Potential of microbial biocontrol for the sustainable management of plant diseases in Scotland: Opportunities and barriers

Project Final Report

Credit: Soo Ann Woo

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Authors: Rees, H. J.[1], Elliot, M.[2], Maloney, K.[3], Degiovanni, H.[1] and Creissen, H.[1]
[1]Peter Wilson Building, King's Buildings, W Mains Rd, Edinburgh, EH9 3JG.
[2]Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh, Scotland, EH3 5LR.
[3]Craibstone Estate, Ferguson Building, Aberdeen, AB21 9YA.

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Content

1 Executive Summary

In recent years, there has been a move by governments to reduce the use of chemicals in agriculture, horticulture, and forestry due to environmental concerns and the impact on worker and consumer safety (Hillocks, 2012; Collinge *et al.*, 2022). As a result of regulation many pesticides have been withdrawn. Of particular concern in Scotland, a high proportion of chemical insecticides are estimated to be at high or medium risk of withdrawal, with six recently withdrawn for use in the UK (Dimmock *et al.*, 2023).

In addition, increasing resistance in the target pest or pathogen is becoming a significant issue (Le Goff and Giraudo, 2019). This is because many of the active substances in the currently available products have the same mode of action (MoA). Therefore, if a pest or pathogen develops resistance to one product, it can show cross-resistance to all related products. Forestry and arable production are particularly exposed to this risk, vegetables and soft fruit less so (Grimmer et al., 2014; Dimmock *et al.*, 2023).

The withdrawal of chemicals has driven an increased interest in using biological controls within integrated pest management (IPM) programmes to fill the gap (Samada and Tambunan, 2020). For clarity, biological control or biocontrol is the process of controlling a pest, disease or weed using another living organism for human benefit (Stenberg *et al.*, 2021). Organisms with biocontrol potential are termed biocontrol agents (BCA). Where microbes, usually bacterial and fungi, have biocontrol potential they are termed microbial biocontrol agents (MBCA).

Through a workshop with stakeholders and a literature review, this study aimed to clarify the current use of MBCA in Scotland, determine the potential for future use, and identify potential opportunities and barriers associated with MBCA. In addition, the current regulations for registering new MBCA in the UK was examined to understand whether it is fit for purpose. The project focused on the use of MBCA to control plant pathogens and comments within this report pertain to that.

<u>Key findings:</u>

- Biocontrol has potential to reduce the long-term negative impacts of chemical pesticides on human, animals, non-target organisms and the environment. Using MBCA can also reduce the harvest interval periods, and potentially have fewer health and safety concerns to users and consumers compared to conventional plant protection products (PPP).

- The public perception of biological control is positive, being viewed as an environmentally friendly option. In addition, MBCA are seen as alternative methods for disease control, especially in cases where resistance has occurred, and conventional chemical control is no longer effective.

- The most commonly used MBCA are *Bacillus* and *Trichoderma* species, with examples of both available in products registered and used in Scotland. These are used for protected crops, e.g., strawberry, lettuce and tomato, for controlling diseases such as mildew and botrytis. This study provides a full list of products registered for use in the UK (and therefore Scotland), including details of the target pathogen and hosts.

- Mycoviruses (viruses that infect fungi, thereby rendering them less pathogenic) could potentially play an important role in disease management, particularly in forestry.

- The Scottish 2020 arable crop pesticide usage survey reported the use of only one biological, a seed treatment applied to oilseed rape, accounting for 0.2% of the total arable treated area. No biologicals were recorded in the most recent pesticide usage in grassland and fodder crop report.

- Respondents to a FERA survey indicated that the crops most favourable to biological products were fruit (54.1 %), salad crops (50.8 %) and vegetables (45.9 %). Arable crops (14.7 %) and potatoes (8.2 %) were considered least favourable. This is generally reflected in the Pesticides Usage in Scotland reports where almost no microbial based plant protection is used in arable and potato production but is more widely used by soft fruit growers.

- Reviews suggest that climate change will not have adverse effects on the potential for MBCA in the next 20 – 40 years in Scotland. It is possible, that with warming temperatures, conditions for MBCA could become more suitable. However, this could also mean more pathogens survive overwinter or expand their host ranges.

- The impact on the natural environment of MBCA should be examined very carefully, particularly the implications of MBCA application on native microbial communities.

- The registration of new MBCA in the UK is currently stifling innovation. Stakeholders widely believed that the current system is not fit for purpose. This is largely because the pre-existing chemical registration process is being used for BCA which means that most of the tests are inappropriate. It currently takes about ten years to register a new product in the UK compared to two years in the US.

- Due to BCA being treated as chemicals, their registration is managed by the HSE using a framework designed for chemical PPP. Stakeholders suggested that Defra (England) and Scottish Government (Scotland) may be better placed to manage aspects of BCA registration because they already have expertise in this area due to their other workstreams (e.g., invasive species, plant health and biosecurity). Therefore, a new regulatory framework tailored to evidencing the benefits and risks associated with BCA should be developed. This would streamline the registration of MBCA whilst providing the highest possible protections for the environment. The new National Action Plan (NAP) for sustainable use of pesticides, due for publication soon, may address some of these issues.

2 Introduction

Biological control or biocontrol is the process of controlling a pest, disease or weed using another living organism for human benefit (Stenberg *et al.*, 2021). According to Stenberg *et al.* (2021), there are four categories of biocontrol:

1. Augmentative – occurs when an organism is applied to achieve temporary control;
2. Classical – refers to an organism which is added to an ecosystem to become permanently established;
3. Conservation – the stimulation of a targeted organism which is already present to improve the biocontrol potential;
4. Natural – relies on biocontrol organisms already present and does not require human intervention.

Organisms with biocontrol potential are termed biocontrol agents. Where microbes, usually bacterial and fungi, have biocontrol potential they are termed microbial biocontrol agents (MBCA).

In general, biocontrol agents (BCA) are considered an environmentally friendly option to controlling plant diseases and allow for reduced chemical inputs in integrated pest management (IPM) programmes. Pathogens are less likely to develop resistance to BCA (Collinge *et al.*, 2022) which can help to prolong the life of conventional pesticides used in IPM, however, this must be monitored as BCA use increases. There are some limitations associated with using BCA including the perception BCA are less reliable and or less efficient than conventional chemistry (Collinge *et al.*, 2022). Given the antagonistic properties of BCA, particularly microbial biocontrol agents, it is important that the risks to native microbial communities are considered to ensure no long-lasting damage occurs. Furthermore, regulatory improvements are required. Regulations are considered complex with little scope to realise the benefits of BCA and could benefit from a more harmonized approach (Ward, 2016).

There are several MBCA commercialised for use worldwide, primarily in the agricultural (followed by horticultural) sectors, and include bacteria, fungi, oomycetes, and bacteriophages that target pests and diseases (reviewed by Sabbahi et al., 2022 and Collinge et al., 2022, respectively). The MBCA used for invertebrate pest control are termed entomopathogens and include nematodes. North America has the largest commercial market for MBCA of invertebrate pests with 73 registered products (Sabbahi *et al.*, 2022). In forestry, biological control of pathogens is rarely used, but some examples have been successful such as the use of mycoviruses to control Chestnut blight in Europe (Prospero *et al.*, 2021). Microbial biological control options are also being explored for the control of invasive non-native species in parts of the UK (e.g., rust fungus for managing Himalayan balsam (Pollard, 2023)), a management option which may be applicable in Scotland. This report will focus on the use of MBCA for the control of plant pathogens.

Microbial biological control agents are playing an increasingly important role in crop protection and are vital components of integrated disease management programmes. In 2020, the PHC2020/09 report (Burnett *et al.*, 2021) highlighted the risk of active substances being withdrawn in Scotland. Across each plant health sector, including agriculture, horticulture, forestry and the natural environment, and subsectors within those groups, significant proportions of commonly used active substances are identified to be of medium or high risk of being withdrawn. Thus, it is essential to consider the potential of biological control within Scotland. However, there are significant barriers and knowledge gaps for the widespread practical use of MBCA in Scotland that need to be addressed.

The objective of this project was to address the current situation of microbial biocontrol in Scotland. Using a literature review and conducting a workshop with plant health stakeholders the project aimed to:

- Understand the current use of MBCA in Scotland.
- Determine the potential for using MBCA in Scotland.
- Identify potential opportunities and risks associated with using MBCA.
- Advise whether the current regulations for MBCA are fit for purpose.

3 Methods

3.1 Workshop

To improve our understanding of current practices, perceptions and regulations regarding MBCA, across plant health sectors we held an in-person workshop with stakeholders at SRUC (Edinburgh) in May 2023.

Potential attendees were approached by members of the project team from their existing networks. Participants were selected to represent each of the four plant health sectors (agriculture, forestry, horticulture, and the natural environment) and also to represent varying roles (e.g., research/consultancy/policy). There was overlap in the roles of some participants (e.g., research and consulting). The workshop comprised several group discussion sessions focused on the following themes:

- Use of MBCA
- Benefits of MBCA
- Limitations of MBCA
- Risks of MBCA
- Knowledge exchange for MBCA
- Regulations of MBCA
- Future direction of MBCA

At regular periods during the workshop a set of prepared questions (Appendix A) were put to participants who responded online using Slido (https://www.slido.com/). In many cases participants had the option to choose multiple answers. The data were captured by the software and made available for our analysis. Flip charts were also used to capture data from the participants. Notes from flipcharts were typed up verbatim, except in the case of repetitions which were grouped together.

Considerations when analysing the data:

- The data only captured the views of those who participated in the workshop; we recognise that there were people who did not or could not engage.
- The results analysed are those received from respondents. With minor exceptions where there were obvious discrepancies (e.g., spelling mistakes), no attempt was made to verify data reported.

3.2 Relevant seminars / events

Two relevant events were also utilised as a source of information on MBCA. FERA Science held a webinar entitled 'Biopesticide – Current Challenges and Future Opportunities' on the 29th March 2023. A recording can be found at this link, and a compilation of the questions addressed in the discussion can be found here. Information from this webinar was used to inform aspects of the literature review from a UK perspective and in the discussion section to compare with perspectives and opinions raised in the workshop at SRUC.

The World BioProtection Forum (WBF) held an event 'Bringing Biopesticide Regulatory Reform to UK Parliament' on the 5th September 2023. This event and WBF's White Paper on biocontrol regulatory systems informed the regulatory sections of this report.

4 Results

4.1 *Literature review*

4.1.1 *What are microbial biological control agents?*

Many definitions of biocontrol exist in the literature, broadly defining biocontrol as the use of a living organism to reduce the population of another (usually harmful) organism (Bale *et al.*, 2008; EMPHASIS, 2016; Prospero *et al.*, 2021; Stenberg *et al.*, 2021; Collinge *et al.*, 2022). Biocontrol agents have been used to target problematic animals, plants, pests, and disease-causing microorganisms. To control plant diseases, biocontrol relies on the use of microbial agents which can directly or indirectly inhibit the growth of a pathogen (Collinge *et al.*, 2022). In some cases, metabolites, plant extracts and other non-living nature-derived substances are used to control diseases, however it is suggested that these are split from BCA and are grouped separately under the umbrella term 'bioprotectants' (Stenberg *et al.*, 2021; Collinge *et al.*, 2022) and therefore did not form part of this review.

4.1.2 *Which microbial biological control agents are used in Scotland?*

Recently, Collinge (*et al.*, 2022) and Prospero (*et al.*, 2021) published reviews on biocontrol of plant diseases, and biocontrol of forest pathogens complete with examples of biocontrol products. A full list of microbial based biopesticides registered by the Health and Safety Executive (HSE) in the UK (including Scotland), can be found in the appendices (Appendix B) with details of target pathogen and hosts. Two important MBCA registered in the UK are *Bacillus* and *Trichoderma* spp.. Serenade® ASO (Bayer) contains the bacteria *Bacillus amyloliquefaciens* (formerly *subtilis*) strain QST 713 and is applied as a foliar spray or seed treatment. Serenade is registered to control *Botrytis cinerea* in protected strawberry, lettuce, tomato, pepper and aubergine crops as well as *Sclerotinia* spp. in lettuce and *Helminthosporium solani* on potato tubers. There are also several Extension of Authorisation for Minor Use (EAMUs) for foliar sprays, root drenches and post-harvest treatments (Bayer, 2023). In addition to Serenade®, Constans WG is another commercially available product that targets sclerotinia disease registered for edible and non-edible crops. Contans WG contains the active spores of the soilborne fungus *Coniothyrium minitans* which directly parasites the Sclerotia (Bayer, 2015) to prevent sporulation of *Sclerotinia* sp. and infection of plants. Trianum-G and Trianum-P (Koppert) are *Trichoderma harzianum* T-22 preparations to be mixed into soil or dispersed in water. Trianum products are used to control soil-borne diseases including *Fusarium* spp., *Microdochium* spp., *Sclerotinia* spp., *Pythium* spp. and *Rhizoctonia* spp. and can be applied to most crops (Koppert, 2023). An overview of products used by participants of the Microbial Biocontrol in Scotland Workshop can be found under section 4.2.2 'Use of microbial biocontrol'.

SASA conducts regular pesticide usage surveys which can be used to give an indication of the use of microbial based bioprotection in Scotland (Wardlaw *et al.*, 2023). Products which have been through the HSE authorisation process are termed 'biopesticides', biologicals that do not require to be authorised are reported as 'biological control agents' in the pesticide usage surveys. An overview of crop groups, and biopesticide & BCA usage is detailed in Table 1. In all soft fruit crops, biological control (BCA and biopesticides) was reported as 31 % of the total.

treated area, although this was mainly using invertebrate predators (Wardlaw *et al.,* 2023). Sixteen new biological products were reported for the first time in the 2022 surveys; these were all BCA or biopesticides with only three microbial based products listed. The changing use of microbial based control (BCA and biopesticides) was compared with surveys from 2018, 2020 and 2022. The area treated with BCA increased dramatically over the years (1,129 ha in 2018; 3,463 ha in 2020 and 11,928 ha in 2022). In 2020 there was a spike in the area treated with biopesticides over the same period (2,921 ha in 2018; 1,211 ha in 2020; 2,781 ha in 2022), with an overall slight decrease in areas treated with biopesticides since 2018 (Wardlaw *et al*., 2023). The main use of biopesticides in soft fruit production was to control botrytis and powdery mildew in strawberry (Wardlaw *et al*., 2023). A breakdown of biopesticide usage was not given in the 2022 report (Wardlaw *et al*., 2023), but use in 2020 surveys also included botrytis and powdery mildew for control in strawberry and other soft fruit crops (Wardlaw *et al*., 2020). See Appendix C for more details of microbial-based biopesticide species used in soft fruit crops and their application rates. In arable crops, the only microbial based product recorded was the use of *Bacillus amyloliquefaciens* (strain MBI600) as a seed treatment in oilseed rape (Davis *et al*., 2023). No biologicals were used in grasslands and fodder crops. (Davis *et al*., 2020). No details of microbial based protection products were reported for outdoor vegetables (MacLeod *et al*., 2021). The HSE define biopesticides as a broad range of products that can be used as PPP, they are split into four categories: pheromones (and other semiochemicals), microbial based products, plant-based products, and other novel alternative products (products that don't fit easily into other specific categories) (HSE, 2023a).

Table 1: Overview of microbial based plant protection usage detailed in the Pesticide Usage Surveys conducted by SASA for soft fruits and arable crops in 2020 and outdoor vegetable and grassland and fodder crops in 2021. Biopesticides and biocontrol were grouped together for arable and grasslands crops.

