Non-synthetic alternatives to complement pest and disease management in mushrooms

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Contents

Public summary

Pests and diseases continue to pose a significant threat to the Australian mushroom industry. The development of an integrated pest and disease management (IPDM) program for mushrooms has been identified as a high priority to enable the adoption of more sustainable practices in mushroom production and reduce the reliance on chemical pesticides. Pesticides options are relatively limited and may be withdrawn any time for regulatory reasons. Repetitive use of the same pesticides can promote the development of resistance in pests/pathogens, rendering control options ineffective. Biorational products are low-impact and can be used in crop protection to prevent or suppress pests and pathogens. Formulations can include live microorganisms (and/or their derivatives) and are best suited for used in an IPDM programme. To evaluate the efficiency of biorational products and biological control agents for use in mushroom cultivation, formulations and agents must be screened according to their mode of action. For microbial products, efficacy towards target pests and diseases can vary significantly among different strains of the same species. It is therefore important to test locally sourced products for efficacy to compare with products based on different strains and formulations overseas.

This project aimed to conduct research and extension activities that will ultimately promote the use of non-synthetic or biological approaches in mushroom IPDM. Primary aims were to (i) review current knowledge on the use of biologicals in mushrooms and identify products that are available to trial in Australia; (ii) conduct experiments with biological agents to ascertain control efficacy; and (iii) deliver communication activities that ensure new knowledge and research outputs from the project are easily accessible.

Tables of biorational agents and constituents were compiled by category, based on what is currently available in Australia and registered with the Australian Pesticides and Veterinary Medicines Authority (APVMA). Prospective biological control agents were then selected for testing in experimental mushroom cultivation trays. Agents were selected based on their accessibility and on the criteria that prior research indicated potential activity against the key pests and pathogens of interest. Following the review, experiments were conducted to screen biologicals for compatibility with mushroom growth, when applied to compost. Where an application rate for mushrooms was not provided, studies were conducted to determine the suitable concentrations for use in mushroom growing.

Trials were then subsequently set up to test candidate products alone or in combination for their efficiency to (i) kill sciarid fly larvae in the bedding, (ii) kill adult flies, or (iii) ascertain activity against mycoparasitic fungi. While sciarid flies were the focus of testing (due to constraints of maintaining different fly species for experimentation), biological products were selected for testing, where there was evidence for potential to control phorid flies as well. Products that demonstrated effect against sciarid larvae and adults included a sustained release (s)-methoprene product, an emulsifiable oil spray-treatment for adult flies, entomopathogenic nematodes when applied at specific crop intervals, and a liquid formulation of *Bacillus amyloliquefaciens* strain QST713; all of which could all be evaluated further as stand-alone agents or in combinations for IPDM, and to assess for effects to mushroom production at scale.

Key recommendations include (i) expanding the testing of biorationals for compatibility with mushroom production and for control efficiency against phorid flies and/or pathogens, since not all products identified could be tested during the project, (ii) evaluating products that showed promise in experiments for feasibility in mushroom production, and (iii) continuing the development of a novel mushroom fly lure, for potential use in mass trapping of flies on farms. The project's primary outcome is that the Australian mushroom industry is better informed on the potential for using biologicals in mushroom growing systems. Additional outcomes achieved include the identification of a range of potential pest and disease control options, and proof of concept data that may enable the development of novel biological pest management methods. Addressing these important knowledge gaps paves the way towards the adoption of more sustainable practices.

Technical summary

The fungal gnat, *Lycoriella ingenua*, and mushroom fly *Megaselia halterata,* are major pest insect species in commercial edible mushrooms worldwide, and in addition to causing crop damage through larval feeding, the flies also vector mycoparasitic fungi that can lead to significant crop losses. Pesticides options are relatively limited, and repetitive use of the same chemistries can lead to the development of resistance in pests/pathogens, risking significant crop damage. By diversifying the options available to growers, and by including biologicals as additional tools for use in IPDM, resistance development may be avoided and crop damage limited in the long-term. Additionally, useful chemistries may be banned for crop protection at any time. The primary aims of this project were therefore to (i) review current knowledge on the use of biologicals in mushrooms and identify products that are available to trial in Australia now; (ii) conduct experiments with biological agents to ascertain control efficacy; (iii) deliver communication activities that ensure new knowledge and research outputs from the project are easily accessible.

Data on prospective biological control agents was compiled and products that are currently available in Australia and registered with the APVMA were identified. Prospective agents were then selected for trial in experimental mushroom cultivation systems conducted in a controlled environment room (CER) at the AgriBio facility (Agriculture Victoria). Agents were selected based on their accessibility in Australia and on the criteria that research literature or industry articles indicated their activity against the key pests and pathogens of interest. Following the review, experiments were conducted to screen biological agents and formulations for compatibility with mushroom growth when applied to compost. Where an application rate for mushrooms was not provided or published, dose-response studies were first conducted, to determine the suitable concentrations for use in mushroom growing substrates. Trials were then set up to test candidate agents alone or in combination, for their efficiency to control sciarid fly larvae in the bedding and/or adult flies, or to compare the antagonistic activity exhibited against mycoparasitic fungi (*Trichoderma* green mould and *Lecanicillium fungicola* dry bubble).

While sciarid flies were the focus of testing in MU22000, mainly due to resource constraints of maintaining the insects in culture, biological products were specifically selected for testing, because scientific literature was found to indicate the potential to control phorid flies with the same or similar agent. There is a degree of complexity and risk involved in the adoption of new biological products, therefore, to reduce risk, experimental research such as the experiments described in this report, is required (i.e. for mushroom crops specifically) as a prerequisite to understand suitability and best practice application strategies. Products that demonstrated effect against sciarid larvae and adults included a sustained release (s)-methoprene product, an emulsifiable oil based spray-treatment for adult flies, entomopathogenic nematodes when applied at specific crop intervals, and a liquid formulation of *Bacillus amyloliquefaciens* strain QST713; all of which could all be evaluated further as stand-alone agents or in combinations for IPDM, and to assess for effects to mushroom production at scale. The results suggest that a second phase of this research is warranted to expand testing for products to control phorid flies. Further research is additionally needed to develop molecular assays to facilitate the testing of biofungicide agents for bioactivity or host-disease response, so that efficacy can be measure when these agents are applied to mushroom compost.

