application: Six residue trials have been undertaken and residue analysis has been completed. Preliminary efficacy work has been completed using an emulsifiable concentrates (EC) formulation, but all the residue work was done using water-dispersible granules, for easier incorporation into gypsum for mixing with the spawn. Laboratory efficacy has been determined using the granules against the two main Trichoderma green mould pathogens on grain spawn.