Crop	Overall Cropped Area (ha)	% of microbial based formulations used	Reference
Soft fruits	2,198	31	Wardlaw *et al*., 2023
Arable	487,389	0.2	Davis *et al*., 2023
Grasslands and forage	4,378,628	NA	(Wardlaw *et al*., 2021)
Vegetable	22,066	NA	(MacLeod *et al*., 2021)

In horticultural crops there are a greater proportion of MBCA used because there are fewer pesticides registered for use. In some cases, there are EAMU's available, but these are not long term and are unlikely to be properly supported in the future now that AHDB, who previously applied for EAMU's, no longer collect a levy from this sector. In agriculture, pesticides are cheaper and more effective which means there is often no great drive for greater uptake of MBCA. However, in some crops, such as potato, there is interest in MCBA where the effective chemistry for some pests and pathogens is no longer available.

A webinar held by FERA on 29th March 2023 entitled 'Biopesticide – Current Challenges and Future Opportunities' discussed the theme of biopesticides from a UK perspective. The results of an industry survey (66 respondents) conducted to understand the opportunities and challenges of using biopesticides were reported in this webinar. It is important to note that the survey reports on biopesticides, not just microbial biological control agents. In the survey, respondents were asked which crops they thought were most favourable to biological products. Responses indicated fruit (54.1 %), salad crops (50.8 %) and vegetables (45.9 %) were perceived to be most favourable. Arable crops (14.7 %) and potatoes (8.2 %) were considered least favourable (Dillon *et al*., 2023). No reasons were given for the perception of host suitability, and in the case of arable crops and potatoes, this perception is reflected in the Pesticides Usage in Scotland reports where almost no microbial based control products are used (Davis *et al*., 2023). In addition, the crop group with the most microbial based control was in soft fruits.

4.1.3 What is the potential for the use of microbial biocontrol agents in Scotland?

In terms of opportunity, clearly, there is scope to increase the use of MBCA in Scotland. A range of options are approved by the HSE for use in the UK, mainly for horticultural followed by agricultural crops (HSE, 2023b). The ten-fold greater usage of MBCA in horticulture and soft fruit compared to arable crops in Scotland is striking. This could be due to 1) high value crops produced by the horticulture sector making MBCA more cost effective, 2) the production within protected environments allowing for more reliable control, or 3) approval of products available across sectors. Identifying the reasons for the striking differences will be required to increase usage across sectors.

Several bacterial and fungal MBCA are commercially available within the UK, particularly in the agricultural and horticultural sectors. Work at the University of Reading has studied the diversity of *Eudarluca carici* species in the UK which are hyperparasites of rusts (Kajamuhan *et al*., 2015), an example of opportunities to expand MBCA use as none are currently registered for rusts in the UK (HSE, 2023b). Some research in the UK is also considering alternative application methods for MBCA. For example, the University of Bristol and the RHS (Royal Horticultural Society) has shown reduced disease incidence of Armillaria root rot when strawberry or privet plants were pre-colonised with endophytic *Trichoderma* spp. (Rees *et al*., 2022) as opposed to application directly to soil (Chen *et al*., 2019) Development of new MBCA and evidence to support their registration may limit options so avenues to expand registration of products to new hosts or diseases needs to be studied to meet the demands of pesticide withdrawals.

Mycoviruses could potentially play an important role in disease management, particularly in forestry. Chestnut blight caused by the pathogen *Cryphonectria parasitica* attacks sweet chestnut trees (*Castena sylvatica*) causing cankers on the bark eventually leading to tree death and resulting in the devastation of chestnut in North America. However, although the pathogen was also found in Europe, the epidemic was less severe due to the presence of *Cryphonectria* hypovirus 1 (CHV-1). This mycovirus acts as a biocontrol agent by reducing growth and sporulation of the pathogen *C. parasitica* (Prospero *et al*., 2021). However, the use of CHV-1 as a MBCA in North America was unsuccessful due to the highly diverse vegetative compatibility groups (Prospero *et al*., 2021). *Cryphonetria parasitica* is present in the UK and work has identified UK CHV-1 infected isolates with potential as a microbial biocontrol measure (Romon-Ochoa *et al*., 2020) but the North American findings highlight how situationally dependent efficacy might be.

New opportunities for use of mycoviruses to control a range of diseases are emerging. Several mycoviruses that confer hypovirulence to *Sclerotinia sclerotia* (Xie and Jiang, 2014) have been identified. Mycoviruses of *Botrytis cinerea* have also been identified, and infection with Botrytis cinerea hypovirus 1 (BcHV1) results in attenuation of virulence in mycelium and significant inhibition of the formation of the infection cushion (Hao *et al* 2018). Mycoviruses of *Fusarium* species, including *F. graminearium, F. poae and F. oxysporum* have been identified. However, only two mycoviruses of *F. graminearum* and one from *F. oxysporum* confer hypovirulence (Li *et al*., 2019). Within *Heterobasidion* spp. a diverse community of mycoviruses that can cross species borders have been found (Vainio and Hantula, 2016). For example, the mycovirus (alphapartitivirus Heterobasidion partitivirus 13) isolated from *H. annosum* can change the morphology of infected cultures *in vitro* and reduce the size of infections in *Picea* trees (Vainio *et al*., 2018a). Isolates of the causal agent of ash dieback, *Hymenosyphus fraxineus* from their natural range in Japan were screened for viruses. Five RNA mycoviruses were found in Japanese isolates but could not be sequenced from European isolates (Shamsi *et al*., 2022). The lack of mycovirus infection in the European population of *H. fraxineus* suggests a potential novel method to control the disease. In the UK there are no registered examples of mycoviruses available for plant disease control, however, there are two viral-based MBCA products available (Appendix B), both for the control of Pepino Mosaic virus (PepMV). A Mild strain of PepMV can be inoculated into plants to protect them from infection caused by a more virulent strain of the same virus, a method of control known as mild strain cross protection or pre-immunisation (Pechinger *et al.*, 2019).

4.1.4 How do environmental factors effect microbial biocontrol agents in Scotland?

The UK Climate Projections (UKCP) are published by the met office and offer scenarios based on different models. In Scotland, temperatures will increase with warmer wetter winters and drier summers (Figure 1) with greater extremes in summer and more extreme rainfall events. In worst-case scenario predictions for Scotland, the mean temperatures throughout the year are predicted to increase by 1 – 2 ^{O}C by 2039 (UKCP, 2023). Rainfall increases are expected to be larger in western Scotland (Adaption Scotland, 2020). The climate change predictions in Scotland (Figure 1) mean there could be an increase in plant diseases. Warmer temperatures in Scotland could lead to expansion of pathogen host ranges and warmer winters could increase the survival of pathogens overwinter (Singh *et al.*, 2023).

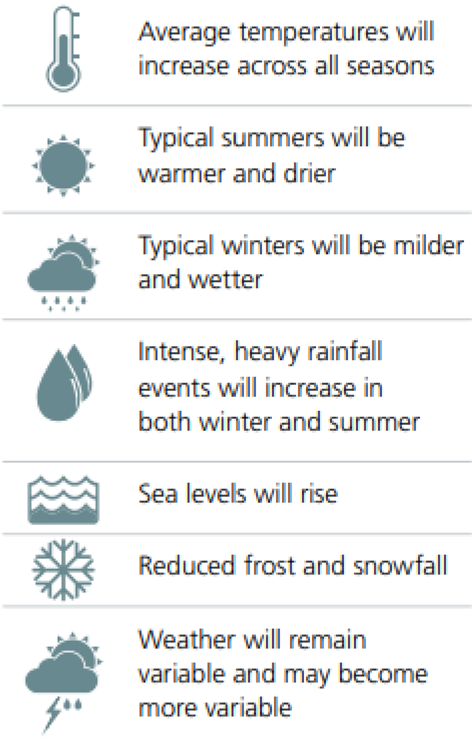

Figure 1: Infographic of climate predictions in Scotland (Adaption Scotland, 2020).

Increases in disease and changes to the Scottish climate could impact the potential use and reliance of MBCA in Scotland. *Trichoderma atroviride* SC1, an isolate with biocontrol potential, is tolerant of a wide range in temperature, growing between 10 ^{O}C and 30 ^{O}C with an optimal temperature of 25 ^{O}C, as tested by Longa *et al.*, (2008) in Petri dishes. Low temperatures (-1 ^{O}C and 5 ^{O}C) induced dormancy of *T. atroviride* however, high temperatures, above 35 ^{O}C for one month, killed the fungus (Longa *et al.*, 2008). It is unlikely that temperatures of 35 ^{O}C for one month will be reached in Scotland, so *Trichoderma* will be able to continue to survive. For use in soil or on plants, temperatures above 10 °C are recommended for Trianum (Koppert, 2023), suggesting the seasonal timeframe for use could be extended in warming climates. One study investigated the effect of temperature and relative humidity (RH) for the biocontrol of Botrytis bunch rot, testing six MBCA. The level of control offered by *Bacillus subtilis* QST 713 and *T. atroviride* SC1 under different conditions is summarised in Table 2, suggesting optimal temperatures of 20 ^{o}C (Fedele *et al.*, 2020). With predictions indicating summers may become drier, this study could give an indication into the efficacy of MBCA potential. Studies have found that some strains of *Trichoderma* spp. can survive and sporulate under osmotic stress and can confer tolerance to water deficiencies in tomatoes (Rawal *et al.*, 2022), suggesting drier weather will not adversely affect efficacy. These studies highlight that in Scotland, climate change will not have adverse effects on the potential for MBCA in the next 20 – 40 years. It is possible that with warming temperatures conditions MBCA could become more suitable.

Table 2: Level of control offered by Bacillus subtilis and Trichoderma atroviride under different conditions.

MBCA	Temperature (°C)	RH (%)	Level of control
Bacillus subtilis QST 713	20	90	Medium-high
T. atroviride SC1	20	90	Medium
Bacillus subtilis QST 713	25	80	Medium-low
T. atroviride SC1	25	80	Medium-low

4.1.5 Review of the benefits of using microbial biocontrol agents

Using MBCA to control plant diseases offers a range of benefits. Biocontrol can reduce the long-term impact of chemical pesticides on humans, animals, non-target organisms and the environment. Using MBCA can also reduce the time between applying Plant Protection Products (PPP) and harvesting crops (the harvest interval), and potentially have fewer health and safety concerns compared to conventional PPP. The public perception of biological control is positive, being viewed as an environmentally friendly option (Collinge *et al.*, 2022). In addition, MBCA are seen as alternative methods for disease control, especially in cases where disease resistance and conventional chemical control are not options (Collinge *et al.*, 2022). MBCA may have benefits where there are multiple hosts to a disease. For example, in the case of controlling *Armillaria* spp., which affects several hundred hosts, neither disease resistance nor chemical control are available. Thus, research has resulted in testing MBCA through endophytic colonisation of plant roots with some success (Rees *et al.*, 2022).

Integrated pest management (IPM) is a system-wide approach using various control strategies (chemical, physical, biological) to create long-term sustainable control measures (Barzman *et al.*, 2015). Barzman et al. (2015), describes key eight principles of IPM which include using non-chemical methods in preference to chemical control, reducing pesticide use and employing anti-resistant strategies. Biological control agents have an important role to play within these principles. Integrating MBCA into an IPM programme for disease control could potentially allow for reduced number of PPP sprays. Contans WG (Bayer) is a MBCA product available to combat Sclerotinia disease, a soilborne pathogen. The fungus, *Coniothyrium minitans,* acts parasitically towards the sclerotia and mycelium of the soilborne pathogen *Sclerotinia* spp. In heavily infected soils, using IPM including multi-crop rotations and in combination with other PPP, can reduce infection (Bayer, 2015). The reduction in use of active substances will help to contribute to slowing the development of pathogen resistance. A further benefit to using MBCA is that pathogens are unlikely to develop resistance to them (Collinge *et al.*, 2022), meaning once registered, a PPP could be effective for an increased number of years, although this should be monitored.

There could be positive impacts on reductions in food waste arising from greater use of MBCA. When using MBCA the harvest interval is reduced compared to conventional chemicals, meaning that diseases can be controlled shortly before harvesting (Collinge *et al.*, 2022; CABI, 2023), which could reduce food wastage or even act as post-harvest control. Many initiatives exist which encourage farmers and growers to use IPM, including but not limited to, the National Action Plan, Sustainable Farming Initiative, Voluntary Initiative and LEAF (Linking Farming and the Environment). An online tool to aid growers with IPM planning and to use more sustainable practices is hosted by the Plant Health Centre (Plant Health Centre Scotland, 2023) and based on work conducted by Creissen *et al.* (2019). An updated version, 'IPM tool' (https://ipmtool.net/)was released in September 2023 as part of a collaboration between

NFU, ADAS and SRUC. These schemes, while not restricted to advocating for biocontrol, could increase the use of MBCA as a way to improve IPM scores and enable reductions in PPP, while providing a source of knowledge exchange and raising awareness of MBCA.

Some species used as MBCA can have multiple benefits. *Trichoderma* species are one of the most common biological control agents but are also known to be plant growth promoters of different parameters including roots, shoots, biomass, and chlorophyll production. Growth promotion by various *Trichoderma* species has been demonstrated in barley (Moya *et al.*, 2020), potato (Hicks *et al.*, 2014) and strawberries (Porras *et al.*, 2007), all of which are important crops in Scotland. *Trichoderma* spp. have also been shown to promote growth in *Pinus sylvestris* var. *mongolica* seedlings (Halifu *et al.*, 2019); this could potentially be applied to the native Scots Pine species, *P. sylvestris*.

Many MBCA are effective against only a limited number of species or are confined to specific species, for example the *Ampelomyces quisqualis* strain AQ10 for control of powdery mildew, and specificity in target is considered to be beneficial, especially in terms of environmental impact. The root rot pathogen *Heterobasidion* sp. can be controlled by the biocontrol agent *Phlebiopsis gigantea* through direct competition (Pratt, 1999). *Conorythium minitans*, a commercial biocontrol against *Sclerotinia* spp. targets only the sclerotia of the *Sclerotinia* spp. (De Vrije *et al.*, 2001) and *Ampelomyces Quisqualis* (AQ10) is specific to powdery mildews (Lewis *et al.*, 2016). When MBCA are highly target specific, their impact on microbial communities is likely to be minimum. Other MBCA are known to control multiple pathogens. *Trichoderma* species are known to control many plant pathogens, including multiple pathogens by the same *Trichoderma* sp. isolate (Harman *et al.*, 2004). The commercialised *Bacillus amyloliquefaciens* strain QST 713 (Serenade, Bayer) can be used against *Botrytis cinerea, Sclerotinia* spp. and *Helminthosporium solani* in a range of crops (Bayer, 2023). The benefit of MBCA products with the ability to control multiple pathogens is multifaceted. From a business perspective, it increases the market size of a product and reduces the number of products that need to be registered. From the perspective of a grower, a MBCA can be bought in bulk and used to treat diseases across different crops or for control of multiple diseases affecting one host.

4.1.6 Are there risks associated with using microbial biocontrol agents?

MBCA may have impacts on non-target species. In cases where MBCA have a wide target range, and antagonistic modes of action against a rage of species, the impact on native microbial communities must be considered. As natural nutrient cyclers, a change in fungal (and bacterial) communities could have a major effect on the environment, or symbiotic relationships such as those formed between plants and mycorrhiza (Brimner and Boland, 2003). In a framework designed to assess safety of MBCA in 1996, Cook *et al.* also suggests competitive displacement is a risk to microbial communities. While written over 25 years ago, the key themes in these reviews are still important considerations today.

Trichoderma spp. are known to antagonise a large range of plant pathogens (Harman *et al.*, 2004), making them popular as MBCA alongside several other examples of MBCA offering broad-spectrum pathogen control. This broad range of antagonism has the potential to antagonise native microbial communities.