A confidential technical report was also submitted with this final milestone for project MU22000 and describes the process for the identification and evaluation of volatile organic compounds (VOCs) as potential commercial attractants for *Lycoriella ingenua* sciarid flies. The results of the experiments conducted and reported on strongly indicate certain VOCs that may be synergistic in action, and competitive with mushroom substrates with respect to attractiveness to fungal gnats. Furthermore, a specific sex pheromone was identified from adult sciarid flies that has not been reported previously in scientific literature. Together, these results warrant further research to determine the best combination of synergistic volatiles, to enable the continued development of a novel lure that may be used in attract-and-kill devices for sciarid pest flies. The scope could be expanded to include phorid flies for the development of an effective combination lure. The development of a lure for sciarid and possibly also for phorid flies could see the mass trapping of flies on farms, which would be beneficial to reduce the overall burden of mushroom flies and the diseases they can vector. Attract-and-kill strategies would offer a practical alternative to direct pesticide use in compost, by luring the flies away from the crop. However, to be effective, a lure must compete with the attractiveness of the compost itself.

Keywords

Integrated pest and disease management; sustainable practices; biopesticides; biorationals; sciarid; phorid flies; biological control; green mould; dry bubble; novel technology.

Introduction

Biologicals

Sustainable agriculture relies on practices and technologies that are effective but have minimal environmental impact. Aside from biorational control products (also called biopesticides), biological approaches broadly include other products such as the 'biostimulants' and 'biofertilizers' that may assist in crop development, promote growth, or suppress disease via competition. Furthermore, other biological approaches include the use of semiochemicals to lure and kill pests away from the crop, or to disrupt mating behaviours.

Apart from testing efficacy, it is also necessary to determine the best application strategy for novel formulations, prior to upscaling to on-farm trials, especially as most commercially available biopesticides have not been developed for use in mushroom cultivation. While lab screening enables the selection of promising candidates to be identified for farm trials, knowledge of the crop system, the pests/pathogens, the microbial community dynamics and formulations themselves, are required to inform the strategy.

Invertebrate pests of mushroom crops

The fungal gnat, *Lycoriella ingenua* Dufour (Diptera: Sciaridae), and the mushroom phorid fly, *Megaselia halterata* (Diptera: Phoridae) are major pest insect species in commercial mushroom production (*Agaricus bisporus*, *Pleurotus* spp.) worldwide, and in addition to causing crop damage through larval feeding, the flies also vector mycoparasitic fungi that can lead to significant crop losses. There is a growing demand for alternative control measures for these pests, since control options are limited and the rise of resistance to the few available measures poses a constant threat to production. Control of flies is generally directed at destructive larval stages; however, pesticide resistance is a serious problem and insecticide efficiency is limited by the difficulty of mixing chemicals into the compost to achieve sufficient contact, without being toxic to the crop. Biologically based insect growth regulators (IGRs) can be used, but other biological protectants may also help to control mushroom flies and larvae, yet research has been limited for mushroom crops specifically. Mushroom growth systems are highly regulated, and this presents an opportunity to support beneficial biological agents that may be added to suppress fly populations; however, it is important to first establish whether the microbes/products available in Australia are compatible with mushroom growth.

Sciarid fly adults and larvae are known to be susceptible, at minimum, to species of insect pathogenic fungi such as *Pandora gloeospora, Beauveria bassiana,* and to *Bacillus thuringiensis israelensis* (*Bti*) (Keil 1991; Andreadis et al. 2016). Both sciarid and phorid flies are also susceptible to strains of insect pathogenic nematodes, *Steinernema feltiae* and *S. carpocapsae*. Recent research on mushroom fly control also showed that BotaniGard, a product based on a formulation of *B. bassiana* isolate GHA, can kill the fly pupae from both species (Andreadis et al. 2021). Although the efficiency of BotaniGard® was not high in these studies, the results indicate that it may be beneficial to screen more isolates of *Beauveria* for their biocontrol potential, because there may be strains that are more virulent against both phorid and sciarid flies. While there is scope to trial locally available insect killing fungal and nematode agents, further research is needed to determine whether these bioprotectants may interact synergistically with other amendments used, such as insect growth regulators, as this has been demonstrated with other insect pests for IPDM (Suarez-Lopez et al. 2022). An integrated approach would facilitate insecticide resistance management, as well as achieve more sustainable practices on farms.

Microbial diseases of mushroom crops

In Australia and worldwide, mycoparasites and bacterial disease can cause heavy losses in commercial mushroom farms. Fungal diseases such as dry bubble (*Lecanicillium fungicola*), cobweb (*Cladobotryum* spp.), wet bubble (*Mycogone perniciosa*), and green mould (*Trichoderma* spp.) impact yield and harvest quality, because they reduce the cropping surface or cause direct damage to caps (Gea et al. 2021). Current measures are mostly preventative and involve intensive hygiene protocols during and after the completion of the crop cycle. Select fungicides are also applied but as is the case in many cropping systems, overuse and dependence on the application of the few fungicides available has promoted the development of resistant disease strains, hence, there is a real need to diversify treatment options and management methods. Alternative control measures may include the use of non-toxic essential oils such as from thyme, mint, or lemon for example, and

other commercially available plant-based formulations, or microbial based biological controls and their derivatives (Gea et al. 2021; Berendsen et al. 2012; Gahukar 2014). Recently, strains of *Bacillus velezensis* isolated from *A. bisporus* casing demonstrated biocontrol activity against the *Trichoderma* pathogen when applied via irrigation (Büchner et al. 2022). Other *Bacillus* strains isolated from casing have also demonstrated biological control against mycoparasites (Stanojević et al. 2019; Pandin et al. 2019; Pandin, Védie, et al. 2018; Clarke et al. 2022). A thorough review of possible biologicals available in Australia, including those used in other crop systems, or those composed in different formulations that haven't been tested, is a necessary first step for the discovery of options and methods that could be trialled.

Understanding how different biological agents work is also important. For example, bacterial agents used in biofungicides work as preventative measures rather than as curative treatments. Beneficial species of the genus *Bacillus* that are used in several biofungicides are naturally associated with mushroom cultivation and can be found in abundance in compost (Pandin, Védie, et al. 2018). When applied early to growing mushroom mycelium (i.e. in phase III), they may be able to prevent fungal disease from establishing. However, they need to be (1) applied before the disease agent is abundant and (2) during conditions that facilitate 'biofilm' formation (Pandin et al. 2019). A biofilm is defined as a community of surface-associated microbial cells that is enclosed in a polymeric substance matrix (Donlan 2002). Essentially, this means that certain microbes can develop a sticky gel-like substance on the surface of the *Agaricus* mycelia or compost material, which provides a protective benefit to the crop. Biofilms house and nurture the bacterial cells, giving them a competitive advantage over other microorganisms, allowing them to grow and collectively respond to potential invaders, including mushroom pathogenic fungi. A study by Pandin et al (2019) demonstrated the protective effect of biofilm formation on mushroom compost and mycelia, by investigating the bacterial growth under using microscopy and by assessing the key genetic activity involved in antagonism. The authors applied the inoculum of the bacterial agents' *Bacillus velezensis** QST713 and *B. velezensis* FZB42 to phase III compost, in combination with the pathogen *Trichoderma aggressivum*. By doing this, they were able to show the physical interaction between the bacteria and fungi and indicate the specific metabolic mode of action against *Trichoderma* that leads to growth inhibition. *Note that *Bacillus velezensis** strains' QST713 and FZB42 are synonymous with *Bacillus amyloliquefaciens* strains' QST713 and FZB42; This species name change is due to the taxonomic reassignment that is enabled by comparing whole genome sequences (Pandin, Le Coq, et al. 2018).