They may also disperse and persist in the environment. *Trichoderma atroviride* SC1 could be isolated from soils a year after inoculation and was recovered at depths up to 0.4 m and spread up to four metres from the original point of inoculation, decreasing in density with distance and depth (Longa *et al.*, 2009). The ability for MBCA, such as *Trichoderma* spp., to disperse such distances from inoculation points and survive without re-inoculation for long-time periods mean that any microbial community shifts have the potential to also be long-lasting.

MBCA may alter the balance of microbial populations. In rhizospheric soils with maize, *T. harzianum* increased beneficial bacterial communities, including acidobacteria, but had no significant effect in fungal communities (Saravanakumar *et al.*, 2017). Fungal and bacterial populations in soils inoculated with *T. atroviride* I-1237 were measured at two sites over nine months. A significant increase in fungal populations was only reported at 3 dpi (days post inoculation) from one site. However, populations of bacteria were significantly increased at 3 dpi at one site and, after 15dpi, a lasting significant increase was seen at the second site (Cordier and Alabouvette, 2009). In contrast, five *Trichoderma* strains (*T. harzianum* & *T. pseudokoningii*) reduced the soil bacterial populations after 21 dpi. Four *Trichoderma* strains significantly increased the root fungal communities, while three strains significantly increased and one significantly reduced the soil fungal communities (Naseby *et al.*, 2000). A 2008 study considered the functional behaviour of microbial communities and found that *T. harzianum* inoculated soils metabolised carbon sources at a lower rate than controls and reported a decrease in metabolic diversity. The authors suggest that the biocontrol *T. harzianum* can stimulate the growth of specific bacterial populations (Gasoni *et al.*, 2008) therefore altering the microbial community. It is clear that changes in microbial communities are highly variable, perhaps as a result of species combinations or environmental factors.

MBCA may also affect the microbial communities present in or on plants. The phyllosphere hosts a community of epiphytic microbes on the aerial tissue of plants. Any MBCA that are sprayed onto plants are likely to come into contact with the phyllosphere microbial communities. *Bacillus thuringiensis* can reportedly cause significant changes to bacterial communities in the phyllosphere of pepper (*Capsicum annuum* L.) (Zhang *et al.*, 2008), while *B. subtillus* reportedly caused no significant differences in phyllosphere bacterial communities of control and inoculated strawberry plants (Wei *et al.*, 2016). Greater diversity of bacterial phyllosphere communities was reported on strawberries grown outside compared to polytunnels and new leaves had higher diversity than old leaves (Wei *et al.*, 2016) highlighting the spatial and temporal differences affecting phyllosphere communities. More research on changes to phyllosphere communities by MBCA is required, especially focusing on fungal communities, while taking into considerations spatial and temporal changes although such changes are rarely observable in most agricultural and many horticultural systems due to the use of broad-spectrum fungicides.

Endophytes form important microbial communities within plants, although exact details of microbial communities and location of colonisation is still poorly understood (Bacon and White, 2016). Endophytic colonisation of plants with MBCA is often seen as a useful tool for control of plant diseases (Latz *et al.*, 2018; Chen *et al.*, 2019; Collinge *et al.*, 2022; Rees *et al.*, 2022). However, inoculation of plants with endophytic MBCA could potentially impact the native communities. Authors Bacon and White (2016) have specified three major functional roles of endophytes; to alleviate abiotic stress of host plants, to defend hosts from biotic stresses, and to support host nutrition. Therefore, if an endophytic MBCA changes microbial composition, it could reduce pathogen attack at the cost of another function to support the plant. A recent study addressed the impact of MBCA on bacterial endophytic communities in Pak choi. *Trichoderma harzianum* decreased the number of dominant bacterial species resulting in increased species variety and a more evenly distributed community of root bacterial endophytes (Gulzar *et al.*, 2023). This change to species diversity could potentially impact the functionality of the endophytic community. It may also be reasoned that endophytic colonisation of MBCA have limited or no effect on soil microbial communities because they are associated within the plant. However, depending on inoculation methods, this might not be the case. *Trichoderma koningiopsis* was inoculated into blueberry seedlings as a spore suspension and results found increased diversity and richness of fungi and bacteria in the soil rhizosphere (Li *et al.*, 2023). Information on microbial communities in plants endophytically colonised through means other than soil drenches are required to determine if endophytic inoculation has less impact on soil microbial communities.

Together, these studies highlight that changes to microbial communities can be species, and even strain specific, but often fungal communities are reportedly more resilient than bacterial communities. Thus, the impact of MBCA inoculation can change the microbial communities and are therefore a risk that must be considered and studied in more detail. A summary of positive and negative aspects to be considered can be found in Table 3.

Table 3: Potential beneficial and negative impacts caused by MBCA.

Positive	**Negative**
Long-term negative impacts on environment and health may be less severe than conventional PPP	Potential for reduced native populations of beneficial or neutral microbial species through directed antagonism or competitive displacement.
Native microbial community recovery often reported in literature	Potential for long-term changes in community structure is poorly understood
MBCA may have other beneficial effects such as improved plant growth	Influence on microbial soil functions is poorly understood
Improved specificity against target pests and pathogens	Unintended control of beneficial endophytes and epiphytes with potential for increased plant stress
Useful in situations with a wide host range for the problem	
Can be used in IPM programmes in combination with other PPP.	Little known or understood about different application methods.

4.1.7 What are the limitations of using microbial biocontrol agents?

There are several factors that create barriers for growers and result in reduced uptake of MBCA. Barriers caused by regulations are discussed in the section below (4.1.8). During the FERA webinar on Biopesticides, survey respondents were asked what the biggest barriers were for using biopesticides. The major concerns were around the efficacy (57.6 %), followed by costs (35.6 %) and integration with conventional PPP (33.9 %). Concerns were also raised around knowledge of how to use biopesticides (22 %), which are viewed as complicated or require specialised equipment (11.9 %). Other concerns were raised about consistency, shelf-life, availability of products, and issues around cost and difficulty of registering a new product (Dillon *et al.*, 2023). Many similar barriers (efficacy, cost, integration with PPP, use, application) were reported by participants at the workshop held for this project (see section4.2.4). In addition, our workshop identified issues around advice, regulations, and availability.

In a review on biocontrol of plant diseases, Collinge *et al.* (2022) highlighted that BCA are often considered less reliable or efficient than conventional control or host resistance, creating a major challenge. To address areas of inconsistency, Collinge *et al.* (2022) speculated that endophytic microbes could play an important role given they are adapted to an endophytic lifestyle and are protected from the external environment, which is an uncontrollable variable sometimes attributed to the unreliability and efficacy challenges of MBCA.

The issues concerning knowledge exchange of BCA are not new, and a review by Barratt *et al.* (2018) suggests practitioners should communicate the benefits associated with biocontrol verbally and in popular publications, not only through scientific publications. They also suggest that the ‘sales pitch’ is changed to focus on outcomes, such as ‘avoiding harm from pesticides’ rather than solely on the pest reduction aspect of the product. These shifts could change the perceptions of biocontrol agents making growers more likely to use them, or at least view them more positively. Many of the limitations, such as cost, specialised equipment and storage requirements could be reduced if overall uptake is increased. Although this is a chicken and egg situation, greater uptake will create a bigger market, therefore driving down costs. However, even if demand were to increase, a previous PHC project (Burnett *et al.*, 2021) identified difficulties in upscaling biological solutions. Growers will also need to invest in storage, equipment and new machinery and need evidence that investment will be worth the associated costs.

4.1.8 Review of the regulatory landscape for microbial biocontrol agents

The registration of new BCA is managed by the Chemical Regulation Division (CRD) of the HSE. This work is guided by the Organisation for Economic Co-operation and Development (OECD) in the form of the 'Working Document to the Environmental Safety Evaluation of Microbial Biocontrol Agents' noted in the Standing Committee on the Food Chain and Animal Health on 28 September 2012, as SANCO/12117/2012 –rev. 0 (OECD, 2012). As the title of this document states, it is a working document and therefore it is not a requirement which has to be followed nor does it provide any mandatory guidance. A review by ADAS in 2011/12 explored barriers to the commercialisation of biopesticides and the scope for strengthening support for biopesticides within current regulatory and other constraints. As a result, HSE created a 'Biopesticide Scheme' which includes reduced fees for biopesticide applications. It is important to note that this scheme was not mentioned by stakeholders that participated in the workshop.

For companies registering new BCA products in GB and NI, considerable frustration exists because the registration process uses the existing chemical pesticide registration for BCA products. The level of control that needs to be achieved for a PPP to gain registration may be unreasonably high for a BCA, which means that it takes many years to get a product to market (World BioProtection Forum, 2023). Developers also argue that chemical products are fundamentally different to biological products, which may already be present in the wider environment, and therefore the registration process should be different (World BioProtection Forum, 2023). However, any registration process has to evidence the benefits and one potential barrier to uptake is a scepticism amongst growers where overblown marketing claims are made, particularly in the areas of biostimulants, with little apparent evidence.

The above regulations date from the UK's EU membership, which ended in 2020, and therefore an opportunity potentially exists to reform the above regulatory framework to allow for BCA innovation in GB and NI.

4.2 Findings from the 'Microbial Biocontrol in Scotland' workshop

Sixteen participants took part in a workshop titled 'Microbial Biocontrol in Scotland' held at SRUC on the 25th of May 2023. Using Slido, participants were asked which sector they worked in and were able to select multiple options. Agriculture was represented by 53 % of participants, followed by horticultural crops (47 %), forestry (27 %) and the natural environment (27 %). Participants were also asked to identify their roles, again with the option to select multiple answers. Sixty percent of participants identified as researchers, followed by consultants (47 %), policy (47 %) and biotech companies (7 %). No growers were present, partially due it being a very busy time of year. All participants had an interest in microbial biocontrol agents (MBCA) and 93 % reported to have direct experience using MBCA or plan to do so in the future.

4.2.1 Workshop participant's general perceptions of microbial biocontrol agents

Participants were asked to rate their opinion of MBCA at the start and end of the day (Figure 2). The opinion rating was based on a 10-point scale where 10 was the most positive perception. Following this, participants were asked to write down their definition of MBCA and then share these in a group discussion. Pests were specifically excluded from discussions. It was clear that there was no standard definition for MBCA within the group. The discussion concluded that MBCA were whole organisms that had a direct effect on disease causing agents. Bacteria, fungi, viruses, oomycetes, and bacteriophages were all considered MBCA. Some participants thought that MBCA should be alive or stimulate resistance, while others did not;

no clear consensus was reached on these aspects. However, it was agreed that microbial bi-products, synthetic RNA, and peptides were not considered MBCA.

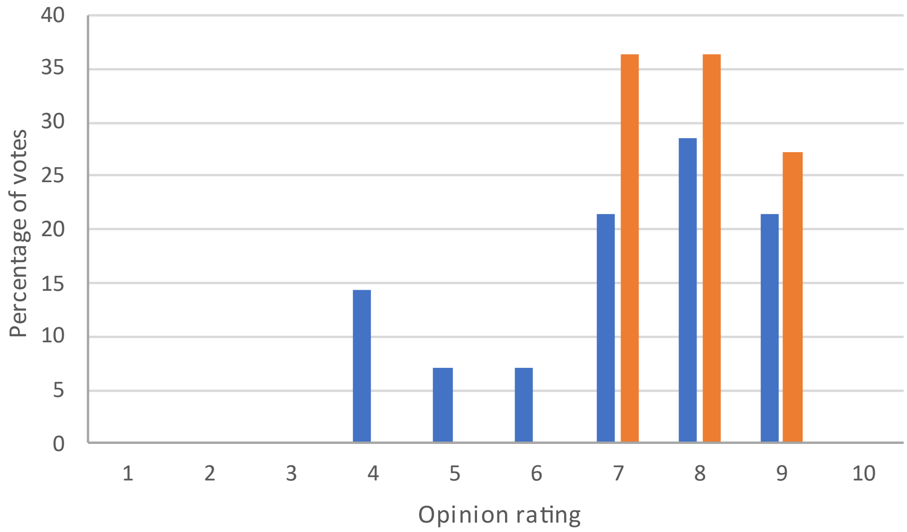

Figure 2 - Opinion poll of participant perceptions of microbial biological control agents at the start (blue) and the end of the day (orange). The poll was based on a 10-point scale where 10 is most positive. The poll was based on responses from 14 and 11 people respectively and the graph represents the percentage of the votes.

The HSE has 38 microbial PPP registered for the UK (see Appendix B for full details). Within this there are 17 fungal, 15 bacterial, five virus and one oomycete products registered. The microbial PPP include agents against pests and diseases, however this project focused on MBCA for plant diseases which reduces the number of products registered for control of plant pathogens. Details of which products were used by participants attending the workshop are given under the relevant sectors below. Methods of MBCA application used are detailed in Appendix D.

Participants were asked to rate their opinion of the efficacy and reliability of microbial biocontrol agents (Figure 3a). Overall, the majority of participants (55 %; 6 individuals) thought that the efficacy and reliability of MBCA was medium on the 5-point scale used. The remaining 45 % (5 individuals) thought that MBCA were slightly better or worse than average. Further to this, participants were also asked to rate how they felt MBCA compared to synthetic PPP (Figure 3b). Again, the majority (70 %; 7 individuals) of participants thought the comparison was medium on the scale used. However, 10 % (1 individual) thought that MBCA did not achieve comparable control to synthetic PPP at all, giving the lowest ranking, and 20 % (2 individuals) thought the comparison between MBCA control was slightly better than the medium ranking.

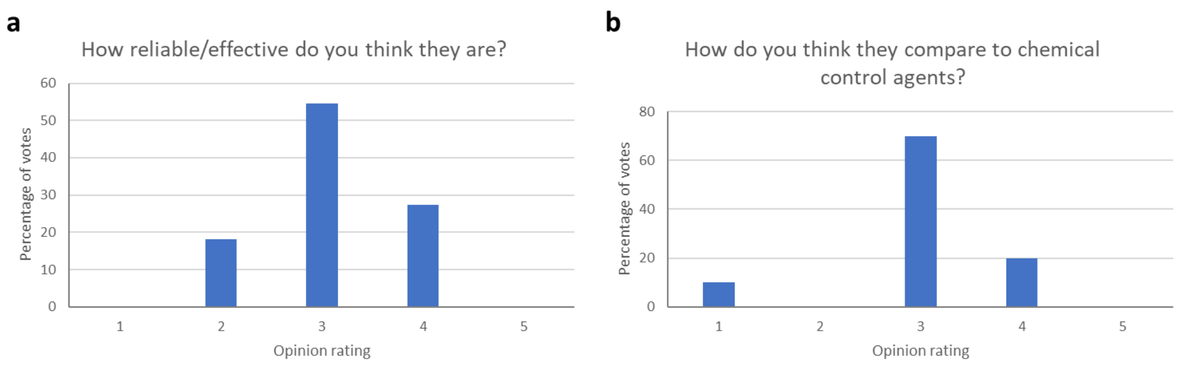

Figure 3 - Opinion poll of participant perceptions on the (a) efficacy and reliability of microbial biological control agents and (b) how MBCA compares to chemical control. The poll was based on a 5-point scale where five is best.

4.2.2 Use of microbial biocontrol by workshop participants

4.2.2.1 Agriculture

A range of microbial biocontrol agents were reported from the agriculture sector (Table 4). Separating the role of the responder was not always apparent given the fact participants could select multiple roles, therefore if someone selected consultant and researcher, it is unknown which aspect of their role the MBCA related to. The majority of MBCA usage reported were bacterial agents.