Testing the efficacy of biofungicides therefore presents various research challenges. While evidence of control can theoretically be measured as the absence of disease in the host crop (*A. bisporus* in this case), in a controlled experiment, the pathogen inoculum must be artificially introduced in sufficient amounts to cause an infection. The inoculation of the disease agent often exceeds the quantity of what would naturally occur, and disease can flourish as a result, counteracting the otherwise preventative activity of the biofungicide. Consequently, directly comparing the effect of a biofungicide with a synthetic fungicide, is often an inappropriate and uninformative exercise, since they tend to have different modes of action.

To determine whether a bacterial agent may be effective, researchers tend to focus on (1) identifying or quantifying specific bioactive products involved in pathogen antagonism (e.g. Clarke et al. 2022), (2) or on quantifying the pathogen-load using cultivation-dependent methods (i.e. by isolating microbial colonies on selective agar), or (3) by using molecular methods to quantify the genetic activity of the biological agent or the host response to disease. However, these approaches all require the development of laboratory diagnostic assays and often, the collection of genomic data, if these don't already exist in the public domain. Such methods are required before an assessment of biocontrol efficacy can be made with biofungicide control agents, however, developing diagnostic methodology is laborious and so was beyond the scope of project MU22000. For this reason, the project objectives were developed to focus mainly on invertebrate pests, which can vector disease agents, and are therefore key in the prevention of disease introduction and spread on farms.

Considering products tested overseas already, certain pesticide safety criteria must be met for APVMA registration, including for organic and biologically derived constituents, and there is a cost associated with the registration process when considering the importation of international products. To gain approval for registration in Australia, well-informed market research and robust efficacy data is required, which frequently presents a barrier to the process and therefore the accessibility of international products to growers is limited. To remedy this, locally sourced products that are already registered but developed for other cropping systems,

should be screened for suitability with mushroom crops, pests and diseases.

Research aims

Based on the above context and identified research gaps, the specific aims of this project were scoped to:

(i) Review non-synthetic control methods for pests and diseases of mushrooms, and other crops, to identify what may be readily available in Australia to trial.
(ii) Evaluate the compatibility of select biopesticides, such as products based on insect pathogenic fungi, bacteria and/or nematode agents, with mushroom (*Agaricus*) growth
(iii) Conduct laboratory trials against targeted pests and pathogens using select biological agents and/or formulations to ascertain efficacy
(iv) Communicate findings and provide educational resources on the use of biologicals to industry

Methodology

The following methodology outlines project activities according to the objectives, that were designed to link with and complement the activities of project MU21007 'Pest and disease management for the Australian mushroom industry'. The project activities are written in order of the main project objectives. A technical summary on scientific methodology is also provided in Appendix 1 of this document.

Objective 1: Review non-synthetic control methods for pests and diseases of mushrooms

Engagement with industry experts. To enable an effective and relevant review of non-synthetic control methods for mushrooms, international research and industry leaders, that were recommended by the Australian Mushroom Growers Association (AMGA), were contacted by Agriculture Victoria project leader, consulted with (virtually) on a regular basis, and additionally, a project reference group (PRG) was formed as mandated. Aside from the official ~6 monthly PRG meetings that were held with the panel of experts, objectives were discussed with the project team of MU21007 as required. International researchers with niche expertise in biological control of pests and diseases of mushrooms, were also consulted with regularly during the project. Farm visits and tours were conducted, and in total this included three mushroom (*Agaricus* bisporus) farms in Victoria and one in South Australia, with an additional two exotic mushroom farms. During these visits, pest and disease problems and control strategies were discussed with the growers, and on three occasions, live flies were also collected for laboratory rearing. A material transfer agreement (MTA) was arranged with a commercial farm in Victoria to enable the ongoing supply of insecticide and fungicide-free phase II compost and commercial *A. bisporus* mushroom spawn, as needed for research purposes; this was arranged for the duration of the project. In November 2022, the project leader visited the Marsh Lawson Mushroom Research Centre (MLMRC) at the University of Sydney to view the facility, to learn about mushroom cultivation research methods, and to discuss potential opportunities for collaboration.

Literature and product review. Reviewed literature included (1) scientific peer reviewed articles, (2) proceedings archived in the International Society of Mushroom Science (ISMS), (3) product technical reports, where available and accessible and (4) articles available on either the AGORA website or from the communications project MU21003. Literature was searched for pertaining to the control of pests and diseases of commercially growing mushrooms (primarily *Agaricus bisporus* but *Pleurotus* research was included if pests/diseases occurred on both crops). Literature on the biology of the key pests and pathogens of mushroom crops in Australia was further reviewed to provide context on the subject matter, enabling capability development of the Agriculture Victoria team. As a result of the review exercise, a search was conducted against the Australian Pesticides and Veterinary Medicines Authority (APVMA) public chemical registration system (pubcris) database, to obtain information on registration status for specific biological control products and/or constituents, where available in Australia.

Tables that provide detail on specific constituents were compiled, these included the type of agent (e.g. whether fungi, bacteria, plant derived), what they have been tested for with respect to (1) compatibility with mushroom growth, and (2) for activity against key fungal pathogens or mushroom fly pests, with supporting references provided. Information provided in the tables also includes the category; the type of product with it's mode of action (MoA) group, as determined by either the global fungicide resistance action committee (FRAC) (https://www.frac.info/) or the insecticide resistance action committee (IRAC) (https://irac-online.org/). Detailed information on the mode of action classification systems can be freely obtained from these websites. Use of the FRAC and IRAC mode of action classification system enabled some products to be excluded from the product list, on account of being synthetic. For example, some insect growth regulators (IGRs) may be considered biorational, due to being plant-derived (i.e. ex neem oil) or because they are analogues of naturally occurring insect juvenile hormones. Other IGRs, however, are not typically classified as biorational, for example, constituents in groups' 15, 16, 17, or 18 which include chemicals which function as inhibitors of chitin biosynthesis. Examples include cyromazine (MoA group 17) and diflubenzuron (MoA group 15), which have both been tested and used against sciarid and phorid mushroom flies (Erler et al. 2011; Navarro et al. 2021) but are not biorational.

Rationale for inclusion. Pertaining to the use of IGRs in mushroom pest control, there have been reports of mycotoxicity against A*. bisporus* mycelium (Navarro et al. 2021; Erler et al. 2011). However, often only the independent action of IGRs is tested in these experiments, where the concentration of the product is relatively

high because it is being used as a stand-alone product to achieve adequate pest control (i.e. the 'silver bullet' approach). In the scenario where an IGR is combined with a compatible agent for additive or synergistic effects in IPDM, a lower concentration of the IGR may be used to achieve pest control. Lower concentrations may mitigate any mycotoxic effects, are more economical, and using a combination of products or strategies have the added benefit of challenging the pest with diverse modes of action, reducing the risk of resistance developing. For these reasons, select biorational IGRs have been included in the bioinsecticide table, since they may be compatible with mushroom growth when the concentration is low. Following an extensive review of the mushroom IPDM literature, no studies were identified that have tested low or sublethal concentrations of any control agent, neither have synergy studies been conducted though they have been suggested (Potočnik et al. 2010). This indicates a knowledge gap and presents an opportunity for future research to focus on product combinations to achieve synergy in a mushroom IPDM program.

Additional notes. Plant essential oils or extracts were predominantly excluded from the list of biorationals compiled, except where a specific product or agent holds a current registration with the APVMA or is well supported by scientific testing. The decision to omit essential oils from this product review was primarily because they have been recently reviewed. For example, tables listing essential oils and effects against mushroom pathogens can be freely obtained from Gea et al. (2021). There are also studies that have tested essential oils as fumigants and/or repellents for sciarid and phorid flies (Choi et al. 2006; Jess et al. 2017; Navarro et al. 2021), although significant variation in efficacy has been reported to date. Plant extracts and essential oils are biorational and may consist of multiple active constituents that could be tested independently to identify the specific potential of the constituents as (1) repellents, (2) insecticidal agents, and/or (3) microbial disease antagonists. For example, diallyl polysulfides (DAS) from garlic are known to be nematocidal (Eder et al. 2021) as well as antimicrobial (Anwar et al. 2016) but are just one of the bioactive constituents that garlic possesses (Jess et al. 2017). Further fundamental research is required to classify the constituents in many plant extracts for their specific effects and mode of action.

Objective 2: Evaluate the compatibility of select biorationals with mushroom (*Agaricus*) growth

Screening for compatibility with mushroom growth. Candidate biological agents identified during the review were prioritized for testing, based on published data that indicated likely compatibility with mushroom cultivation. Toxic effects to mushroom growth were subsequently assessed for *in vitro* using 'race tube assays' with fresh phase II-end compost. The aim of these assays was to measure the vegetative growth of *Agaricus bisporus* mycelium during exposure to constituents and formulations, and/or to different concentrations of constituents, to ascertain the potential for detrimental impacts to mushroom growth. However, beneficial growth-promotion effects were also measured and where observed, the experimental assays were then repeated on another occasion, and with a different batch of compost, to ensure valid and robust data analysis. The objective of testing was to eliminate from further experimentation any products that are not suitable for mushroom cultivation.

Biological products that were identified in the review process, were subsequently tested for compatibility with mushroom growth using race tube assays, following the suggestion of the MLMRU project (MU21004) team at the University of Sydney. These assays are a standard way to test effects to growth, involving *A. bisporus* spawn grain applied to commercially prepared phase II-end compost. Race tube assays were conducted in plant growth chambers at the AgriBio Centre in Bundoora (Agriculture Victoria); these chambers allowed for temperature and humidity control to simulate the phase III conditions. By this method, two commercially available bacterial biofungicide strains and their commercial formulations were tested. Additionally, a bacterial isolate strain (*Paenibacillus* sp.) that is not yet commercialized but is under development, was tested. Bioinsecticidal products were also investigated for compatibility with mycelial growth. Insecticidal 'potassium salts of fatty acids' (insectical soap) were tested in combination with an oil-based adjuvant, as recommended on the label; the commercially available *Beauveria bassiana* strain and formulation product were tested in two different concentrations, and a sustained release (s)-methoprene product was tested at two concentrations. Finally, a combination of *B. bassiana* and sustained release (s)-methoprene were tested together, to see if the direct mixing of products could cause any impact to mushroom growth. For more detailed methodology refer to Appendix 1.

Objective 3: Conduct laboratory trials against targeted pests and pathogens to ascertain efficacy

Research activities to support product testing. Products and/or constituents were shortlisted for further experiments, following the compatibility tests. To accomplish experiments with insects, live sciarid flies and phorid flies were collected from three farms in Victoria, and methods were developed to facilitate ongoing fly maintenance. A protocol for maintaining sciarid flies in continuous culture was successfully developed. Although published methodology can be referred to for both insect species, protocols relied on a constant supply of fresh insecticide-free mushroom compost, which restricted the culturing of flies on project MU22000 to one species due to resource constraints. The rearing protocol developed by the Agriculture Victoria research team involved rearing sciarid larvae on an oyster mushroom grain spawn, wheat bran, and vermiculite mix. This method of rearing flies provided the advantage of allowing fly larvae to be easily observed during development and easily transferred to experimental containers in known quantities. The oyster substrate could be prepared in-house at Agriculture Victoria, and using this also proved effective at reducing weed mold contamination, which could impact downstream experiments. Daily maintenance involved assessing cages for population density and determining when fresh substrate was required. The approach used enabled the tracking of fly lifecycles with ease, which was necessary to conduct efficient screening of biological control agents under reproducible conditions. Notably, oyster grain-spawn proved not to be a suitable rearing substrate for phorid mushroom flies.

Experiments with flies involved applying microbial agents and formulations to (1) vermiculite, (2) compost and/or (3) casing material, with and without *Agaricus* mushroom spawn. The focus of the research was on assessing larvicidal effects and at concentrations that were deemed tolerable for the crop. To assess biofungicides, dual culture competition assays were conducted in the laboratory, using nutrient agar media and/or casing material. Applications were tested in accordance with the specific mode of action for the different biological agents used, and practical advice was sought from the PRG expert panel to develop the experiments effectively. While the intention was to use lab experiments to identify a product for further testing at the Marsh Lawson Mushroom Research Unit (MLMRU), the MLMRU was out of action from November 2023 to ~May 2025, and so following consultation with the project reference group, the decision was made to instead scale-up experiments in the controlled environment room (CER) at the AgriBio research facility (Agriculture Victoria). Refer to the technical report in Appendix 1 for a full description of the experiments.