Table 4: Microbial biological control agents used by participants working in the agriculture sectors.

Microbial biocontrol agent	**Host**	**Disease**
Bacillus subtilis	Unspecified	*Sclerotinia*
Bacterial strains (Mix of commercially available products and research materials)	Unspecified host 'Field trials'	Unspecified
Idriella bolleyi	Wheat	Take-all Stem base diseases Root diseases
Pseudomonas sp.	Unspecified	*Rhizoctonia* sp.
Serenade® ASO (*Bacillus subtilis* strain QST 713)	Barley	Foliar disease
Serenade® ASO (*Bacillus subtilis* strain QST 713)	Cereals	Mildew
Various	Unspecified	*Phytophthora infestans*

4.2.2.2 Horticulture

A range of MBCA products were reported from participants in the horticulture sector (Table 5) where fungal biocontrol agents were most frequently reported. As with the agriculture sector, the role of the responder was not always apparent given the fact participants could select multiple roles, therefore if someone selected consultant and researcher, it is unknown which aspect of their role the MBCA related to. A mix of fungal and bacterial MBCA were reported in the horticultural sectors.

Table 5: Microbial biological control agents used by participants working in the horticulture sector.

Microbial biocontrol agent	**Host**	**Disease**
Aureobasidium sp.	Apples Cherries Plums	Unspecified
Bacillus sp.	Unspecified	*Botrytis* sp.
Botector® (*Aureobasidium pullulans*)	Soft fruit Top fruit	Unspecified
Chromobacterium sp.	Strawberries	Unspecified
Trianum® (*Trichoderma harzianum* Rifai T-22)	Multiple	*Pythium* sp. *Fusarium* sp.
Trichoderma sp.	Apples	Unspecified
Trichoderma spp.	Privet & strawberry	*Armillaria mellea*
Vintec (*Trichoderma atroviride SC1*)	Apple	Apple scab
Vintec (*Trichoderma atroviride SC1*)	Stone fruit	Bacterial canker

Market drivers were also mentioned, for example, McCain's is moving towards a regenerative agriculture framework, with all farms to be enrolled at some level by 2023. As part of their ongoing research and development they are testing biocontrol as well as tillage regimes and fumigation alternatives (McCain's, 2021). In relation to this, the Environmental Impact Quotient (EIQ) which measures the environmental impact of chemicals does not count microbials in the equation (Corredor, 2023).

4.2.2.3 Forestry

Two MBCA's were reported from the forestry sector (Table 6); *Phlebiopsis gigantea* (PG) suspension and sweet chestnut mycoviruses. *Phlebiopsis gigantea* is used as a biocontrol agent of *Heterobasidion* species in *Pinus* species from the UK forestry sector. The commercially produced product, PG Suspension, is a concentrated suspension of *P. gigantea* oidia that are diluted and applied to *Pinus* spp. stumps after felling to outcompete the root-rot pathogen *Heterobasidion* spp. (Pratt, 1999). Although it shows promise as a biocontrol agent for an important root pathogen, PG Suspension is currently only used in Thetford Forest (K. Tubby, personal communication). Research is currently underway to extend the use of PG suspension in the UK. The sweet chestnut mycovirus, *Cryphonectria hypovirus* 1 (CHV-1), is used commercially in Europe (Rigling and Prospero, 2018) to control sweet chestnut blight (*Cryphonectria parasitica*) and current research is underway for its use in the UK, although it is not currently approved.

Table 6: Microbial biological control agents used by participants working in the forestry sector.

Microbial biocontrol agent	**Host**	**Disease**
Mycovirus	Sweet chestnut	Sweet chestnut blight
PG suspension *(Phlebiopsis gigantea)*	Pine trees	*Heterobasidion* root & butt rot

4.2.2.4 Natural Environment

No participants in the workshop reported use of microbial biocontrol agents for the natural environment.

4.2.3 Benefits of microbial biocontrol agents perceived by workshop participants

The benefits of MBCA were discussed amongst participants and were widely agreed upon. To summarise the discussion, participants felt:-

- MBCA are target specific, therefore causing less environmental damage.
- Using MBCA is also considered an important strategy to aid resistance management in plant diseases.
- Reducing the use of synthetic chemicals and their constituent formulas, which can contain other potential contaminants such as microplastics, also reduces the environmental impact of PPP.
- MBCA were considered to cause less stress to plants, are not considered to bioaccumulate and are compatible with organic practices.
- MBCA products usually have low residue the harvesting process can be made more efficient, reducing the harvest interval period and food wastages (especially in soft fruit crops).
- Using MBCA, or sustainable agricultural practices is seen to be 'fashionable' with political and consumer backing.
- Using MBCA could reduce EIQ.

4.2.4 Limitations to uptake of microbial biocontrol agents discussed by workshop participants

Limitations to increasing the use of MBCA are barriers that occur at various stages from discovery to commercial use. Participants selected barriers which might limit uptake of MBCA that were important in their sector (Figure 4). Multiple barriers could be selected. In many cases participants worked across multiple sectors and so determining the main barriers within sectors was not possible. The major barrier to uptake of MBCA was the availability of advice and guidance (77 %). This was followed by the regulations (69 %), evidence of efficacy/safety (69 %) and cost (62 %). When given the option to expand on other barriers, reference was made to lack of understanding from agronomists/advisers, and lack of understanding of the mode of action for MBCA. Concerns were raised regarding the compatibility of MBCA with other PPP and the cost of registering MBCA as PPP, something perceived as not being a commercially viable option, especially for smaller companies. Concerns were raised over the market acceptability of MBCA and the opinion that parts of the industry have vested interest in pesticide sales. In table discussions with small groups of participants raised concerns over the reliability of MBCA and the year-to-year consistency, but also highlighted that conventional fungicides can be variable too. The understanding of why and when MBCA fail to provide expected levels of control is low so making improvements in consistency and efficacy hard to achieve.

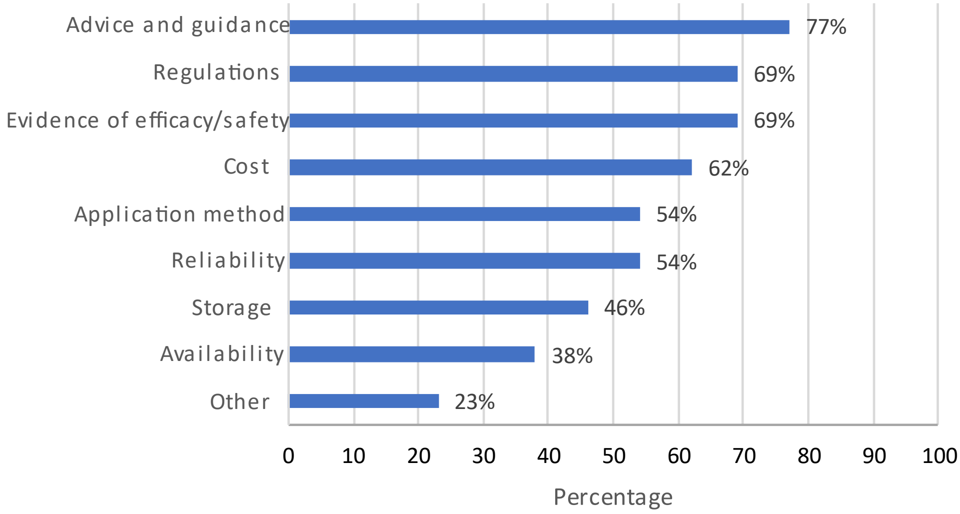

Figure 4 - Barriers to the uptake of MBCA. Participants were able to choose multiple barriers they felt were important for uptake of MBCA in their sector.

After discussing the major barriers/limitations to uptake of MBCA, discussions were held to identify the major barriers and potential strategies to overcome them. These were broken into three main categories: practicalities, evidence base and regulations.

4.2.4.1 Practicalities

To overcome the practicalities of increasing uptake of MBCA participants felt that more focus on research and development is required and costs need to be brought down. Improvements should address:

- Compatibility of MBCA with IPM (e.g., other PPP, cultivars, rotations, and biodiversity schemes)
- Integration of MBCA with decision support systems (DSS)
- Reliability and consistency of MBCA
- Cost of developing, registering, and applying MBCA (improving accessibility)
- Storage of products, including KE around potential contamination and shelf life
- Information on handling of MBCA products (e.g., sprayers, mixing, application timing)
- Improved advice available to users and consultants
- Aligning ease of use and costs of MBCA with chemical controls
- Persistence and viability of MBCA
- Concerns regarding the biosecurity of trading commercial products
- Improved availability of products in each sector

4.2.4.2 Evidence base

The main theme when discussing evidence for MBCA was the lack of a bigger picture. There is plenty of research into novel MBCA products under controlled conditions, however there is limited research that continues this into field trials and these trials often highlight inconsistencies. Products that do show promise often struggle to be taken up by companies to become commercialised. This is partially due to costs of registration, and the UK being a small market.

Participants felt that there was very little evidence available for efficacy of MBCA in a Scottish context. Suggestions were made that global datasets should be available for the use of MBCA in different climates to indicate products or species worth trialling. Information included would indicate efficacy under different climatic conditions (temperature, humidity, precipitation), cropping systems, target pathogens and hosts. This should also include information on ecotoxicology testing, products successfully used globally or field trial data. Such a resource would allow potential MBCA products that are suitable to local crops and growing conditions to be readily identified and tested under field conditions.

4.2.4.3 Regulations

Participants, including representatives from MBCA development companies (e.g., Koppert), raised concerns about the regulation of MBCA including the cost of registering products and the time taken for the registration process to be completed. As discussed in the workshop and at the World BioProtection Forum event (5th Sept 2023), it currently takes approximately 10 years to complete a new MBCA registration in GB and NI. This has led to a number of companies having to lay-off their staff between the development and registration stages whilst they wait for a product to be approved. The same process takes approximately two years in the United States and 18 months in Brazil. This has made GB and NI a commercially unattractive place for companies to register new products and therefore innovation moves abroad.

Participants described a lack of clarity in the regulation system, with possible differences between Scotland, England, and GB as well as uncertainty in post-Brexit regulations. For example, exotic organisms need to be tested in each GB country and can receive different regulations. Many people raised concerns about the regulation system for MBCA which is based on the same process as the registration of active substances. This means that many questions are not relevant to MBCA and requires biological efficacy to be proven. For example, “would a product be harmful to native fauna?” is an irrelevant question if the product already exists in the wider environment in the UK, which may apply to an MBCA. Therefore, the experiments to prove whether this is the case are unnecessary if the two species already coexist in the environment. Under current regulations, these tests still have to be carried out. The environmental risk assessment is also set up for regulation of chemicals making it hazard based. The feeling this created among participants was that registering biological control products was not considered as important as new chemical products.

In addition, MBCA regulations are currently administered by the HSE (Chemical Regulation Division). It was suggested that some aspects of MBCA regulation might be more suited to the expertise of Defra staff to align MBCA with other environmental work areas (e.g., plant health, biosecurity, invasive species, etc.). The relevant parts of the HSE website were also described as ‘clunky’ and difficult to navigate for those interested in registering or researching new products.

To improve regulations more training and education for regulators was recommended to improve the process. Clarity and guidance are required for regulations, especially considering grey areas, such as registration of biostimulant products that are not considered PPP, potentially making them unregulated although this is beginning to change due to greater awareness within the regulatory body. It was also suggested that MBCA products should be fast-tracked if they have been registered in another country or a globally harmonised data package should exist for all registered MBCA.

4.2.5 Risks of microbial biocontrol agents perceived by workshop participants

To understand whether there is a perception of risks associated with MBCA a discussion was held with a basic, short introduction to avoid biasing opinion. To open the discussion, a poll was conducted to find out the opinion of participants (Figure 5), this was then repeated at the end of the day to determine if opinions changed. The overall view before the discussion was that participants felt too little is known to decide how harmful or risky MBCA can be. Around 20 % of the participants thought that MBCA was either beneficial for the environment or not harmful with only 7 % considering that MBCA might do some harm. After the discussion the overall view was still that we do not know enough to decide, although there was a drop from

50 % to 42 %. There was a slight drop in the number of people that thought microbials were beneficial and an increase in the number of people that thought MBCA were either not harmful or caused some harm after the detailed discussions.

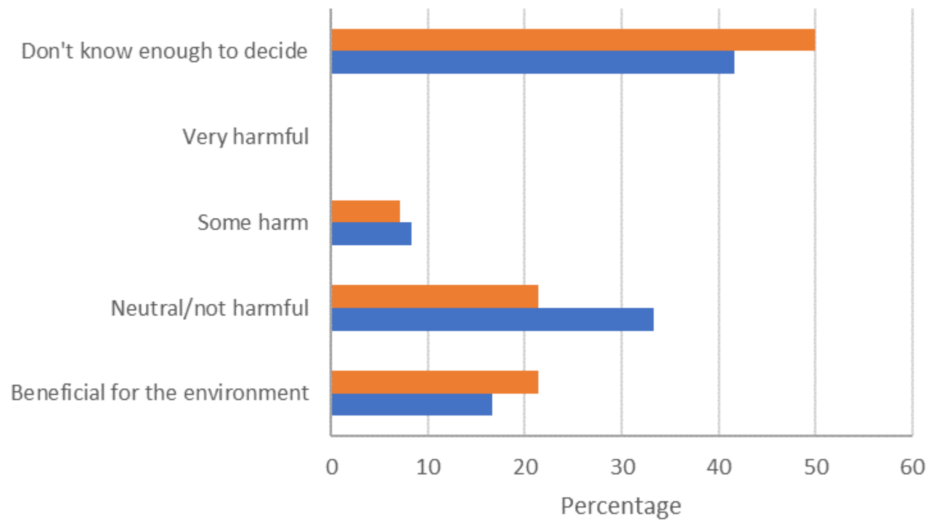

Figure 5 - Perceptions of the risks associated with MBCA before (orange) and after (blue) detailed discussions. Participants were able to choose one option.

Discussions on the risks associated with MBCA focused on a lack of knowledge on the topic. Participants felt that research towards new biocontrol agents often overlooks risks because there is a biased aim to find a novel control agent. It was widely reasoned that we have been putting active substances into the environment for so long that we do not have a baseline to compare natural microbial communities in soil systems. Due to regulatory processes single strains of microbial organisms are registered and used for commercial products. Discussions also covered how flooding the environment with one genotype of an organism could potentially influence naturally occurring strains. Concerns were raised about how likely introduced strains of biocontrol agents would be able outcompete naturally occurring variants. The possibility of genetic recombination by introduction of microbials was also raised as a concern as well as potential influences on larger organisms. Many of the ecotoxicology tests are not fit for purpose and based on tests of species, such as springtails, that are microbivores.

Participants who felt microbials did not have a significant impact on the environment suggested that environmental/soil disturbances (such as flooding events, ploughing, pH changes, or fertiliser additions) can have a major effect on soil systems, soil health, and therefore potentially also on microbial communities. Therefore, they felt that when any focus on the potential risks associated with using MBCA is considered, it should be based on the wider functionality of soils. It is also important to determine how quickly soils and microbial communities will recover after application of MBCA to assess the risks.

4.2.6 Participants experience and opinion of knowledge exchange for microbial biocontrol agents

The availability of advice and guidance was perceived as the biggest barrier to using more MBCA (Figure 4). To understand the issue in more detail we had a discussion in two parts. The first part addressed what information was available and where some of the major gaps are. The most common place to find information about MBCA was either directly from the manufacturer (including videos to advertise products on YouTube), the product labels, information sources online, grey literature and scientific papers. Several participants thought that information databases need to be improved. Criticisms suggested that databases are not easy to search, are disjointed and decentralised. To improve this an easy to search centralised database might provide stakeholders with the information necessary for improved knowledge and understanding about using biocontrol agents. Lack of information on Scottish conditions for MBCA was again highlighted in this discussion. For more detailed information about the current information sources available and the gaps identified by participants at the workshop see details in Table 7.