Objective 4: Communicate findings and provide educational resources

Industry articles, webinar and podcast. Three industry articles were prepared for MushroomLink magazine with the assistance of the communications project team (MU21003). The first industry article was published in March 2023 and was titled ‘Sustainable Pest and Disease Management’, which was written by and in collaboration with the MU21003 team. Two further industry articles, rather than factsheets, were subsequently drafted; these articles allow for detailed reporting on the research and educational content produced during MU22000. A second article may be published in the Winter edition of MushroomLink magazine in 2025 and pertains to the finalized ‘biorational’ product review, which presents information on how to generally use different biological agents for mushroom pest and pathogen management. A third article has been drafted and will be submitted to MushroomLink later in 2025, subject to obtaining approval for publication from stakeholders.

Two grower workshops/educational webinars were scheduled for the project. Working with the MU21003 MushroomLink team, the first was delivered as a webinar, which was published in November 2023 and is available on YouTube. This presentation focused on the process of finding and reviewing new biorational products, and discussed the approach used to conduct research on integrated pest and disease management tools. The second was delivered by MushroomLink as a podcast interview with the project leader of MU22000 and was published in April 2025. The podcast discusses some of the experimental research outcomes from the project, with a particular focus on bioinsecticides and biocontrol agents for mushroom flies.

Farm visits and extension activities: The MU22000 project leader accompanied team members’ from

MU21007 and MU22003 (PhD program to study viruses associated with *Agaricus* mushrooms in Australia, led by Agriculture Victoria) to visit two farms in Victoria. The goal was to tour two different farms, discuss the different growing strategies, and to ascertain opportunities to receive materials for the development of novel (disease) diagnostics methods. A video conference call was held in 2023 with mushroom growers in NSW, to discuss methods to measure the effects of biological control agents (i.e. mites, entomopathogenic nematodes) on farms; the call was organized by the MU21007 project team. Throughout the project, farms were contacted privately, usually by email initially, to gather information on their use of biological products that are permitted for use on farms or to collect flies and materials.

Conferences: The biennial AMGA conference was attended in October 2022 and 2024. These conference events provided the opportunity to engage with growers and industry stakeholders including local and international experts, researchers and industry leaders. In 2024, a presentation was delivered at the AMGA conference by MU22000 project leader Dr. Aimee McKinnon titled "Investigating biorational agents to complement mushroom pest and disease management". The presentation focused on providing an overview of the project and achievements. In February 2024, Dr. Aimee McKinnon attended the joint 20th International Society of Mushroom Science Conference and the 26th North American Mushroom Conference. A conference presentation was delivered titled "Investigating biorationals for mushroom integrated pest and disease management". The presentation focused on some of the novel experimental research conducted to date, specifically pertaining to the control of mushroom sciarid fly larvae (*Lycoriella ingenua*). The presentation was well received, and attendance provided an opportunity to connect with international mushroom researchers and meet face-to-face with some of the project reference group members.

Results and discussion

Biorational product review

Microbial biofungicides: There is potential to incorporate more biofungicides into mushroom cultivation. In the product review, products that have been reported to have activity against key fungal pathogens of mushrooms are listed (**Table 1**); all but two are registered with the APVMA to date. These two agents are included in Table 1, because they could be proposed for the registration process if not underway. One of these agents, for example, is *Bacillus amyloliquefaciens* subsp. *plantarum* D747, which has been recently tested against the fungus that causes wet bubble disease, *Hypomyces perniciosus*, with promising results reported (Navarro et al. 2023).

Bioinsecticides: In contrast with the biofungicides, biological options are limited for the control of invertebrate pests of mushrooms. To expand on what could be tested, 'soft' insecticides based on bacterial products may be considered, or certain insect growth regulators that are plant-derived and/or analogues of naturally occurring hormones (**Table 2**). Aside from *B. thuringiensis* subsp. *israelensis* serotype H14, which is a bacterial agent currently permitted for use in mushroom cultivation, there are other products that are originally derived from bacteria, such as Spinetoram and Spinosad, both of which have been reported to have insecticidal activity against mushroom (sciarid) flies. Of the options available in Australia, there is one product based on the insect pathogenic fungus *Beauveria bassiana*, South African strain PPRI 5339 that is currently registered with the APVMA. Other commercial strains of *B. bassiana* have been tested for use against mushroom flies (Andreadis et al. 2021; Andreadis et al. 2016) and have been used on farms in the UK and Europe. However, strains of *B. bassiana* can vary widely in their control activity and so it is important to screen strains for virulence against the target pest, and to not assume broad-spectrum control is possible.

Bioinoculants and Biological Controls: Bioinoculants are microbial products that are not intended for pest or disease control, however, frequently these products contain bacterial agents that have the capacity to suppress pests or pathogens. They may be marketed as 'soil conditioners' or microbiome-amendment products, analogous to pro-biotics for crop health. Because bioinoculants are not intended for pest/disease control, they do not need to be registered with the APVMA (or other regulatory body). They are often formulated in low quantities of the inoculum, making the product more economical to mass produce but also limits control potential. Examples of bioinoculants that contain agents with the ability to prevent or suppress pests and diseases can, however, be found in **Table 3**. For instance, the bioactivity of *B. amyloliquefaciens* FZB42 (Table 3), has been tested for activity against *T. aggressivum* in compost, as mentioned previously.

There is also a product that combines three species of insect pathogenic fungi, which is marketed as a soil-health product. This is because these fungi species, as well as being able to kill a wide range on insect pests, can sometimes form beneficial associations with plant roots and may assist in nutrient acquisition and stress tolerance (McKinnon et al. 2023). Whether these species/strains are beneficial for mushroom crops and have the capacity to control associated pests, has not been tested to date.

Unlike microbiological control agents, macro biological control agents such as predatory mites and entomopathogenic nematodes do not currently require APVMA registration. These beneficial agents work upon release into compost and/casing substrates, where they move, hunt for and attack their prey. In the case of the entomopathogenic nematodes (EPN), it is important to understand that they do not harm the crop, unlike the crop-parasitic nematode species. They are formulated in dehydrated cellulose which dissolves quickly in water and can provide nutritional support to the nematodes. The application of EPN and predatory mites for sciarid and phorid fly control has been well studied (Jess and Schweizer 2009; Rinker et al. 1995; Shamshad, Clift, and Mansfield 2008; Scheepmaker et al. 1998; Navarro et al. 2021; Jess and Bingham 2007). Internationally, products have been developed for mushroom cultivation, and these are reportedly routinely implemented for on-farm control, overseas.