Table 7: Current sources of information for MBCA and gaps identified by participants for better knowledge exchange.

Current information sources	**Gaps**
Manufacturers' representatives and updates to consultants	Application methods
Liaison Database (includes pesticides and EAMUs). Operated by FERA	Centralised easy-to-use database
Manufacturers YouTube channels	Compatibility standards
European Agricultural Knowledge and Innovation Systems project	Digestible customer friendly information (infographics, You Tube, QR Codes)
James Hutton Institute	Growers and sellers knowledge of availability
Health and Safety Executive (HSE) Database (hard to search website)	Impartial advice
Online information	Information on Scottish conditions
Conferences	Manufacturers have data on other crops but can only show ones the product is registered for
International Organization for Biological Control (IOBC)	Platform for stakeholders using MBCA to share experiences (trusted source)
Company representatives	Regenerative Agriculture needs to include biocontrol
Demonstrations - yearly reviews of products	
Justitute AK13 Project	
Legal Advice	
Manufacturers	
Product labels	
Scientific papers (often behind a paywall)	
Twitter	
Big investment coming to Scotland (accreditation and training)	

Following this, participants were asked to discuss what they thought the advantages and disadvantages of three different formats of knowledge exchange would be. The suggested formats of knowledge exchange were 1) websites or online factsheets; 2) training and webinars and 3) an advisory service. Information obtained from flip charts used to capture the data in the session are summarised in Appendix E, Appendix F and Appendix G respectively and briefly outlined below.

Using the internet to host information about MBCA was seen to have great potential. Many companies already have websites that provide information about products available, and some include factsheets on MBCA products. Websites are also able to reach a wider audience than paper-based information sources (e.g., magazines, conference proceedings, peer reviewed paper). It was also noted that company websites are considered trusted sources and represent the industry standard. While many company websites are open access, some information is behind a paywall, for example CABI and peer-reviewed papers. In some cases, databases are not well connected and information within websites and factsheets can be hard to find, especially information regarding application and storage.

Delivering training in person and through webinars were thought to have many advantages and disadvantages. Generally, online training through webinars is freely available and easily accessible (can also be accessed in-field). Training events are also important for certification standards (e.g., LEAF, Red Tractor, BASIS). However, there are some caveats. Ingrained behaviour and generational differences can be hard to change, and this is unlikely to be solved by training. Often training events can result in an overloading of information and they are not treated as formal qualifications so attendees can be at risk of receiving a patchy understanding. Especially in the case of free training, questions were raised as to who provides this, although it is most likely a representative from an MBCA producer.

Clients with access to an advisor can develop personal relationships with the advisor and receive tailored advice. Advisors should provide clients with trusted and accurate advice and should have legislated liability. However, advisors may have some liability for failure which will limit the advice they are prepared to give. Some advisors may also have a conflict of interest (i.e., commission). Often these services are underfunded, and participants raised questions as to where funding for advice on MBCA would come from.

4.2.7 Next steps to improve uptake of microbial biocontrol agents

To close the workshop, each participant was asked to write down what the next steps are under three main categories: practicalities, evidence base and regulations. These are summarised below.

4.2.7.1 Practicalities

- Enhance perception of MBCA.
- We need a mindset shift away from chemicals due to the environmental damage caused.
- Better understanding of how to deploy novel materials in crop protection programmes and integrate them into decision support systems.
- MBCA needs to be presented to the public in a positive way to avoid issues that arose with GM perceptions.
- Evidence of efficacy.
- Provide non-biased information for growers.
- Improved sources of information and independent advice.
- One centralised/connected database or website for information.
- Integrate MBCA into agriculture, forestry, horticulture, and natural environment training.
- Increased training/education and KE using demonstrations, field trials, network of users.
- MBCA use is too complex at the moment.
- We need a way to get new MBCA to the market faster.

4.2.7.2 Evidence

- We need non-biased evidence, fit for Scotland.
- Better funding for applied research to allow more field-based trials (inconsistencies between field- and lab- based studies).
- Avoid reinventing the wheel by accepting data from compatible countries.
- Utilise existing body of evidence (using data from other countries).
- Availability and connection between sources that are regularly updated.

- Good sound supporting technical data/evidence on efficacy and use of materials (peer reviewed preferably)
- Standardized common information that every "microbial product" should have.
- Create a centralised database that includes information on the availability, use, compatibility, application of MBCA products for ease of use.

4.2.7.3 Regulations

- Develop a robust definition for MBCA.
- Clarification of regulations
  - Transparency of timeframe and cost expectations.
  - Updated web-based guidance on data requirements.
  - On-call assistance/advice from HSE.
- We need regulatory framework dedicated to MBCA.
- Review and reform of regulations/legislation/policy around MBCA.
  - Ensure testing for new MBCA is relevant (decoupled from chemical testing requirements).
  - Take the opportunity to build on legislative work done elsewhere.
- Have support in getting MBCA to market.
- Government support of regulations to encourage application of MBCA.

4.3 Case studies

Three case studies have been selected from different plant health sectors to showcase the use of MBCA. The first case study details the control of Heterobasidion root rot in Forestry. The second covers control of Armillaria root rot, which is an important disease in ornamental gardens, to the home gardener, orchard growers and foresters. Finally control of sclerotinia disease is provided as a case study for biocontrol of fungal pathogens in vegetable production. In these examples, all control options are presented to highlight the effectiveness of an integrated approach. The case studies have been broken down to provide an overview of the disease, review options for silvicultural or cultural control, chemical and biological control and conclude with novel control options being considered.

4.3.1 Case study 1: Controlling Heterobasidion root rot in forestry.

4.3.1.1 Heterobasidion root rot

Pathogens in the genus *Heterobasidion* cause Heterobasidion root rot, which is an important forestry disease in the UK, Europe, and North America. Figures from 2005 estimate that economic losses in Europe are € 800 million annually (Asiegbu *et al.*, 2005). The impact of Heterobasidion root rot can be more severe in parts of mainland Europe than in the UK due to this country's long history of preventative treatment. A failure to control the disease would increase inoculum (spore) levels, and levels of infection in future forest rotations. *Heterobasidion* species predominantly affect conifers including *Pinus, Picea*, *Abies, Pseudotsuga menziessii* and *Juniperus* (Asiegbu *et al.*, 2005), all important to commercial Scottish forestry and Scotland's natural environment. Infection within native Scots pine could be devastating to native Caledonian pinewoods.

Heterobasidion spp. infection can cause mortality in young *Pinus* trees and significant decay in other conifers, including *Abies, Picea, Pseudotsuga menziesii*, decreasing timber value and, where infection is found around the root zone, increasing risk of wind throw (Honkaniemi *et al,* 2017). The external symptoms of Heterobasidion root rot are typical of root rot diseases, showing symptoms such as resin exudation from the stem and crown deterioration. Internal decay begins at the base and basidiocarps (Figure 6) may also be noted in late-stage infections (Asiegbu *et al.*, 2005). There are two main routes for pathogen infection. The primary mode of infection is via aerially disseminated basidiospores, which land on freshly cut stump surfaces, but can also result from infection of wounds on the roots and stems of host plants. Basidiospores are predominantly released from fruit bodies throughout the spring through to the autumn, with inoculum levels falling when temperatures are below 5 ^{O}C and above 35 ^{O}C. Once infection has established within a stump or tree, the pathogen can spread through root-to-root contact, infecting adjacent healthy trees (Asiegbu *et al.*, 2005; Garbelotto and Gonthier, 2013).

Figure 6: Heterobasidion annosum from a lodgepole pine and Sitka spruce clear fell site in Caithness, Scotland. (a) basidiocarps growing on felled stumps, (b) underside of H. annosum basidiocarps. Image credit: Katherine Tubby, Forest Research.

4.3.1.2 Silvicultural control

Conifer species have varying susceptibility to the pathogen and broadleaf trees are relatively unsusceptible. Therefore, focusing on species that are more resistant to the pathogen or planting in species mixtures can help to reduce disease incidence and slow the spread of disease through a crop (Asiegbu *et al.*, 2005). Felling operations can also be conducted during winter periods when the fungus is less active (Asiegbu *et al.*, 2005; Garbelotto and Gonthier, 2013) although in Britain's temperate climate *Heterobasidion* species can sporulate all year round. Crop thinning operations, which are commonly employed to improve timber form and yield, can also be reduced or carried out as late as possible in a rotation to reduce levels of infection. Stump excavation to remove infected material before replanting the next generation of trees can be effective, but it can also be more costly than chemical or biocontrol options, is labour intensive and is only feasible on relatively level, easily accessible sites (Cleary *et al.*, 2013). One study has investigated fire as a method for disease control in *Pinus mugo* forests. Fire reduced the survival of *Heterobasidion* species on some sites, being most effective on sandy soils (Lygis *et al.*, 2010).

4.3.1.3 Chemical control

As *Heterobasidion* species primarily enter a forest through cut stumps, it is possible to prevent infection by applying treatments to stumps during or shortly after felling. Chemical treatments include urea and borate compounds. Borates interfere with metabolic processes in basidiomycetes, preventing spore germination (Asiegbu *et al.*, 2005). While urea does not directly affect the growth of *H. annosum,* it causes a significant rise in pH and ammonia content in sapwood which prevents growth of the pathogen and can be maintained for several months in warm and humid weather (Johansson *et al.*, 2002). However, borate and urea can damage ground vegetation surrounding treated stumps (Westlund and Nohrsted, 2000). Urea also has significant impacts on stump fungal communities (Vasiliauskas, *et al.*, 2004) and there can be environmental concerns when applying nitrogenous compounds to forest ecosystems.

4.3.1.4 Biological control

The biological control of *Heterobasidion* species is one of the few success stories for biocontrol of forest pathogens. The fungus *Phlebiopsis gigantea* has been used as a biocontrol agent against *Heterobasidion* spp. since the early 1960's (Pratt *et al.*, 2000). *Phlebiopsis gigantea*, like *Heterobasidion* spp., is a wood rotting basidiomycete that can colonise pine, although *P. gigantea* is primarily a saprophyte and directly competes with *Heterobasidion* spp. for the same ecological niche i.e., recently cut stumps (Pratt, 1999).

In Europe, *P. gigantea* is available in three commercial formulations (Table 8; (Pratt *et al.*, 2000)). In the UK, Forest Research produces PG Suspension which has been through several product iterations: originally, inoculated pinewood blocks were sent out to foresters who shook them in water to release oidia which were then painted or sprayed onto pine stumps. Since the 1970's however, PG Suspension has been sold as a concentrated suspension of oidia in a sucrose solution that can be packaged in small sachets to protect from contamination (Pratt *et al.*, 2000). The isolates of *P. gigantea* in the product originate from natural, unmodified colonies and spore trapping carried out in British forests. In a 30-year period to 1991, 10 isolates of *P. gigantea* were used (Pratt *et al.*, 2000) but more recently, European and British pesticide regulations have limited the product to just a few, named, molecularly characterised isolates. PG suspension sachets contain up to 500 million spores which are diluted in water and must be applied within an hour of felling (Forest Research, 2023). Application of stump treatments using mechanical harvesting machinery can result in less than the desired 100 % coverage. However, biological stump treatments have the advantage of being able to grow laterally across the stump surface to close small gaps in coverage, something not possible with the alternative chemical products (Tubby *et al.*, 2008).

Table 8: Summary of commercialised Phlebiopsis gigantea–based biocontrol products in Europe used against Heterobasidion root rot (adapted from Pratt et al., 2000).

Product	**Formulation**	**Target host**	**Shelf life**	**Storage temperature**
PG Suspension	Sucrose spore suspension	Pine	6 months	3-5 ^{O}C
IBL	Sawdust/Fungus mix	Pine	1 year	4 ^{O}C
Rotstop	Spores mixed with silica	Pine & Norway spruce	18 months	≤8 ^{O}C

4.3.1.5 Future direction

While reports suggest that *P. gigantea* offers effective control of Heterobasidion root rot on *Pinus* species, its current restriction to this tree species limits the use of PG suspension to *Pinus* spp. plantations. Currently, PG suspension is only used in Thetford Forest in southeastern England (K. Tubby, personal communication), possibly due to close ties with Forest Research; Thetford Forest has been using PG suspension in *Pinus* spp. for the past 30 years (Forest Research, 2023). Further research is needed to improve performance on some of the UK's other more diversely planted conifer plantations together with an examination of other barriers to wider usage, which might include product cost, ease of use and the complex and expensive statutory pesticide re-registration process which, combined with the small market, make the product investment unattractive (K. Tubby, personal communication). Increased awareness among foresters through knowledge exchange and training could increase the uptake of PG Suspension. Given that the product is produced by Forest Research (2023), large increases in demand might face issues with upscaling production, as highlighted by Burnett *et al.* (2021). Looking to the future, ongoing research has identified a number of mycoviruses naturally present in *Heterobasidion* species that could offer further biocontrol options (Vainio and Hantula, 2016; Vainio *et al.*, 2018b). Based on the success of controlling *Cryphonectria parasitica* (sweet chestnut blight), a forest pathogen in European chestnuts, the potential for mycovirus control of Heterobasidion root rot in plantations should be considered.

4.3.2 Case study 2: Controlling Armillaria root rot in ornamental horticulture and forestry.

4.3.2.1 Armillaria root rot

Armillaria species are an important root rot pathogen in forestry, horticulture, and ornamental gardening. Common names include the honey fungus or bootlace fungus. There are between 50 – 60 species worldwide (Koch and Herr, 2021) with five species recorded in the UK (Perez Sierra *et al.*, 1999; Koch *et al.*, 2017). The most common are *A. mellea, A. gallica* and *A. ostoyae*. In the UK, *A. mellea* is primarily a necrotrophic pathogen, while *A. gallica* is more commonly an opportunistic pathogen or saprophyte (Rishbeth, 1982). Research suggests *A. mellea* is more likely to spread between hosts in garden situations than *A. gallica* (Drakulic *et al.*, 2017). *Armillaria ostoyae* was generally considered a pathogen of coniferous trees (Rishbeth, 1982; Blodgett and Worrall, 1992), however recent evidence shows that, in garden settings, it has no preference over gymnosperms (Cromey *et al.*, 2019). Symptoms are typical of root rot pathogens with wilting, stunted growth, crown dieback, and stem bleeding (Figure 7a). Below the bark a white mycelial matt or mycelial fan (Figure 7b) characteristic of *Armillaria* infections can be found in heavy infections (Baumgartner *et al.*, 2011). Honey coloured fruiting bodies (Figure 7c) can be found in autumn and generally signify heavy infection within the host (Fox, 2000a). Heavy infections weaken plants, eventually leading to plant death.

Figure 7: Symptoms of Armillaria sp. infection; a) bleeding trunk on larch; b) mycelial fan underneath bark; c) Armillaria mellea fruiting bodies. Image credits: Helen Rees

Armillaria mellea has a host range of > 500 species (Raabe, 1962). Infection causes serious losses in forestry (Wargo and Shaw, 1985), vineyards (Baumgartner and Rizzo, 2001; Aguín-Casal *et al.*, 2004) and stone-fruit production (Baumgartner and Rizzo, 2001). It is also a major issue for home gardeners; since records began in the mid-90's, *Armillaria* has been the top enquiry to the RHS advisory service (RHS, 2023a).

There are a range of methods by which *Armillaria* spp. can infect new host plants. Infection can spread from infected plants to healthy plants via root-to-root contact or via rhizomorphs, a characteristic feature of *Armillaria* species (Baumgartner *et al.*, 2011; Heinzelmann *et al.*, 2019). Rhizomorphs, which are melanised fungal structures (Heinzelmann *et al.*, 2019) can grow up to a metre a year through the soil (Redfern, 1973) and are able to survive on small fragments of wood (Redfern, 1973; Perez Sierra and Gorton, 2005). The role of basidiospores in *Armillaria* infection cycles is poorly understood (Heinzelmann *et al.*, 2019). Baumgartner *et al.* (2011) reported that the role of basidiospores in new infections is limited. However, recent work by the RHS (2023) suggests that basidiospores have a greater role than previously thought. In the area surrounding RHS Wisley in Surrey, studies were undertaken to assess genetic variation in populations of *Armillaria* collected by the RHS Advisory service. The samples were all from gardens, which are considered highly disturbed habitats and therefore at high risk to new infection. Within the *Armillaria* isolates studied, there was high genetic diversity suggesting that new populations also occurred via spore dispersal.

4.3.2.2 Silvicultural control

Due to the biology of the disease, *Armillaria* infections are very difficult to control. Silvicultural control options can be used to limit the severity of *Armillaria* infection. Site choice consideration are important, particularly where susceptible hosts were previously grown leaving an established inoculum source in the soil (Sherman and Beckman, 1999). In forests, planting bare-root stock can significantly increase susceptibility to *Armillaria* infection compared to seed planting (Hagle and Shaw, 1991) potentially caused by damage during transplantation. Due to the large host range of *Armillaria*, there is limited choice of resistant or partially resistance species. Douglas Fir is considered to have some resistance against *A. ostoyae* and is therefore recommended in the UK for sites with a high risk of infection (Hagle and Shaw, 1991). *Prunus* species such as cherry, almond, and peach are often grown with rootstocks from plum which has more resistance to *Armillaria* attack (Fox, 2000b). Cromey *et al.* (2019) recently assessed the susceptibility of UK garden plants to *Armillaria* infection. Examples of garden plants with some level of resistance to infection included *Catalpa*, *Sarcococca*, and *Vaccinium*.

Stump removal is currently one of the most popular methods of control for *Armillaria* infections. This does not allow for complete eradiation of disease, but can prevent build-up of inoculum (Fox, 2000b). In forestry, stump removal is considered more effective than chemicals, although it is more expensive (Vasaitis *et al.*, 2008). Given *Armillaria* can survive on small pieces of root or woody material as a source of inoculum for up to 12 years (Reaves *et al.*, 1993), removal of most belowground biomass will reduce the energy available and therefore reduce the lifespan and incidence of disease (Fox, 2000b). The RHS also recommends stump removal of infected areas and advises a period of fallow for one year (RHS, 2020). This method is expensive and labour intensive, but in small amenity or high value sites it is a viable option to reduce potential inoculum sources. In high value plantations, such as vineyards, kiwifruit and coffee plantations, trenches can be dug to isolate areas of infection. Trenches are often around one metre deep and sometimes lined with plastic before being backfilled (Hagle and Shaw, 1991; Fox, 2000b). This process is labour intensive and needs to be managed regularly (Fox, 2000b), but is a tool that can be used to manage small disease outbreaks.

4.3.2.3 Chemical control

Chemical control was historically used to control *Armillaria* infections, especially in high value crops. Soil fumigants, especially methyl bromide (CH_3Br) and carbon disulphide (CS_2), were routinely used against *Armillaria* infection (Baumgartner *et al.*, 2011). Soil fumigants were popular because they provided good penetration of soil where *Armillaria* was present, however, fumigation of soil was not target-specific (Hagle and Shaw, 1991). Due to negative environmental impacts and safety issues, soil fumigation has since been banned (Ristaino and Thomas, 1996; Baumgartner *et al.*, 2011), leaving limited options for control that are based on physical removal of infected plants.

4.3.2.4 Biological control

Many studies have been conducted to identify putative biocontrol agents of *Armillaria mellea*. Saprobic fungi including *Hypholoma fasciculare, Ganoderma lucidum, Schizophyllum commune, Phanerochaete velutina and Xylaria hypoxylon* have shown antagonism towards *A. mellea in vitro* and on wood blocks (Cox and Scherm, 2006). A commercial bacterial soil inoculant (Vesta; Biologically Integrated Organics, Inc., USA) added to the irrigation system in vineyards has shown to significantly improve yields of infected vines but it did not reduce the symptom development or mortality of infected vines (Baumgartner and Warnock, 2006). The mycophagous nematode, *Aphelenchus avenae*, has been shown to reduce the mortality of ponderosa pine seedlings which were inoculated with *Armillaria* (Fox, 2003). In Australian karri forests termites have been suggested as a potential biocontrol agent as they feed on decayed wood (Robinson and Smith, 2001), but there has been no further research into this subject.

Trichoderma species are most frequently referred to as potential biocontrol agents of *Armillaria* (Otieno *et al*., 2003; Raziq and Fox, 2003; Kwaśna *et al*., 2004; Baumgartner and Warnock, 2006; Chen *et al*., 2019; Rees *et al*., 2022). Using woody segments of tea plants *Trichoderma* spp. were assessed as antagonists of *Armillaria*. *Trichoderma longibrachiatum, T. harzianum* and *T. koningii* could colonise tea stem sections, even if *Armillaria* was present and were able to deter the growth of *Armillaria* where they were already established (Otieno *et al*., 2003) demonstrating potential biocontrol through competition.

Research for biocontrol of *Armillaria* has focused on endophytic *Trichoderma* in more recent years. Endophytes for biocontrol are better protected from environmental factors (Collinge *et al*., 2022) and can act through antibiosis, competition, induced disease resistance and mycoparasitism (Latz *et al*., 2018), which are all modes of action reported in *Trichoderma* species. Using scanning electron microscopy, *Trichoderma* isolates were shown to mycoparasitise *Armillaria* rhizomorphs (Asef *et al*., 2008) and studies of rhizomorph production in wood-billet assays found rhizopspheric *Trichoderma* isolates could reduce *Armillaria* growth (Kwasana *et al*., 2004). Isolates from rhizomorph-associated soil have been endophytically inoculated to provide some protection against *Armillaria* infection in Turkey oak (Chen *et al*., 2019). Endophytic isolates of *Trichoderma* in healthy plants inoculated into strawberry and privet roots can also afford some control against *A. mellea*, with variation found between isolates and species of *Trichoderma* (Rees *et al*., 2022).

4.3.2.5 Future direction

There has been revived interest in developing control measures against *Armillaria*, however progress is slow. Plant based assays using strawberry as a model organism take a minimum of five months to complete (Rees *et al*., 2022) and for meaningful experiments using larger and more relevant hosts longer timeframes are required to track disease development in saplings. Research in the UK is being conducted by the RHS, University of Bristol and Bartletts Tree Experts with some collaborations at the University of Birmingham.

Work at Bartletts Tree Experts and the University of Reading has recently considered biochar for control of *Armillaria* infection, however due to issues with inoculation (another challenge when working with *Armillaria* species), experiments remained inconclusive and further study was recommended (Hailey, 2021). To further assess the potential for *Trichoderma* as a control measure for *Armillaria* several questions need to be addressed. Firstly, the mode of action needs to be better understood. Evidence suggests *Trichoderma* parasitises *A. mellea* hyphae (Dumas and Boyonoski, 1992; Rees *et al*., 2021). *Trichoderma* isolates also produce a range of enzymes that could be important in control (Rees, 2021). Current research suggests that *Trichoderma* spp. can change the pH of surrounding media, which could play a role in *Armillaria* disease control (Drakulic, 2023). Endophytic *Trichoderma* spp. can induce resistance in plants (Harman *et al*., 2004) and this should be assessed where *Trichoderma* is being used for biocontrol. Much of the experimental work using endophytic *Trichoderma* spp. is conducted with sterile or commercial growing media and the impact of microbial communities in soils on efficiency of biocontrol needs to be understood. If commercialised, *Trichoderma* could be used as a protectant, inoculated endophytically into plants for establishment into areas at high risk of *Armillaria* infection. Finally, the genomes of many *Armillaria* species have now been published (Wingfield *et al.*, 2016; Sipos *et al.*, 2017) and an agrobacterium-mediated transformation protocol for *Armillaria* is established (Ford *et al.*, 2016). This opens new avenues to understanding *Armillaria* species as pathogens and developing novel tools for control. Armillaria root rot is most often a serious problem in parks, gardens, arboreta, and commercial orchards/vineyards (Cromey *et al.*, 2019) where there is high disturbance and plant stress. In the UK, Armillaria root rot poses the most significant threats in private gardens and has been the most common disease identified by the RHS advisory service for over the last 20 years (Cromey *et al.*, 2019), therefore, there are limited funding opportunities. Research is being conducted by the RHS to investigate MBCA in garden settings (Drakulic, 2023) which will require knowledge exchange with members of the public to raise awareness if a suitable product is commercialised.

4.3.3 Case study 3: Controlling Sclerotinia disease with <u>Coniothyrium minitans</u>

4.3.3.1 Sclerotinia disease

Sclerotinia disease, caused by the fungus *Sclerotinia sclerotiorum* and other species of *Sclerotinia,* is a major pathogen of many economically important crops worldwide (McQuilken and Chalton, 2009; O'Sullivan *et al.*, 2021). In Scotland, the major hosts of Sclerotinia disease include oilseed rape, potatoes, peas, beans, carrots, and lettuce (FAS, 2023). In the UK, Sclerotinia disease of carrots is estimated to cause an annual loss of over £6 million to growers (Mcquilken, 2011).

Sclerotinia species produce melanised sclerotia (Figure 8a) which protect the pathogen from desiccation during periods of dormancy. The sclerotia can survive in soil and stubble for up to five years and under favourable conditions apothecia (Figure 8b, c) will develop. Wind-dispersed spores are released from apothecia under suitable conditions; typically, 15 – 25 ^{O}C with high humidity for a period of 48 hours. Infection starts on flower petals and /or senescing leaves resulting in water-soaked spots or grey-brown lesions. Infected plant leaves which fall or lodge lower into the canopy of plants can become a source of infection to other plants. Heavily infected plants can develop a white cotton-like growth on the stems (Figure 8d) which is followed by the formation of sclerotia. Often Sclerotinia disease is patchy within a field with losses greatest during cool, wet, and humid conditions (O'Sullivan *et al.*, 2021).

Figure 8: Images of Sclerotinia sp. and disease symptoms; (a) sclerotia of Sclerotinia sp., (d) germinated sclerotia producing apothecia, (c) apothecia of Sclerotinia sp. in soil and (d) Sclerotinia disease symptoms on lettuce. Image credits: Madhavi Dassanayaka (a & d), Eirian Jones (b - c).

4.3.3.2 Cultural control

Crop rotation, plant density, canopy management and stubble management are important cultural control methods for Sclerotinia disease (O'Sullivan *et al.*, 2021). In the UK short term rotations with susceptible crops should be avoided and growing cereals could reduce the build-up of inoculum. In areas with a history of sclerotinia disease carrots could be planted late, with some evidence to show that crops sown in May have less disease than those shown in March and April. Due to the biology of the disease, canopy management can be an effective way to offer some control. Reduced crop density can create more space within the canopy and excessive nitrogen application should be avoided because it can lead to a dense canopy (Mcquilken, 2011). To prevent carryover of disease stubble management practises can be employed; burning off crop residue and encouraging sclerotia to rot down through irrigation could be effective (O'Sullivan *et al.*, 2021), however crop burning in the UK is banned.

4.3.3.3 *Chemical control*

Fungicides can be used to reduce the inoculum of *Sclerotinia* spp. present on a site (O'Sullivan *et al.*, 2021). In the UK fungicide sprays against sclerotinia disease should be used before the canopy has closed to protect senescing leaves at the base of canopy (Mcquilken, 2011), and because once symptoms have developed the efficacy of fungicides reduces (O'Sullivan *et al.*, 2021). Fungicides with mixed modes of action should be used because while sclerotinia is a homothallic pathogen, there have been some cases where strains with reduced sensitivity have been found (O'Sullivan *et al.*, 2021).

4.3.3.4 *Biological control*

Coniothyrium minitans strain CON/M/91-08 (DSM 9660) is registered as Contans WG in the UK as an MBCA for use on edible and non-edible crops (HSE, 2023b). The SASA pesticide usage survey for outdoor vegetables in 2020 did not encounter the use of any biological control (biopesticide or BCA) (MacLeod et al., 2021). The pesticide usage statistics provided by FERA (2024) show that Contans WG was applied to 151 ha of outdoor vegetables in 2021 (85 ha in 2017), and 158 ha of protected edible crops in 2017. Contans WG is applied and mixed into the soil using conventional equipment (Bayer, 2015) and directly parasitises the sclerotia of *Sclerotinia* species. This soil application means that mycoparasitism occurs before apothecia germinate and release spores (De Vrije *et al.*, 2001). Studies have found that parasitism by *C. minitans* can occur within 8 weeks (Jones *et al.*, 2004). However, there are suggestions in France that efficacy of *C. minitans* varies depending on the susceptibility of *S. sclerotiorum* strains (Nicot *et al.*, 2016). In carrots, the use of Contans WG was shown to be effective at reducing the viability of sclerotia in disease plants and on crop debris (McQuilken and Chalton, 2009). Some research also suggests that conidia of *C. minitans* can be dispersed through water splash and by soil fauna (De Vrije *et al.*, 2001). Commercial formulations (Agrimm Technologies, NZ) of *C. minitans* and *Trichoderma* spp. isolates including *T. hamatum* have also been shown to offer control against *S. minor* in field trials and for disease in lettuce (Rabeendran *et al.*, 2006).

4.3.3.5 *Future direction*

There are suggestions that mycoviruses found within *Sclerotinia* species can reduce stem rot disease in *Brassica napus* (Xie and Jiang, 2014). Going forward, it will be important to determine how extensive the use of Contans against sclerotinia disease is in the UK. It appears Contans WG is not used in Scotland, but pesticide usage reports from FERA show that it is being used in the UK. An important next step would be determining the perceived effectiveness, and how to increase use in the future. It will also be important to fully understand how MBCA of Sclerotinia disease fits into IPM programmes to encourage greater uptake.

4.3.4 *Summary of case studies*

Heterobasidion is a root rot pathogen important in the UK, European and North American forestry sectors. Infection by *Heterobasidion* spp. can result in mortality of young *Pinus* trees and significant decay of other conifers. A lack of Disease Control could increase inoculum levels and therefore infection in future forest rotations (Asiegbu *et al.*, 2005). Chemical, cultural and MBCA control options are summarised in Table 9. In the UK, MBCA for *Heterobasidion* spp. is only used in Thetford Forest (K. Tubby, personal communication). There are opportunities, with increased knowledge exchange, that PG suspension usage could be increased in forest systems of the UK.

Armillaria spp. or honey fungus is an important root rot pathogen affecting most trees and shrubs; it is a particular concern for gardeners in the UK. *Armillaria* spp. spread through root-like structures called rhizomorphs in the soil, by root-to-root contact and production of basidiospores from the fruiting bodies in autumn (Baumgartner *et al.*, 2011). Chemical, cultural and MBCA control options are summarised in Table 9. In the future, a *Trichoderma*-colonised plant that is safe to plant in areas where plants were removed due to *Armillaria* infection could be sold to gardeners. This is ongoing research, which if successful, will develop a product that will require effective knowledge exchange with members of the public to allow successful uptake. Increased costs of such a product are likely, but in gardens and amity setting such costs may be justified.