Experimental results summary

Screening for compatibility with mushroom growth. Results of testing different biologicals for their effect on mushroom mycelium growth demonstrated the importance of assessing different concentrations, especially when an application rate for use in mushroom crops is unknown. Furthermore, the growth experiments showed the importance of testing biological agents and formulations separately and for mushroom crops specifically, since formulations have generally been developed for application to plant cropping systems. The growth of mushroom mycelium was positively affected by an application of the 'potassium salts of fatty acids' insecticidal soap product, when combined with the emulsifiable oil adjuvant. A potentially positive effect of the oil-based formulation was additionally indicated for the product based on *B. bassiana* PPRI 5339, which contains spores of entomopathogenic fungus in emulsifiable oil suspension. When not formulated in oil, the *B. bassiana* isolate may have had an antagonistic effect to mycelial growth but this was not observed when the product was used, suggesting a nutritional effect on both fungi. As a result of conducting compatibility experiments, all efficacy tests used low-impact concentrations of the biologicals to support mushroom growth, meaning that the optimal efficacy possible against the target pest/pathogens was not assessed for any agent but rather what the crop could tolerate.

Research activities to support product testing. In trials conducted in a controlled environment room with phase III compost and casing, a 48 % reduction in the quantity of adult sciarid flies that emerged was observed from the low-dose sustained release (SR) (s)-methoprene-treated casing, compared to the control casing. This shows the sensitivity of sciarid fly larvae to the SR (s)-methoprene product alone, and the suitability of the formulation in casing specifically, owing to the high-water holding capacity of the substrate.

Combination experiments: The results of the smaller-scale vermiculite bioassay that tested a combination of the SR (s)-methoprene product with the insect-killing fungus *Beauveria bassiana* strain PPRI 5339, the *B. bassiana* product initially caused high mortality against larvae/pupae and adult flies. However, it was not effective against larvae when mixed with casing. This may be due to the lack of suitability of the formulation, the strain, and/or the application. In an experiment that tested spraying adult flies with *Beauveria bassiana* strain PPRI 5339 and/or a 2% emulsifiable oil adjuvant, on average, 95% of flies were killed on immediate contact with the spray application, regardless of the treatment used, which suggests that either a 2% emulsifiable oil adjuvant (EOA) solution is insecticidal at the rate applied, and/or that flies are highly susceptible to the action of the misting/fogging. Of the 5% of flies that survived the spray application, the control flies sustained for longer than the *B. bassiana* treated flies, but the results were not significantly different between treatments. However, the *B. bassiana* treatment affected fly fecundity; flies that survived the initial exposure to the spray treatment were able to oviposit (lay eggs) into rearing substrate provided for them in the cages. More flies emerged from substrates that were provided to the control treated flies, indicating that flies undergoing infection by *B. bassiana*, laid less eggs.

In the first experiment conducted with entomopathogenic nematodes (EPN) applied to phase II-end compost, no flies emerged from the nematode-treated containers, suggesting high efficiency of the product in compost, whereas 40% of the flies that had been transferred in known quantities (as larvae) emerged from the control group containers. No weed moulds or mushroom fungal disease was observed with the introduction of the EPN formulation, suggesting that the cellulose-based product is suitable for use and may be effective if used in multiple applications, as has been previously recommended by Penn State University research. We found that EPN are effective when applied at appropriate intervals during the cropping process, such as (1) during early-stage phase III growth (i.e. < 12 days post spawn run or before complete colonization of *Agaricus*), and (2) following casing but prior to knotting and pinning of the mycelium, as that renders the casing substrate too water repellent for the nematodes.

All bacterial biofungicide isolates tested in dual-culture experiments antagonised the growth of *T. aggressivum* and *L. fungicola*. These pathogenic fungal colonies displayed the characteristic "square" pattern of growth and growth was stunted, which demonstrated a clear zone of inhibition between the fungi and the bacterial colonies. When applied to casing, viable colonies of the *L. fungicola* pathogen were not able to be reisolated and quantified from the substrate in any treatment or control using cultivation-dependent methods, suggesting a quantitative molecular method may be more appropriate to monitor outcomes of inoculation, as was indicated in the literature review of the research and described in the introduction of this report.

Table 1. List of biofungicide products which contain biorational microorganisms, and/or bioactive substances that have been reported to have activity against mushroom pathogens.

Category MoA (FRAC)	Agent	Species	APVMA Registration	APVMA Permit for mushrooms	Tested for*	Reference
Biofungicide, Not classified	Potassium salts of fatty acids -		63223, 82672	-	-	-
Biofungicide, Not classified	Fungi - yeast	*Aureobasidium pullulans* DSM 14940; DSM 14941	82495	-	*Pleurotus ostreatus* and *Trichoderma* sp.	doi.org/10.1016/j.biocontrol.2019.04.016
Biofungicide, BM02	Bacteria	*Bacillus amyloliquefaciens** QST 713	82243, 83292	-	*Agaricus bisporus* and *Trichoderma* spp., *Lecanicillium fungicola*	doi.org/10.1111/1462-2920.14765, doi.org/10.1111/aab.12048, doi.org/10.1016/j.jbiotec.2018.04.014, doi.org/10.1007/s10658-022-02482-1, doi.org/10.1128/aem.00327-19 doi.org/10.1016/j.cropro.2023.106530
Biofungicide, BM02	Bacteria	*Bacillus amyloliquefaciens** MBI 600	82600, 82243	**PER91265**	*A. bisporus* and *Trichoderma* spp.	doi.org/10.1111/1462-2920.14765, doi.org/10.1016/j.cropro.2019.104944
Biofungicide, BM03	Bacteria	*Bacillus amyloliquefaciens** subsp. *plantarum* D747	*Not Registered*	-	*A. bisporus* and *Trichoderma* spp.	https://teagaschorticultureportal.ie/table-view/mushrooms/
Biofungicide, BM02	Bacteria	*Streptomyces griseoviridis* K61	*Not Registered*	-	*A. bisporus* and *Lecanicillium fungicola*	doi.org/10.1111/1462-2920.14765; Beyer, D. M., Pecchia, J. A., Paley, K. (2016).
Biofungicide, BM02	Bacteria	Streptomyces lydicus WYEC 108	64384	-	*A. bisporus* and *Lecanicillium fungicola*	Product technical guide for Actinovate https://biosolutions.novozymes.com/
Biofungicide, 46	Plant extract	Extract from Melaleuca alternifolia (tea tree)	63549	-	*A. bisporus* and *Cladobotryum dendroides*	doi.org/10.1016/j.cropro.2009.07.016

Table 2. List of bioinsecticide products and insect growth regulators which contain biorational microorganisms, and/or substances that have been reported to have activity against mushroom flies.

Category MoA (IRAC)	Agent	Species	APVMA Registration	APVMA Permit for mushrooms	Tested for*	Reference
Bioinsecticide, UNF	Fungi	*Beauveria bassiana* strain PPRI 5339	85696, 80782	-	Isolate GHA: *A. bisporus* and sciarid/phorid flies	doi.org/10.1016/j.biocontrol.2016.09.003 doi.org/10.1080/09583157.2021.1926427
Bioinsecticide, 11a	Bacteria	*Bacillus thuringiensis subsp. israelensis* serotype H14	52642	**PER87515**	*A. bisporus, Pleurotus* sp.; sciarid/phorid flies	doi.org/10.1093/jee/77.2.473 doi.org/10.1093/jee/84.4.1180 doi.org/10.3390/insects12090786
Bioinsecticide, 5	Bacterial product	Spinetoram	64109	-	*Agaricus bisporus* and sciarid flies	doi.org/10.3923/pjn.2014.50.55 doi.org/10.1093/jisesa/iey082
Bioinsecticide, 5	Bacterial product	Spinosad	93096	-	*Agaricus bisporus* and sciarid flies	doi.org/10.3923/pjn.2014.50.55 doi.org/10.1093/jisesa/iey082
Bioinsecticide, Not classified	Potassium salts of fatty acids (insecticidal soap)	C7-C20	39752	-	-	-
Insect Growth Regulator, Un	Plant extract	Azadirachtin, extract from neem	61980, 63139	-	*A. bisporus* and sciarid/phorid flies	doi.org/10.2298/PIF1902111D doi.org/10.3390/agronomy11101958 doi.org/10.1002/ps.1658
Insect Growth Regulator, 7C	Juvenile hormone analogue	Pyriproxyfen	67264	-	*A. bisporus* and sciarid/phorid flies.	doi.org/10.1603/ec10292 doi.org/10.3390/agronomy11101958 http://www.jstor.org/stable/27896075
Insect Growth Regulator, 7A	Juvenile hormone analogue	(S)-Methoprene	59560, 82315	-	*A. bisporus* and sciarid/phorid flies.	doi.org/10.3390/agronomy11101958 doi.org/10.1603/EC10292 http://www.jstor.org/stable/27896075

Table 3. Products that are not registered with the Australian Pesticides and Veterinary Medicines Authority (APVMA) but contain biorational microorganisms, and/or bioactive substances or biocontrol agents, that could be tested for potential activity against mushroom pests and disease pathogens.

Category	Agent	Species	Tested for*	Reference
Bioinoculant	Bacteria	*Bacillus amyloliquefaciens* FZB 42	*A. bisporus* and *Trichoderma* spp.	doi.org/10.1128/AEM.00327-19
Bioinoculant	Bacteria	*Bacillus subtilis*	-	-
Bioinoculant	Bacteria	*Azotobacter vinelandii*	*Pleurotus* and *Trichoderma* spp	doi.org/10.4489/MYCO.2005.33.1.019
Bioinoculant	Entomopathogenic fungi	*Beauveria bassiana, Metarhizium anisopliae, and Lecanicillium lecanii* (strains not identified)	*B. bassiana* isolate GHA: *A. bisporus* and sciarid/phorid flies	doi.org/10.1016/j.biocontrol.2016.09.003 doi.org/10.1080/09583157.2021.1926427
Biocontrol agent	Predatory mite	*Hypoaspis* sp.	*A. bisporus* and sciarid/phorid flies	doi.org/10.1079/ber2003286
Biocontrol agent	Entomopathogenic nematode	*Steinernema carpocapsae*	*A. bisporus* and phorid flies	doi.org/10.1079/ber2003286
Biocontrol agent	Entomopathogenic nematode	*Steinernema feltiae*	*A. bisporus* and sciarid flies	doi.org/10.1079/ber2003286

Outputs

Table 4. Output summary

Output	Description	Detail
Biorational product list and final report	Provided as tables for public dissemination; Final report document	Output available in this final report; product names have been omitted. Product tables also to be published in one of the industry articles in MushroomLink Magazine, Winter edition (in press)
Final technical report delivered	A document that details the research conducted to test the viability and efficiency of selected biorational products against mushroom pest and fungal pathogens	Supplied as Appendix 1 with the final report (embargoed to allow time for publication in a scientific journal)
IP register	A confidential document that identifies the novel IP produced under project MU22000 that could be commercialized	Supplied as a confidential appendix to Hort Innovation with MS190 (Appendix 2)
Technical report on novel IPM technology	A confidential document that details the development of a novel IPM tool intended for potential mushroom fly pest trapping	Supplied as a confidential appendix to Hort Innovation with MS190 (Appendix 3)
1 webinar delivered (instead of a workshop)	A recorded video presentation titled	Published as a MushroomLink webinar; available on YouTube: Https://www.youtube.com/watch?v=NQGTiLU7F7o
1 podcast delivered (instead of a workshop)	A recorded audio interview with Jenny Ekman (MU21003); titled 'Complementing management with biological agents - Aimee McKinnon'	Published as a MushroomLink podcast; available on Spotify and on the MushroomLink website: https://www.mushroomlink.com.au/resources-1/complementing-management-with-biological-agents-aimee-mckinnon
3 x industry articles produced	Article 1 (published): Sustainable Pest and disease management Article 2 (drafted and in press): Biological control of pests and diseases – A review of products potentially useful for mushrooms Article 3 (drafted, pending approval): Preventing disease with	Supplied as appendices to Hort Innovation with MS190; Available through MushroomLink as published (Appendix 4,5,6)

	biofungicides: How to conduct a farm trial (tentative title)	
Industry conference (AMGA) presentation	Presentation (oral) delivered October 2024 and titled: Investigating biorational agents to complement mushroom pest and disease management	https://www.amgaconference.com.au/dr-aimee-mckinnon-2/
1 x Manuscript for publication	Draft article for publication in a scientific journal, abstract supplied only, titled: Investigating combination bioinsecticides for integrated control and resistance management of the fungus gnat, *Lycoriella ingenua*	Supplied as an abstract, in a confidential appendix to Hort Innovation with MS190 (Appendix 7)
Conference proceedings	Oral presentation delivered February 2024 and titled: Investigating biorationals for mushroom integrated pest management	Supplied as an abstract, in an appendix to Hort Innovation with MS190 (Appendix 8)

Outcomes

Table 5. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Growers, consultants, and industry stakeholders are better informed on the potential of biorationals (non-synthetic alternatives) to complement pest and disease management practices in Australian mushrooms	Aligns with Mushroom Fund current SIP outcome 2.5 (IPDM with biologicals), and 2.4 (biosecurity preparedness)	Information publicly disseminated via the final report, webinar, podcast, industry articles and conference presentations; Research discussed with project reference group members, as well as the MU21007 pest and disease project team.	Meeting minutes from PRG discussions, feedback on activities requested in follow-up emails with PRG chair (all feedback is detailed in documents of meeting minutes supplied to Hort Innovation as confidential appendices in previous milestone reports for MU22000).
Availability of new knowledge for next phase project	Aligns with Mushroom Fund current SIP outcome 2.6 (research capacity developed)	Information publicly disseminated via the final report, with technical summaries (embargoed but will be made available May 2026). Research progress and proposals for future R&D investment presented to the Hort Innovation SIAP in May 2024.	An opportunity has been created and with further investment, could see the continued development of novel IPM tools that will promote effective biological pest management in Australian mushrooms. Alternate mushroom casing substrate casing/blends with alternative materials (MU24001) approved – some ideas contributed to the SIAP in May 2024, may have supported the project concept note for project MU24001.
Research capability developed	Aligns with Mushroom Fund current SIP outcome 2.6 (research capacity developed)	Scientific research conducted to a high standard enabling the drafting of a complete package of research for publication in an international peer reviewed journal	Supplied abstracts of (1) conference proceedings and (2) an original research article to be submitted for publication