Sclerotinia disease is a major pathogen of many economically important crops in Scotland including oilseed rape, potatoes, beans, carrots, and lettuce (FAS, 2023). *Sclerotinia* spp. produce sclerotia that can survive in the soil for many years before the fruiting bodies develop and disperse spores that lead to new plant infections. Chemical, cultural and MBCA control options are summarised in Table 9. Although the MBCA Contans WG is commercially available in the UK, it is not used in Scotland and the SASA pesticide usage survey for outdoor vegetables in 2020 did not encounter the use of any biological control (biopesticide or BCA) (MacLeod *et al.*, 2021). Going forward, it will be important to determine how much Contans is used in the UK to identify where barriers are, and how usage could be increased.

*Table 9:*Summary of *control* options for *Heterobasidion* spp., *Armillaria* spp. and *Sclerotinia* spp. discussed in case studies.

Disease	**Pathogen**	**Host**	**Cultural control options**	**Chemical control options**	**MBCA**	**Mode of action by MBCA**
Heterobasidion root rot	*Heterobasidion* spp.	Conifer species	• Species mixture (broadleaf and conifer) • Felling time • Delayed thinning	Urea and borate control options	*Phlebiopsis gigantea* / PG suspension	Direct competition with pathogen
Armillaria root rot	*Armillaria* spp.	Trees and shrubs	• Removal of infected plants • Fallow	Not available	Endophytic colonisation with *Trichoderma* spp.	Unknow, potentially mycoparasitism
Sclerotinia disease	*Sclerotinia* spp.	Vegetable crops including oilseed rape, potatoes, beans, carrots, and lettuce	• Crop rotation • Canopy management • Stubble management • Reduced planting density	Chemical control is available in the UK	*Conorythium minitans* / Contans WG	Direct parasitism of sclerotia

5 Discussion

5.1 Defining Microbial biocontrol

By reviewing the literature and talking to stakeholders it is clear that there is no fully agreed definition for microbial biocontrol. Often biological control (biocontrol) is only considered to be the control of pest populations using invertebrates. There are many variations of the definition, but recent reviews have helped to reach a certain conclusion for biocontrol; 'Biological control or biocontrol is the process of controlling a pest, disease or weed using another living organism for human benefit' (Stenberg *et al.*, 2021; Collinge *et al.*, 2022). As suggested in these reviews, non-living but nature-derived substances used in sustainable control for plant pathogens are classed as bioprotectants which was something that participants agreed on in the workshop held for this project. To ensure that the use of biocontrol for plant diseases is optimised a clear definition needs to be universally adopted. In Scotland, it would aid clarity in knowledge exchange activities around MBCA if the definition above is deployed consistently.

5.2 Overview of the use of microbial biocontrol agents in Scotland

Almost all the participants at the workshop had direct experience using microbial biocontrol. This was likely to be the case given attendance was optional and by invitation to stakeholders from the Plant Health Centre and project team networks and a bias towards those who were likely to be able to contribute opinions on the use of MBCA. Data from Scotland's Pesticide usage surveys suggest there is limited use of biological control for plant disease in agriculture and vegetable production (Davis et al., 2020; MacLeod et al., 2021). This is reflected in the list of MBCA used by participants working in the agricultural sectors where *Bacillus*-based Serenade® is the only commercially available product on the market of the examples listed. In contrast, many more examples of MBCA were given by participants working in the horticultural sector. The pesticides usage surveys also suggest that, within the soft fruit industry, MBCA is used to control some diseases, although predators of pests play a bigger role under biocontrol agents (Wardlaw et al., 2020). Of the participants present at the workshop for this project, those in horticulture worked predominantly within the orchard fruit and ornamental sectors. No biologicals were encountered in the Scottish outdoor vegetable crop surveys (MacLeod *et al.*, 2021), so the extent to which Contans WG is used cannot be determined. There were no representatives from Bayer or vegetable growers present at the workshop, so we were unable to gain a better understanding. During the webinar held on biopesticides, Dillon *et al.* (2023) reported that there is a perception that cereals and potatoes crops are not favourable for using biopesticides as PPP. A key target for knowledge exchange will be to address this perception and identify reasons for it.

There are few examples of MBCA used in arable agriculture or cereal production. The HSE pesticides register (HSE, 2023b) does not explicitly list cereals as registered crops for *Trichoderma* (grapes are the only outdoor crops for which it is approved) or *Bacillus* based products, although for the latter approvals exist for a range of broad acre crops including oilseed rape, combinable peas and potatoes. Clearly more MBCA options, with evidence on modes of action and efficacy, would increase options available and it is positive that a project within the Scottish Government's Environment, Natural Resources and Agriculture Strategic Research Programme (SRP) is considering the use of MBCA in IPM programmes. Use of MBCA alone or in combination with reduced rate fungicides or other alternatives like elicitors is being trialled in spring barley at sites across Scotland. This will help to give important data for MBCA use in the Scottish climate, a concern raised during the workshop. As identified in the workshop knowledge exchange will be key to increasing awareness and uptake of MBCA. Using data from the SRP project will add to the evidence base in Scottish situations. Initiatives to encourage sustainable farming could also play an important role in increasing the use of MBCA in the agriculture sector.

Identifying novel MBCA could open new avenues for control, especially of diseases in difficult situations such as forests. In forestry, mycoviruses are showing good potential to control plant pathogens. The CHV-1 mycovirus if *Cryphonectria parasitica* (chestnut blight) has been successful in Europe and current research is taking this forward for the UK to control chestnut blight.

Climate change is likely to result in many changes to our environment. Scotland will be warmer with wetter winters and drier summers (Adaption Scotland, 2020; UKCP, 2023). It is likely that there will be more plant diseases. Plants could become more stressed and therefore more susceptible to disease. However, studies show that many MBCA have optimal temperatures between 20 ^{O}C to 30 ^{O}C suggesting the efficacy of MBCA products might improve.

5.3 Overview of the benefits and opportunities presented by microbial biocontrol agents

A range of benefits are offered by MBCA, these primarily relate to their use in sustainable production. An overview of the benefits discussed in this report are summarised as follows:

- MBCA can reduce the reliance on chemical pesticides and reduce the long-term impact on humans, animals and non-target organisms resulting in improved environmental profiles.
- MBCA generally have a positive public perception and are viewed as an environmentally friendly option.
- MBCA can reduce harvest interval periods and be integrated into disease management programmes.
- MBCA can be target specific and can be used with multiple plant hosts.
- MBCA can potentially reduce microplastics and other pollutants often contained within the constituent formulas of chemical pesticides that have an additional environmental impacts.
- Some MBCA have additional benefits to plants, including improved plant growth.

As evidenced by the table in Appendix B, there are several MBCA products that are registered for use in the UK. However, in soft fruit crops microbial based control represents 31 % of treated area (Wardlaw et al., 2020), although this is mainly to target pests, rather than diseases, and microbial-based control represents only 0.2 % of treated area in arable crops (Davis et al., 2020). Clearly there is opportunity to increase the usage of MBCA across sectors in Scotland. Given the role MBCA can play within IPM programmes, they present a way to reduce the use of chemical pesticides to move towards a sustainable solution and still provide suitable plant protection.

5.4 The barriers to overcome to achieve the potential of microbial biocontrol agents

A lack of awareness and knowledge came up repeatedly as a barrier. In the workshops for this project, the most important limitation to uptake was 'advice and guidance'. Dillon *et al.* (2023) reported that lack of knowledge was the second most important barrier to uptake of biopesticides. To overcome barriers for the use of biological crop protection, Dillon *et al.* (2023) ranked 'more research and advice on IPM' as the priority. Effective knowledge exchange will be very important to help realise the potential of MBCA.

Based on the webinar held by Dillon *et al.* (2023) and questions presented to participants at the workshop within this project, the perception of efficacy by growers is seen to be a barrier, with strong and ingrained perceptions that MBCA has poorer performance than fungicides.

However, when participants at the workshop were asked of their opinion, most people perceived the efficacy of biocontrol to be equivalent when compared to chemical control. It is possible that participants from this project had a biased opinion, given many work closely with MBCA and growers would respond differently. Efficacy and reliability are major issues that must be overcome to increase use. Growers want products that will offer consistent and reliable control of plant diseases in new products (such as MBCA), while overlooking that conventional pesticides can also be variable but are much more consistent than MBCA. Studies should focus on factors impacting efficacy of MBCA, such as environmental conditions and application timing (e.g., temperature). Different application methods may also need to be developed and a key gap is how they will integrate into pesticide programmes. Changing perceptions around efficacy and control levels and highlighting research attempts to close the gap will help to begin overcoming some of these barriers.

5.5 Overview of the barriers to increasing use of microbial biocontrol agents

The risks of using microbial biocontrol are important considerations to make and are summarised here:

- There are risks that disease control could be more variable, and training and knowledge exchange is clearly required so that users understand how and where they may be applied to minimise this risk.
- MBCA are generally comprised of one genotype that is inundated into an environment and could have adverse effects on microbial communities present in soils and associated with plants.
- MBCA may persist and disperse within native microbial communities.
- There is limited knowledge on the impact of MBCA in the microbial communities.
- Concerns about upscaling BCA for broad acre use were raised in previous PHC projects (Burnett *et al.*, 2021). Production and formulation are often more complicated and expensive so broader scale usage may take a while to achieve. This is likely to apply across sectors where increased use could put pressure on production systems.

In the literature many studies have addressed the effect of MBCA on microbial communities. Research that was considered in the literature review often reported that microbial communities recovered after application of MBCA. However, in some cases contradictory reports are made such as significant increases versus significant decreases in bacterial populations (Naseby et al., 2000; Cordier and Alabouvette, 2009). Generally speaking, changes to communities are only studied for a matter of months and generally no longer than a year so the long-term impact or changes to communities is unknown. In the majority of cases, researchers addressing the impact of MBCA on microbial communities do so in tandem with disease control and could potentially have a bias in their perception of risk. Most studies compare treated and untreated soils, rather than attempt to find a baseline microbial community. This seems appropriate given it is a realistic reflection of the current microbial communities living in soils. Future studies should relate changes in microbial communities to environmental factors (e.g., temperature, flooding/drought events) and agronomic practices (e.g., tillage, fertilising, fallow). A detailed long-term and impartial assessment using updated molecular tools should be conducted for the risks posed by MBCA on microbial communities. This is particularly important for sensitive sites such as Botanic Garden collections or for use in nature reserves and Sites of Special Scientific Interest.

5.6 *Overview of the requirements for regulatory reform of microbial biocontrol agents*

A concern that the current method of regulating and authorising MBCA is not fully fit for purpose was expressed by participants. There are currently approximately 400 BCA products registered for use in the United States, compared to approximately 120 in Europe (WBF, 2022). This disparity is largely due to the different regulatory processes (Frederiks & Wesseler, 2019). Some products which likely have BCA activity are registered through the less onerous biostimulants route, with disease control claims made in marketing information rather than on label. This likely adds to the confusion and scepticism that surrounds the use of alternative PPP products. Credible data of efficacy will be required to evidence label claims, but the threshold of efficacy and reproducibility required for chemical PPP registration may be too high and inappropriate for BCA. With regards to assessing their impact on the environment, key to the development of a new system would be to move away from treating BCA in the same way as chemicals during the registration process. This is because some of the species used as BCA are already present in the wider environment, therefore their effects on native wildlife are already well understood.

Post-Brexit, GB and NI have an opportunity to develop a more streamlined registration system which provides the strongest possible environmental protections whilst supporting innovation in BCA. A legacy of the EU system is that the HSE are responsible for both chemical and BCA registration. It was argued during the workshop that BCA registration should fit in line with other environmental work areas (e.g., plant health, biosecurity, invasive species, etc.). Possibly a revised system, developed specifically for MBCA, that combines the work of HSE, Scottish Government and Defra and other environmental work areas would be a pragmatic way forward.

5.7 *Conclusions on the opportunities and limitations for the microbial biocontrol agents in Scotland*

Microbial biocontrol has the potential to play an important role in sustainable plant protection within Scotland but it is evident that at present uptake is very low, even although commercial options are available, and despite a body of research and development activity. To fully realise the potential of MBCA in Scotland activity needs to address:

- Information and research on the availability, efficacy, and cost of products for common pathogens.
- Research into potential risk to native microbial communities from the use of MBCA, particularly over a long-time frame.
- Advice tailored for Scottish climates.
- Research and development to improve the efficacy of MBCA products.
- Collaboration to develop a tailored registration process.

Lack of awareness and a need for advice on MBCA were identified as factors in limiting uptake. To this end some key points are suggested:

- Clearly define the term microbial biological control.
- Create a centralised database for MBCA to include information on availability, use, compatibility, application etc.
- Provide impartial guidance on use of MBCA products, including information on availability, compatibility, application, etc.
- Provide impartial advice with focus on Scotland.

Microbial biocontrol agents have the potential to reduce the long-term negative impacts of chemical pesticides on human, animals, non-target organisms and the environment. Using MBCA can also reduce the harvest interval periods, and potentially have fewer health and safety concerns to users and consumers compared to conventional PPP. The public perception of biological control is positive, being viewed as an environmentally friendly option. In addition, MBCA are seen as alternative methods for disease control, especially in cases where disease resistance has occurred.

Use of MBCA gives growers the opportunity to improve their environmental profiles and reduce the rate or number of applications of chemical control when following integrated control programmes. In addition, MBCA often offers more specific control against pathogens compared to chemical control. For example, *Ampelomyces quisqualis* gives specific control against powdery mildew. Reviews suggest that climate change will not have adverse effects on the potential for MBCA in the next 20 – 40 years in Scotland. It is possible, that with warming temperatures, conditions for MBCA could become more suitable. However, this could also mean more pathogens survive overwinter or expand their host ranges.

The workshop held for this project identified enthusiasm for the use of MBCA among participants. The participants were keen to engage, to share experiences and brainstorm ways to improve the experience for MBCA uses. Among consumers, MBCA receives a positive attitude, and this can be used as an opportunity to encourage uptake of MBCA by more growers. In protected crops there are already many examples of the use of MBCA; in strawberries *Ampelomyces 39uisqualis* (strain AQ10), *Aureobasidium pullulans*, and two *Bacillus* species are used against various pathogens. Many more commercial MBCA are available and registered within Scotland providing excellent opportunity to increase use within all plant health sectors.

To fully realise the potential of MBCA some barriers must be addressed. Firstly, there needs to be improved advice and guidance regarding MBCA. This should encompass information on the use and availability of MBCA for pathogens and plant hosts. There is a poor understanding amongst users of the compatibility of MBCA with conventional PPP programmes that can be addressed through directed KE activities. There is a need for specialist equipment for using MBCA products and investment to help growers increase their use of MBCA and to support upscaling the production of MBCA products. A number of research gaps have been identified in this project that will help to address some concerns raised around MBCA products.

Stakeholders widely believed that the current regulation system for MBCA is not fit for purpose. This is largely because the pre-existing chemical registration process is being used for BCA which is means that most of the tests are inappropriate. It currently takes about ten years to register a new product in the UK compared to two years in the US. Due to existing experts within environmental work areas (e.g., plant health, biosecurity, invasive species, etc.), stakeholders suggested that they may be better placed than HSE to manage aspects of BCA registration. Finally, an agreed definition of MBCA must be accepted to avoid confusion. We suggest that the definition proposed by Stenberg *et al.*, (2021) and adopted by Collinge *et al.* (2022) is used: 'Biological control or biocontrol is the process of controlling a pest, disease or weed using another living organism for human benefit'.

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7 Appendices

Appendix A: Prepared Slido questions put to participants during the workshop. Slido questions allowed multiple or single choice answers, ratings, and produced word clouds.