Monitoring and evaluation

Table 6. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
1. Effectiveness: To what extent has the project achieved its expected outcomes?	The MU22000 project (i) improved industry, grower, and researcher knowledge on biorationals for mushroom pest and disease management; (ii) tested the efficacy of biorationals for potential industry uptake* in Australia *Note that Industry uptake is dependent on product efficacy and gaining APVMA permits for use in Australian mushroom farms. Applying for permits was not an objective of project MU22000	Products that are (1) registered with the APVMA and are (2) biorational, have been developed for use in plant crops. However, mushroom crops are biologically and agronomically unique. Biorational options are more likely to be effective and thus successfully adopted by growers when R&D investment is first adequately directed towards the innovation of novel IPDM tools. These tools should be developed for use in mushroom production and in Australia.
2. How relevant was the project to the needs of intended beneficiaries?	The project contributed new knowledge on the development and evaluation of biorationals for mushroom IPDM. The information is provided in public reports and articles, and in confidential technical reports - which contain commercially sensitive data.	The development of novel IPM tools for mushroom production will promote effective biological pest management in Australia; Several products tested that are not currently in use could be implemented with next-stage investigation.

3. How well have intended beneficiaries been engaged in the project?	Growers/consultants/AMGA members have been engaged with in the conferences, PRG meetings, private meetings, farm visits and via articles aimed at improving knowledge of biorationals for IPDM The agreed level of communication (articles/fact sheets and workshops/webinars) have been delivered	Webinar/podcast content was delivered instead of workshops, as this was recommended as the most accessible form of engagement and can be listened to at any time. Industry articles were prepared instead of factsheets, as the information required some educational content and not just 'how to' instructions. MushroomLink magazine articles enabled the delivery of more comprehensive information.
4. To what extent were engagement processes appropriate to the target audience/s of the project?	The project engaged with industry levy payers through a variety of means (emails, video calls, progress meetings, face to face meetings on farms, conference and round-table presentations, industry articles, webinar/podcast publications).	Feedback on engagement and extension activities could be assessed and provided as part of the comms project, if not already, but this is not information supplied to date.
5. What efforts did the project make to improve efficiency?	The biorational product review compiled as planned. PRG meetings were held but were largely virtual, given that many members were located internationally. "In vivo" experiment(s) were not conducted at the Marsh Lawson Mushroom Research Centre, as it was out of order. Trials that were undertaken have been effectively communicated through industry articles, final reports and conference presentations.	Experiments that assess biologicals or insecticides with fly pests must be done in cages, to enable adequate quantification of effects to fly populations. Robust data is required for the purpose of registration/approval and permits, and this usually cannot be achieved on farms without installing cages, emergence traps or using whole rooms with strict exclusion procedures and sufficient experimental replication (which is not practical). The MLMRU can, however, be used to assess the impact of novel products on mushroom production and quality. Experiments conducted in project MU22000 pave the way for products to be tested at the MLMRU for effects to production, but this fell outside of the scope of the current project which focused on efficacy to target pests and/or pathogens.

Recommendations

Insights, opportunities, and gaps in research were identified throughout the course of project MU22000 by the Agriculture Victoria research team. Specifically, these ideas include:

1. **Testing the efficiency of biological products**: In the biorational product review, agents and constituents were identified but not all were tested for compatibility and efficacy. Further research is recommended to evaluate more of these products for potential mushroom pest and disease control.
2. **Testing combinations of products at the MLMRU**: Products that demonstrated effect in experiments including (1) sustained release (s)-methoprene, (2) an emulsifiable oil spray-treatment for adult flies (3) entomopathogenic nematodes applied at key crop intervals, and (4) the new liquid formulation of *Bacillus amyloliquefaciens* strain QST713, could all be evaluated further as stand-alone agents and/or in combinations, to assess for effects to mushroom production.
3. **Improve the mass trapping of flies on farms**: An opportunity has been created under project MU22000 with the preliminary development of a novel mushroom fly lure. This lure has shown early stage but highly promising fly trapping efficacy, indicating that a novel technology could be developed further for commercialization, with the ultimate purpose to use in biologically based 'attract-and-kill' devices for mushroom IPDM.

More broadly, the following recommendations could add significant value and/or help to future-proof the Australian mushroom industry, paving the way for increased adoption of sustainable pest and disease management:

4. **Develop novel diagnostic methods**: Testing the efficacy of microbial agents to prevent mushroom pathogen activity requires quantitative assays, however, there is no effective methodology available in the public domain to support this approach. For a robust assessment of microbial biofungicides, molecular diagnostic methodology could be developed to enable specific and viable microbial agents to be quantified after application to mushroom crops, and/or the bioactivity measured; this would enable disease *prevention* to be properly assessed.
5. **Future proofing pest and disease management**: Testing of microbial control agents and bioinoculants should also be conducted in alternative casing substrates for pest and disease preparedness, and to better understand the role of applying microorganisms in mushroom production.
6. **Generate information on pest species impact**: The distribution of mushroom fly pest species in Australia is not well described. To remedy this, the association of either sciarid or phorid fly pests with different mushroom production systems and farms could be evaluated (i.e. to species level classification). This may be accomplished with high-throughput insect DNA based identification. Knowledge on invertebrate species distributions and diversity across Australian farms can inform (1) effective pest and disease management, (2) biosecurity preparedness and (3) market research for crop protection - allowing financial impact assessments of new technologies to be conducted. The latter being necessary to facilitate importation/registration of effective international products that are currently not accessible for Australian growers.
7. **Develop novel biological technologies in and for Australia**: Mushroom crops are not plants. Mushrooms are biologically and agronomically unique from other crops, and biorational options are more likely to be effective when these tools are developed for specific use in mushroom production. Innovative research is required to enable the development of novel microbial control agents, as well as entomopathogenic nematode species and strains, and bioinoculants/biofertilisers; for sustainable mushroom production in Australia.

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Intellectual property

Project IP is detailed in the 'IP Register' document which has been submitted as a separate appendix (Appendix 2) to this report.

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