Question	**Answer Type**
Which sector(s) do you work in? (select all that apply)	Multiple choice (Multiple answer)
What is your role? (select all that apply)	Multiple choice (Multiple answer)
What is your opinion of microbial biological control agents?	Rating (1-10)
Do you use/recommend microbial biological control?	Quiz (Single choice)
If yes, tell us about the agent(s) you use/recommend specifying the agent, crop and disease. For examp‘e: 'Contans WG (*Coniothyrium minitans*)/ carrot/ Sclerotinia dis’ase'	Word cloud
How much experience do you have using microbial control agents?	Quiz (Single choice)
How do you prefer to apply microbial agents?	Word cloud
How reliable/effective do you think they are?	Rating (1-5)
How do you think they compare to chemical control agents?	Rating (1-5)
What barriers do you find prevent greater uptake of MBCA? (select all that apply)	Multiple choice (Multiple answer)
What other barriers do you think prevent greater uptake?	Word cloud
Do you consider there to be any environmental effects associated with the application of MBCA?	Quiz (Single choice)
What is your opinion of microbial biological control agents now?	Rating (1-10)
Do you consider there to be any environmental effects associated with the application of MBCA?	Quiz (Single choice)

Appendix B: Details of microbial biocontrol products registered for plant pathogens in the UK. Details include MBCA, product name, manufacturer, target pathogen and plant host. Information collated from the HSE pesticides register (HSE, 2023b), *BPDB: Bio-Pesticides DataBase (Lewis et al., 2016) and individual product labels.*

MBCA species	Product name	Manufacturer	Target organism	Host
Ampelomyces quisqualis strain AQ10	AQ10	Fargo	Powdery mildew	Aubergine (protected), courgette and summer squash (protected), cucumber (protected), melon (protected), pepper and chilli (protected), strawberry (protected), tomato (protected), winter squash and pumpkin (protected)
Aureobasidium pullulans strain DSM 14940 and DSM 14941	• Blossom Protect • Boni Protect	• Masstock Arable (UK) Ltd • Nufarm UK Ltd	*Erwinia amylovora*	Apple (outdoor); medlar (outdoor); pear (outdoor); quince (outdoor)
Aureobasidium pullulans strain DSM 14940 and DSM 14941	Botector	Nufarm UK Ltd	*Erwinia amylovora*	Strawberry, table grapes, wine grapes
Aureobasidium pullulans strain DSM 14940 and DSM 14941	BOTECTOR	Nufarm UK ltd	*Erwinia amylovora*	Apricot, cherry, peach and nectarine, plum, strawberry, table grapes, wine grapes
Bacillus amyloliquefaciens D747	Amylo-X WG	Certis Belchim	Powdery mildew; *Sclerotinia* spp.; *Botrytis cinerea*	Aubergine (permanent protection with full enclosure), blackberry (permanent protection with full enclosure), blackcurrant and redcurrant (permanent protection with full enclosure), blueberry (permanent protection with full enclosure), chic–ry - witloof (permanent protection with full enclosure), courgette and summer squash (permanent protection with full enclosure), cress (permanent protection with full enclosure), cucumber (permanent protection with full enclosure), endive (permanent protection with full enclosure), gooseberry (permanent protection with full enclosure), 'amb's lettuce (permanent protection with full enclosure), land cress (permanent protection with full enclosure), lettuce (permanent protection with full

MBCA species	Product name	Manufacturer	Target organism	Host
				enclosure), loganberry and rubus hybrid (permanent protection with full enclosure), melon (permanent protection with full enclosure), mushroom (permanent protection with full enclosure), pepper and chilli (permanent protection with full enclosure), raspberry (permanent protection with full enclosure), red mustard (permanent protection with full enclosure), rocket (permanent protection with full enclosure), spinach (permanent protection with full enclosure), spinach beet (permanent protection with full enclosure), strawberry (permanent protection with full enclosure), tomato (permanent protection with full enclosure), watercress (permanent protection with full enclosure), watermelon (permanent protection with full enclosure), winter squash and pumpkin (permanent protection with full enclosure)
Bacillus amyloliquefaciens strain FZB24	Taegro	Syngenta UK ltd	Powdery mildew; Downy mildew; early blight; *Botrytis sp.*	Aubergine (permanent protection with full enclosure), courgette and summer squash (permanent protection with full enclosure), cucumber (permanent protection with full enclosure), lettuce (permanent protection with full enclosure), melon (permanent protection with full enclosure), pepper and chilli (permanent protection with full enclosure), strawberry (permanent protection with full enclosure), table grapes (outdoor), tomato (permanent protection with full enclosure), watermelon (permanent protection with full enclosure), wine grapes (outdoor)
Bacillus amyloliquefaciens strain MBI600	Serifel	BASF plc	*Rhizoctonia solani; Pythium ultimum; Fusarium* spp.	Aubergine (permanent protection with full enclosure), blackberry (protected), blueberry (protected), choi sum (permanent protection with full enclosure), endive (permanent protection with full enclosure), 'amb's lettuce (permanent protection with full enclosure), lettuce (permanent protection with full enclosure), loganberry and rubus hybrid, oriental

MBCA species	Product name	Manufacturer	Target organism	Host
				cabbages (permanent protection with full enclosure), pepper and chilli (permanent protection with full enclosure), raspberry (protected), rocket (permanent protection with full enclosure), spinach (permanent protection with full enclosure), spinach beet (permanent protection with full enclosure), strawberry (protected), tomato (permanent protection with full enclosure)
Bacillus amyloliquefaciens strain MBI600	Integral Pro	BASF plc	*Phoma lingam*	Oilseed rape (winter)
Bacillus amyloliquefaciens strain QST 713	Harmonix Turf Defense	• Bayer CropScience Ltd • 2022 Environmental Science FR SAS	*Microdochium nivale* ; *Anthracnose*; Dollar Spot disease	Managed amenity turf
Bacillus pumilus QST2808	Sonata	Bayer CropScience Ltd	Powdery mildew	Aubergine (permanent protection with full enclosure); blackberry (protected); blackcurrant and redcurrant (protected); cucumber (permanent protection with full enclosure); endive (protected); lamb's lettuce (protected); pepper and chilli (permanent protection with full enclosure); raspberry (protected); strawberry (protected); tomato (permanent protection with full enclosure)
Bacillus subtilis strain QST 713	• Serenade ASO • Solani	• Bayer CropScience Ltd • Russell IPM ltd	*Botrytis cinerea; Sclerotinia* spp.*; Helminthosporium solani*	Aubergine (permanent protection with full enclosure); lettuce (permanent protection with full enclosure); pepper and chilli (permanent protection with full enclosure); potato; strawberry (protected); tomato (permanent protection with full enclosure)
Candida oleophila strain O	Nexy 1	• BioNext sprl • Agrauxine	*Botrytis cinerea; Penicillium expansum*	Apple (post-harvest use); pear (post-harvest use)

MBCA species	**Product name**	**Manufacturer**	**Target organism**	**Host**
Coniothyrium minitans strain CON/M/91-08 (DSM 9660)	• Contans WG • Lalstop Contans WG	• Bayer CropScience Ltd • Danstar Ferment AG	*Sclerotinia sclerotiorum; Sclerotinia minor*	All edible crops; all non-edible crops
Gliocladium catenulatum	• Prestop • Prestop Mix	• Everris Ltd • Danstar Ferment AG • Fargro Ltd	*Botrytis cinerea; Didymella bryoniae; Mycospharella* sp.; *Pythium* spp.; *Fusarium* spp.; *Phytophthora* spp.; *Rhizoctonia* spp.	All edible crops (protected); all non edible crops (protected); strawberry
Mild Pepino Mosaic Virus isolate VC1 and VX1	V10	Valto B.V.	Pepino mosaic virus	Tomato (permanent protection with full enclosure)
Pepino mosaic virus strain CH2 isolate 1906	PMV-01	De Ceuster Meststoffen nv	Pepino mosaic virus	Tomato (permanent protection with full enclosure)
Phlebiopsis gigantea	PG Suspension	Forest Research	*Heterobasidion annosum*	Farm forestry (stump); forest (stump); forest nursery (stump)
Pseudomonas chlororaphis MA 342	Cerall	Koppert (UK) Ltd	Seed-borne fungal diseases such as *Fusarium* spp.; *Tilletia caries*; *Septoria nodorum*	Rye (seed); triticale (seed); wheat (seed)
Pseudomonas Sp. DSMZ 13134	Proradix	SP Sourcon Padena GmbH	*Rhizoctonia solani*	Potato (seed)
Pythium oligandrum M1	Polyversum	• De Sangosse SAS • BIOPREPARÃTY; spol. s r.o.	*Alternaria* spp.; *Botrytis cinerea; Fusarium* spp.; *Phoma* spp.; *Phytophtora* spp.; *Pythium* spp.;	*barley (spring), barley (winter), durum wheat, oats, oilseed rape, rye, triticale, wheat (spring), wheat (winter)*

MBCA species	Product name	Manufacturer	Target organism	Host
			Sclerotinia sclerotiorum; Rhizoctonia solani	
Streptomyces griseoviridis strain K61	• LALSTOP K61 WP • Mycostop	Danstar Ferment AG	*Fusarium; Pythium; Phytophthora; Alternaria; Rhizoctonia*	all edible crops (protected), all edible crops (protected, seed), all non edible crops (protected), all non edible crops (protected, seed), angelica (protected), aubergine (protected), balm (protected), basil (protected), bay (protected), bulb onion (protected), caraway leaves, celery leaves (protected), chervil (protected), coriander leaves (protected), courgette and summer squash (protected), cress (protected), cucumber (protected), dill leaves (protected), edible flowers (protected), endive (protected), fennel leaves (protected), garlic (protected), he–rb - other (protected), hyssop (protected), 'amb's lettuce (protected), land cress (protected), lettuce (protected), lovage leaves (protected), marjoram (protected), melon (protected), mint (protected), oregano (protected), ornamental plant production (before planting), ornamental plant production (protected), parsley (protected), pepper and chilli (protected), rocket (protected), rosemary (protected), sage (protected), salad burnet (protected), salad onion (protected), savory (protected), shallot (protected), spinach (protected), spinach beet (protected), sweet cicely, sweetcorn (protected), tarragon (protected), thyme (protected), tomato (protected), watercress (protected), watermelon (protected), winter squash and pumpkin (protected)
Trichoderma asperellum strain T34	T34 BIOCONTROL	Fargro Ltd	*Fusar 52 uisqualioru m* f.sp.*dianthi; Pythi um*	Aubergine (permanent protection with full enclosure); ornamental plant production; pepper and chilli (permanent protection with full enclosure); tomato (permanent protection with full enclosure)

MBCA species	Product name	Manufacturer	Target organism	Host
Trichoderma atroviride strain SC1	Vintec	• Belchim Crop Protection Ltd • Certis Belchim B.V.	ESCA diseases *Phaeomoniel la chlamydospore; Togninia minima*.	Wine grapes (outdoor)
Trichoderma harzianum strain T22	Trianum G;	Koppert (UK) Ltd	*Pythium* spp.*; Rhizoc tonia* spp.*; Fusarium* spp.*; Scler otinia* spp.*; Microdochium* spp.	Baby leaf crops; cucumber (protected); dw53uisquench bean (protected); lettuce (protected); ornamental plant production (protected); pepper and chilli (protected); tomato (protected)
Trichoderma harzianum strain T22	Trianum P	Koppert (UK) Ltd	*Pythium* spp.*; Rhizoc tonia* spp.*; Fusarium* spp.*; Scler otinia* spp.*; Microdoc hium* spp.	Aubergine (protected); baby leaf crops; cucumber (protected); lettuce (protected); ornamental plant production (protected); pepper (protected); tomato (protected)
Verticillium alobo-atrum	Dutch Trig	BTL Bomendienst B.V.	Ophiostoma ulmi; Ophiostoma novo-ulmi	Amenity vegetation

Appendix C: Microbial -based biopesticides used in soft fruits for disease management as reported in 2022 pesticide use survey, detailing area (ha) and weight (kg). Adapted from Wardlaw et al., *2023.*
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Biological control agent	**Crop**	**ha**	**kg**
Ampelomyces quisqualis strain AQ10	Strawberry	239	10
Aureobasidium pullulans	Strawberry	107	27
Bacillus amyloliquefaciens strain D747	Strawberry		34
Bacillus subtilis strain QST 713	Strawberry	1,618	124
Cerevisane *(saccharomyces cerevisiae* strain *LAS 117)*	Strawberry	38	27
Gliocladium catenulatum strain J1446	Strawberry	49	79
Trichoderma harzianum	Strawberry	2	<0.05
Bacillus amyloliquefaciens strain D747	Raspberry	8	3
Bacillus subtilis strain QST 713	Raspberry	135	11
Aureobasidium pullulans	Other soft fruit	3	1
Bacillus pumilus strain QST 2808	Other soft fruit	1	6
Bacillus subtilis strain QST 713	Other soft fruit	3	14

Appendix D: Application methods of MBCA used by participants.

Application methods
As per the label recommendations
Commercial forestry equipment (helicopter, harvest machinery, knapsack sprayers)
Drench
Drip irrigation
Early spray application
Endophytic colonisation
Fertigation
Foliar sprays
Formulated products
Granular formulations
High volume spray
Mixed into growing media
Seed treatment
Soil drench
Tried using conventional pesticide applicators.

Appendix E: Advantages and disadvantages of using a website or online factsheets to disseminate information on the use of microbial biocontrol agents.

Advantage	**Disadvantage**
Websites	
Company website databases are available	Databases are not connected
Websites generally reach wider audience than paper (e.g., magazines, conference proceedings, peer reviewed paper)	Needs to be managed well with regular updates
Open access (company websites)	On some company websites it is still hard to find label information
Web format searchable and AI integration in websites	Searchability within websites can be poor
Factsheets	
Trusted source	Digital accessibility (including neurodiversity)
Industry standard	Pay walls (e.g., CABI, peer review papers)
Information can be condensed into best practice information	Application/storage and other information not always on factsheets
	Awareness of who produces factsheets

Appendix F: Advantages and disadvantages of using training and webinars to disseminate information on the use of microbial biocontrol agents.

Advantage	**Disadvantage**
Accessible (webinars can be joined in-field)	Information overload in short space of time at training events
On-farm training popular with farmers	Patchy understanding (no formal qualification from many training events)
Cross group communication; Pull farmers, horticulture sector and amateurs at training events	Ingrained behaviour and generational differences are not easily addressed at training events
Webinar training can be freely available	Who is providing the training?
Provide training for certification standards (LEAF, Red Tractor, BASIS)	Hard to reach wider audiences, especially at in person events
Face to face training builds professional relationships and enhances engagement	Webinars are impersonal and engagement is harder online

Appendix G: Advantages and disadvantages of using an advisory service to disseminate information on the use of microbial biocontrol agents.

Advantage	**Disadvantage**
Personal relationship with client to give tailored advice	Adviser only as good as the information they get (impartiality)
Advisors should give trusted and accurate advice	Not all advisers are independent and might have a conflict of interest (incentivised advice (i.e. commission))
Legislated impartiality	Adviser takes some liability for failure – may limit the advice they are prepared to give
	Underfunded resulting in dis-joined provision of advice
Who would fund an independent service? Government? Devolved administrations/councils?	

Plant Health Centre
c/o The James Hutton Institute
Invergowrie,
Dundee, DD2 5DA

Tel: +44 (0)1382 568905

Email: Info@PlantHealthCentre.scot
Website: www.planthealthcentre.scot
Twitter: @PlantHealthScot